转基因技术在大豆育种中的应用

练 云[1],梁慧珍[1],余永亮[1],王树峰[1],位艳丽[1],董 薇[1],张孟臣[2],蒋春志[2]

(1.河南省农业科学院 经济作物研究所,国家大豆改良中心郑州分中心,河南 郑州 450002; 2.河北省农林科学院 粮油作物研究所,国家大豆改良中心石家庄分中心,河北 石家庄 050031)

摘 要:获得转基因植株是植物基因工程的基础,但是由于转化效率较低,在转基因的过程中通常需要一个有效筛选细胞和组织的手段,于是选择标记基因被广泛应用。然而,选择标记基因的使用,存在生物安全性和环境安全性隐患。因此,建立一个安全高效稳定的大豆转化体系,是改善大豆产量、品质、抗逆能力,培育新型大豆品种的保障。该文综述了新型标记基因在大豆育种上的应用及转基因技术在大豆和其它作物育种中的应用。

关键词:大豆;选择标记基因;遗传转化;转基因技术

中图分类号:S565.1 **文献标识码**:A **文章编号**:1000-9841(2011)02-0333-04

Application of Transgenic Technology in Soybean Breeding

LIAN Yun[1], LIANG Hui-zhen[1], YU Yong-liang[1], WANG Shu-feng[1], WEI Yan-li[1], DONG Wei[1], ZHANG Meng-chen[2], JIANG Chun-zhi[2]

(1. National Soybean Improvement Center Zhengzhou Sub-center, Institute of Economic Crops, Henan Academy of Agricultural Sciences, Zhengzhou 450002, Henan; 2. National Soybean Improvement Center Shijiazhuang Sub-center, Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang 050031, Hebei, China)

Abstract: A prerequisite for plant genetic engineering is to produce transgenic plants. Selectable marker genes have been widely used in plant transformation because of the low efficiency of transgenic integration. However, the use of selectable marker genes would affect the safety evaluation of transgenic plants and there are perceived risks in bio-safety and environmental safety. An effective soybean transformation system is necessary to breed new soybean varieties in improving the yield, quality and resistance. In this review, the new selectable marker genes in soybean breeding and transgenic technology in crops breeding were summarized.

Key words: Soybean; Selectable marker genes; Genetic transformation; Transgenic technology

1 新型标记基因的应用

最常用的选择标记基因编码的蛋白是抗生素或除草剂,通过赋予转化细胞抗生素抗性或除草剂抗性,从而使转化细胞能在含有一定浓度的抗生素或除草剂的培养基中生长,非转化细胞被杀死,这样得到的转基因植株中除了目的基因,还包含标记基因,目前解决这一问题有 2 个策略:一是从转基因植物中去除或分离出标记基因,获得无筛选标记转基因植株,主要技术有共转化法[1-2]、MAT 载体系统[3-4]、重组定位系统[5]等,但这些方法在实际应用中,要得到转基因作物,目前还比较困难;另一个策略是使用抗生素和除草剂抗性之外的标记基因,近年来,已研究出一些安全标记基因,比如从可食用的植物中分离出来的选择标记基因,这些标记基因没有抗生素和除草剂抗性,不用担心由于"基因逃逸"而产生临床应用抗生素失效或由于产生"超级杂草"而破坏生态平衡问题,相对来说对生物是安全的。

目前,非抗生素类选择标记性基因已广泛应用于转基因领域。Rao 等用非抗生素选择剂 s-(2-氨基乙基)L-半胱氨酸(AEC)对大豆体细胞进行筛选,得到了转 *DHPS* 基因的转基因大豆[6];苄嘧磺隆(BSM)是一个 SU-type ALS 抑制剂,Pornprom 等将 BSM 用于大豆体细胞胚分裂增殖过程中所用的培养基中,证实体细胞的生长完全受到抑制[7];乙酰乳酸合成酶基因(*ALS*)是支链蛋白质合成通路中的第 1 个常见酶,该基因中特定氨基酸突变可以提高对抑制乙酰乳酸合酶类除草剂的抗性,如磺酰脲类和嘧啶羧酸类除草剂。Kawai 等从抗除草剂的水

收稿日期:2010-11-23

基金项目:引进国际先进农业科学技术计划资助项目(2009-Z34,2010-Z34);国家转基因重大专项资助项目(2008ZX08004-005,2009ZX08004-001B,2009ZX08018-001B)。

第一作者简介:练 云(1978-),女,博士,助理研究员,主要从事大豆遗传育种工作。E-mail:lianyun262@gmail.com。

通讯作者:梁慧珍(1968-),女,博士,研究员,主要从事大豆遗传育种与品质改良研究。E-mail:lhzh66666@163.com。

稻愈伤中分离出了 1 个两点突变的 *ALS* 基因,并已证明转化有将第 95 位的甘氨酸突变成丙氨酸的乙酰乳酸合酶基因的水稻植株表现出对嘧啶羧酸类除草剂的抗性,表明该基因在转基因水稻中可以用作选择标记基因[8]。Tougou 等证明转化定点突变的 *ALS* 基因的转基因大豆,在 T_1 代用嘧啶羧酸类除草剂筛选显示出抗性,遗传分析表明 *Os-mALS* 基因在大豆中能够正确表达,表明 *Os-mALS* 基因也可以作为选择标记基因在转基因大豆中进行应用[9]。突变的 *ALS* 基因在其它作物中也被用作筛选标记基因[10-11]。

2 转基因技术在大豆及其它作物育种中的应用

转基因技术在大豆上的应用,通常是用来改善大豆种子的成分,比如积累亚麻酸[12]或 a-生育酚[13],去除大豆的过敏蛋白抗原[14]或去除植酸[15-16]等。由于农杆菌介导方法具有转化效率相对较高、T-DNA 插入相对稳定[17]、目的基因能够稳定表达、转基因后代表型能恢复正常[18]等优点,因此,农杆菌介导的大豆高效转化方法多有报道[19-22],使用 *hpt* 作为筛选标记,将大豆矮化病毒(*SbDV*)基因导入大豆,得到了大豆矮化抗性的转基因大豆[23-24]。农杆菌介导的遗传转化在其它作物中也得到了广泛应用。比如,在提高棉花抗虫方面:表达 2 个基因(*Bt* + *CpTI*, *Bt* + *GNA*, *Bt* + *sck*)和 3 个基因的(*Bt* + *CpTI* + *GNA*)转基因棉花相继报导[25-29];将来自拟南芥的乙烯受体突变基因 *etr*1-1 转入开花植物文心兰和齿舌兰中,减少它们对外源乙烯的敏感性,以延长开花时间,转化效率达到 1.3%~2.7%[30];利用广泛种植的粮食作物来生产重组药品有可能污染人类食物链[31],用檀香胚性细胞悬浮系培养表达系统重组蛋白显得非常重要[32],Shekhawat 首次通过农杆菌介导的胚性细胞悬浮系将外源蛋白(β-glucuronidase,β-葡萄苷酸酶)在檀香中高水平稳定表达[33];Polin 首次将草酸氧化酶通过转基因手段导入栗属物种[34]。

在植物转化中,根据所用受体类型,转基因方法可以分为三大类:一、以外植体为受体的基因转化方法,包括农杆菌介导法、基因枪法、超声波介导法;二、以种质系统为受体的基因转化方法,包括子房注射法、花粉管通道法;三、以原生质体为受体的基因转化方法,包括聚乙二醇法、电击法、脂质体法。其中,以外植体为受体的转化方法应用较为广泛,由于原生质体培养难度大、再生频率低、而且再生植株的遗传稳定性差等缺陷,因此以原生质体为受体的基因转化方法未被广泛使用。另外,起始于 20 世纪 50 年代初的植物花药培养,在许多作物尤其是油菜、大白菜等十字花科作物育种工作中占有重要地位,花药培养是获得单倍体及纯合体的有效途径。20 世纪 60 年代在烟草、水稻、小麦、玉米等重要农作物中都相继获得了单倍体植株。到目前为止,中国已有 40 多种植物用花药培养方法获得了花粉植株,主要有大麦、小麦、玉米、水稻等[35-39],但花药培养在大豆上的研究却很少。大豆花药培养最早由 Ivers 等[40]开展起来,并首次建立了小孢子发育与小花特征相对应的取材标准,认为长度 2.5 mm 的小花是分离花药的合适材料,此时 30% 的花药处于四分体后期,70% 的花药处于单核期,但只获得了体细胞愈伤组织。母秋华等[41]诱导出了花药愈伤组织,但没有分化出芽。简玉瑜等[42-43]和尹光初等[44-45]分别对 B5 培养基进行了研究改良,在此基础上愈伤组织诱导率有了较多提高,并分化出了少量绿芽和几棵再生植株。刘德璞等[46]首次尝试了利用花粉培养并诱导出了单倍性的愈伤组织;叶兴国等[47],经过染色体观察,开始产生的愈伤组织大多为体细胞愈伤组织,染色体数 2n = 40 条,淡黄色、结构松散、形状不规则、增殖较快;30 d 后产生的愈伤组织大多为单倍体愈伤组织,染色体数 2n = 14 ~ 26 条,乳白色、结构致密、形状似球、增殖较慢。花药培养作为一种育种手段已在许多作物上取得了成功,但是近十多年来,关于大豆花药培养方面的研究很少,如果能将这项技术成功应用于大豆,将会加速大豆新品种的选育过程,缩短育种年限和提高育种效率。

建立一个高效稳定的大豆转化体系,是许多致力于大豆遗传改良的科学家深入研究的课题之一。关于大豆遗传转化在不同的 DNA 输送方法和采用不同受体材料方面有诸多报道,包括:基因枪法配合茎顶端分生组织[48]、胚性细胞悬浮系[49-52]、根癌农杆菌介导的子叶节[53-54],尽管各种方法都取得了一定的成就,但具体到每一种方法在时间、成本和转化效率方面都有各自的缺陷。水稻细胞悬浮培养结合超声波方法进行转化[55]的成功应用,为大豆通过悬浮细胞培养进行转化提供了借鉴,该方法的优点在于操作简便、启动成本低、具有扩大规模的潜力;Khalafalla MM 通过比较超声波微创法(WSS)和基因枪法转化大豆的转化效率,得到的结论是 WSS 转化效率较高[56],因此,超声波微创法可望成为大豆转化的另一个有效的方法。

3 大豆遗传转化存在的问题及展望

在农杆菌介导的大豆遗传转化中,主要采用以

子叶节为外植体的遗传转化方法,尽管大豆转基因技术体系已经建立,但与其它作物尤其是模式作物相比,由于大豆组织培养过程中的植株再生比较困难,导致遗传转化频率低,严重制约着转基因育种效率,因此,其转化技术及发生机理还需进一步探讨。另外,花粉培养是在花药培养的基础上发展起来的技术,它对单细胞育种实践及植物遗传工程具有重要意义。目前,转基因大豆的主体是抗除草剂品种,今后,抗虫、改善营养成分将是转基因大豆的重点,只有建立良好的大豆再生体系,并结合适宜的转化方法,才有可能使转基因技术在大豆的性状改良、新品种的培育中发挥作用。

参考文献

[1] Daley M, Knauf K R, Summerfert K R, et al. Co-transformation with one *Agrobacterium tumefaciens* strain containing two binary plasmids as a method for producing marker-free transgenic plants [J]. Plant Cell Reports, 1998, 17:489-496.

[2] Donaldson P A, Simmond D H. Susceptibility to *Agrobacterium tumefaciens* and cotyledonary node transformation in short-season soybean[J]. Plant Cell Reports, 2000, 19:478-484.

[3] Ebinuma H, Sugita K, Matsunaga E, et al. Systems for the removal of a selection marker and their combination with a positive marker[J]. Plant Cell Reports, 2001, 20:383-392.

[4] Endo S, Sugita K, Sakai M, et al. Single-step transformation for generating marker-free transgenic rice using the ipt-type MAT vector system[J]. Plant Journal, 2002, 30: 115-122.

[5] Song H Y, Ren X S, Si J, et al. Construction of marker-free GFP transgenic tobacco by Cre/lox site-specific recombination system [J]. Scientia Agricultura Sinica, 2008, 41(10):2973-2982.

[6] Rao S S, Mamadou L, McConnell M, et al. Non-antibiotic selection systems for soybean somatic embryos: the lysine analog aminoethyl-cysteine as a selection agent [J]. BMC Biotechnology, 2009, 9: 94.

[7] Pornprom T, Usui K, Ishizuka K. Growth inhibition and acetolactate synthase activity of soybean seedlings and suspension-cultured cells treated with bensulfuron-methyl[J]. Weed Biology Management, 2005, 5:150-153.

[8] Kawai K, Kaku K, Izawa N, et al. A novel mutant acetolactate synthase gene from rice cells, which confers resistance to ALS-inhibiting herbicides[J]. Journal of Pesticide Science, 2007, 32: 89-98.

[9] Tougou M, Yamagishi N, Furutani N, et al. The application of the mutated acetolactate synthase gene from rice as the selectable marker gene in the production of transgenic soybeans[J]. Plant Cell Reports, 2009, 28:769-776.

[10] Ganapathi T R, Higgs N S, Balint-Kurti P J, et al. Agrobacterium-mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB) [J]. Plant Cell Reports, 2001, 20:157-162.

[11] Ray K, Jagannath A, Gangwan S A, et al. Mutant acetolactate synthase gene is an efficient *in vitro* selectable marker for the genetic transformation of *Brassica juncea* (oilseed mustard) [J]. Journal of Plant Physiology, 2004, 161:1079-1083.

[12] Park J, Lee Y K, Kang B K, et al. Co-transformation using a negative selectable marker gene for the production of selectable marker gene-free transgenic plants[J]. Theoretical and Applied Genetics, 2004, 109:1562-1567.

[13] Vidal J R, Kikkert J R, Wallace P G, et al. High-efficiency biolistic co-transformation and regeneration of 'Chardonnay' (*Vitis vinifera* L.) containing npt-II and antimicrobial peptide genes [J]. Plant Cell Reports, 2003, 22:252-260.

[14] Eckert H, La Vallee B, Schweiger B J, et al. Co-expression of the borage Delta 6 desaturase and the *Arabidopsis* Delta 15 desaturase results in high accumulation of stearidonic acid in the seeds of transgenic soybean[J]. Planta, 2006, 224:1050-1057.

[15] Eenennaam A L V, Lincoln K, Durrett T P, et al. Engineering Vitamin E content: from *Arabidopsis* mutant to soy oil[J]. The Plant Cell, 2003, 15:3007-3019.

[16] Herman E M, Helm R M, Jung R, et al. Genetic modification removes an immunodominant allergen from soybean[J]. Plant Physiology, 2003, 132:36-43.

[17] Shi J, Wang H, Schellin K, et al. Embryo-specific silencing of a transporter reduces phytic acid content of maize and soybean seeds [J]. Nature Biotechnology, 2007, 25:930-937.

[18] Nunes A C, Vianna G R, Cuneo F, et al. RNAi-mediated silencing of the myo-inositol-1-phosphate synthase gene (*GmMIPS*1) in transgenic soybean inhibited seed development and reduced phytate content[J]. Planta, 2006, 224:125-132.

[19] Olhoft P M, Flage L E, Donovan C M, et al. Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method[J]. Planta, 2003, 216(5):723-735.

[20] Zeng P, Vadnais D A, Zhang Z, et al. Refined glufosinate selection in Agrobacterium-mediated transformation of soybean [*Glycine max* (L.) Merrill][J]. Plant Cell Reports, 2004, 22(7):478-482.

[21] Paz M M, Shou H X, Guo Z B, et al. Assessment of conditions affecting Agrobacterium-mediated soybean transformation using the cotyledonary node explant[J]. Euphytica, 2004, 136(2):167-179.

[22] Sato H, Yamada T, Kita Y, et al. Production of transgenic plants and their early seed set in Japanese soybean variety, Kariyutaka [J]. Plant Biotechnology, 2007, 24:533-536.

[23] McCabe D E, Swain W F, Martinell B J, et al. Stable transformation of soybean (*Glycine max*) by particle acceleration[J]. Biotechnology, 1988, 6: 923-926.

[24] Finer J J, McMullen M D. Transformation of soybean via particle bombardment of embryogenic suspension culture tissue [J]. In vitro Celluar and Developmental Biology - Plant, 1991, 27:175-182.

[25] El-Shemy H A, Khalafalla M M, Wakasa K, et al. Reproducible transformation in two grain legumes-soybean and azuki bean-using different systems [J]. Cellular & Molecular Biology Letters, 2002, 7:709-719.

[26] El-Shemy H A, Teraishi M, Khalafalla M M, et al. Isolation of soybean plants with stable transgene expression by visual selection based on green fluorescent protein [J]. Molecular Breeding,

2004, 14:227-238.

[27] Khalafalla M M, Rahman S M, El-Shemy H A, et al. Optimization of particle bombardment conditions by monitoring of transient sGFP(S65T) expression in transformed soybean[J]. Breeding Science, 2005, 55:257-263.

[28] Parrott W A, Hoffman L M, Hildebrand D F, et al. Recovery of primary transformants of soybean[J]. Plant Cell Reports, 1989, 7:615-617.

[29] Yan B, Srinivasa R M S, Collins G B, et al. Agrobacterium tumefaciens-mediated transformation of soybean [*Glycine max* (L.) Merrill.] using immature zygotic cotyledon explants[J]. Plant Cell Reports, 2000, 19:1090-1097.

[30] Terakawa T, Hisakazu H, Masanori Y. Efficient whisker-mediated gene transformation in a combination with supersonic treatment [J]. Breeding Science, 2005, 55:456-358.

[31] Khalafalla M M, El-Shemy H A, Rahman S M, et al. Efficient production of transgenic soybean[*Glycine max* (L). Merrill] plants mediated via whisker-supersonic (WSS) method[J]. African Journal of Biotechnology, 2006, 5:1594-1599.

[32] Tougou M, Furutani N, Yamagishi N, et al. Development of resistant transgenic soybeans with inverted repeat-coat protein genes of soybean dwarf virus[J]. Plant Cell Reports, 2006, 25:1213-1218.

[33] Tougou M, Yamagishi N, Furutani N, et al. Soybean dwarf virus-resistant transgenic soybeans with the sense coat protein gene[J]. Plant Cell Reports, 2007, 26:1967-1975.

[34] Liu H K, Chao Y, Wei Z M. Effficient Agrobacterium tumefaciens-mediated transformation of soybeans using an embryonic tip regeneration system[J]. Planta, 2004, 219:1042-1049.

[35] 魏凌基,王咏星,张薇. 大麦花药离体培养及植株再生研究初报[J]. 石河子农学院学报, 1995, 32 (4):60. (Wei L J, Wang Y X, Zhang W. A preliminary report on the study of Hordeum Sativum Jess' anther *in vitro* culture and plantlet regeneration[J]. Journal of Shihezi University, 1995, 32 (4):60.)

[36] 韩晓峰,陶丽莉,殷桂香,等. 基因型和环境条件对小麦花药培养效果的影响[J]. 作物学报, 2010, 36(7): 1209-1215. (Han X F, Tao L L, Yin G X, et al. Effect of genotype and growing environment on anther culture in wheat[J]. Acta Agronomica Sinica, 2010, 36(7):1209-1215.)

[37] 隋新霞,樊庆琦,李根英,等. 小麦花药培养研究进展[J]. 麦类作物学报, 2005, 25 (4):127-131. (Sui X X, Fan Q Q, Li G Y, et al. Review on wheat anther culture[J]. Journal of Triticeae Crops, 2005, 25(4):127-131.)

[38] 付迎军. 玉米离体花药培养体系的建立[J]. 延边大学农学学报, 2004, 26(1):1-5. (Fu Y J. System establishment of maize anther culture *in vitro*[J]. Journal of Agricultural Science Yanbia University, 2004, 26(1):1-5.)

[39] 李艳萍. 水稻花药培养与花培育种研究[J]. 天津农业科学, 2004,9(4): 36-39. (Li Y P. Rice anther culture and anther-culture breeding[J]. Tianjin Agricultural Sciences, 2004, 9(4): 36-39.)

[40] Ivers D R, Palmer R R, Fehr W R. Anther culture in soybean [J]. Crop Science, 1974, 14:891-893.

[41] 母秋华, 杨玉环,张三顺. 花药培养学术讨论会文集[C]. 北京: 科学出版杜, 1977: 302-303. (Mu Q H, Yang Y H, Zhang S S. Seminars corpus on anther culture[C]. Beijing: Science Press,1977: 302-303.)

[42] 简玉瑜, 罗希明, 赵桂兰, 等. 花药培养学术讨论会文集[C]. 北京: 科学出版杜, 1977:209-211. (Jian Y Y, Luo X M, Zhao G L, et al. Seminars corpus on anther culture[C]. Beijing: Science Press, 1977: 209-210.)

[43] 简玉瑜, 孙玉华,陈永祥,等.大豆花药培养的研究[J]. 吉林农业科学,1980(2):54-61. (Jian Y Y, Sun Y H, Chen Y X, et al. Study on another culture of soybean[J]. Journal of Jilin Agricultural Sciences, 1980(2):54-61.)

[44] 尹光初, 李学湛,朱之垠, 等. 大豆花粉育株的研究[J]. 黑龙江农业科学, 1981(1):12-14. (Yin G C, Li X Z, Zhu Z Y, et al. Study on pollen sterile of soybean[J]. Journal of Heilongjiang Agricultural Sciences, 1981(1):12-14.)

[45] 尹光初, 朱之垠,徐振, 等. 大豆花粉植株的诱导及其雄核发育的研究[J]. 大豆科学, 1982, 1(1): 69-75. (Yin G C, Zhu Z Y, Xu Z, et al. Studies on induction of pollen plant and their androgenesis in *Glycine max* (L.) Merr[J]. Soybean Science, 1982, 1(1):69-75.)

[46] 刘德璞, 赵桂兰. 大豆花粉离体培养获得愈伤组织[J]. 大豆科学, 1986, 5(1):49-55. (Liu D P, Zhao G L. Calli were induced from anthers of soybean[J]. Soybean Science, 1986, 5 (1):49-55.)

[47] 叶兴国, 王连铮. 大豆花药培养研究进展[J]. 大豆科学, 1995, 14(4): 349-354. (Ye X G, Wang L Z. Advances of anther culture in soybean[J]. Soybean Science, 1995, 14(4):349-354.)

[48] Li R, Ma Z, Wang Q, et al. Identification and screening on insect resistance of *Bt/CpTI* transgenic cottons[J]. Plant Genetic Resources, 2005, 6:409-413.

[49] Wu X, Wang J, Zhu Z, et al. Study of transgenic cotton carrying *Bt-CpTI-GNA* genes[J]. Cotton Science, 2005, 17:353-359.

[50] Guo J, Zhu X, Guo W, et al. Inheritance analysis and resistance of the transgenic cotton harboring *Bt* + *sck* double genes to *Helicoverpa armigera*[J]. Cotton Science, 2007, 19:88-92.

[51] Li F F, Wu S J, Chen T Z, et al. Agrobacterium-mediated co-transformation of multiple genes in upland cotton[J]. Plant Cell Tissue Organ Culture, 2009, 97:225-235.

[52] Raffeiner B, Serek M, Winkelmann T. *Agrobacterium tumefaciens*-mediated transformation of *Oncidium* and *Odontoglossum orchid* species with the ethylene receptor mutant gene *etr*1-1[J]. Plant Cell Tissue Organ Culture, 2009, 98:125-134.

[53] Hileman B. Prodigene and Starlink incidents provide ammunition to critics[J]. Chemical Engeering News, 2003, 81:25-33.

[54] Murphy D J. Improving containment strategies in biopharming[J]. Plant Biotechnology, 2007, 5:555-569.

[55] Shekhawat U K S, Ganapathi T R, Srinivas L, et al. Agrobacterium-mediated genetic transformation of embryogenic cell suspension cultures of *Santalum album* L.[J]. Plant Cell Tissue Organ Culture, 2008, 92:261-271.

[56] Polin L D, Liang H, Rothrock R E, et al. Agrobacterium-mediated transformation of American chestnut [*Castanea dentata* (Marsh.) Borkh.] somatic embryos[J]. Plant Cell, Tissue and Organ Culture, 2006, 84:69-78.