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农杆菌介导大豆子叶节遗传转化体系的优化

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摘要: 利用农杆菌菌株EHA105, 对17个栽培大豆品种的大豆子叶节进行了侵染, 从大豆基因型、氯气灭菌时间、外植体活力、侵染浓度、侵染时间、共培养时间等方面进行了优化。结果表明: 氯气灭菌14~18 h, 可在不影响大豆种子活力的同时最大限度的减少污染; 暗处理1 d的外植体活力高于光照处理5~7 d的外植体; 菌液浓度 (OD_{600nm}) 在0.8~1.0且侵染浓度 (OD_{600nm}) 为0.6~0.8时GUS瞬时表达率最高; 适宜的侵染时间和共培养天数分别为30 min和4 d。在上述优化研究基础上, 形成一套综合的转基因体系, 该体系的最高转化率可达3.33%。不同处理下的GUS瞬时表达率以及17个大豆品种的丛生芽诱导率综合评价显示, 适宜转化的基因型为TL-1、HC-3、HC-6、Williams 82。

Abstract: In the present study, cotyledon nodes obtained from 17 soybean cultivars were infected with Agrobacterium tumefaciens EHA105. Subsequently, we optimized the soybean genotype, chlorine sterilization time, explant state, vitality of bacterial strain, concentration of infection liquid, infection time and co-cultivation time. As a result, when the chlorine sterilization time was 14-18 h, the minimal pollution and the highest seed vigor was observed. The vitality of overnight-treated explants in the dark were better than those germinated in the light for 5-7 d. The highest GUS transient rate was achieved when the bacterial concentration at OD_{600nm} was 0.8-1.0 and the infection liquid at OD_{600nm} was 0.6-0.8. We also found that the best infection and co-cultivation time were 30 min and 4 d, respectively. Based on the above optimized research, we established a comprehensive transgenic system which resulted in the maximum transformation efficiency of 3.33%. For the comprehensive evaluation about the transient GUS expression under different treatments and the shoot induction rate of 17 soybean varieties, TL-1, HC-3, HC-6 and Williams 82 were the ideal genotypes for transformation.

参考文献/References:

- [1] McCabe D E, Swain W F, Martinell B J, et al. Stable transformation of soybean (*Glycine max*) by particle acceleration [J]. Nature Biotechnology, 1988, 6(8): 923-926.
- [2] Finer J, McMullen M. Transformation of soybean via particle bombardment of embryogenic suspension culture tissue [J]. In Vitro Cellular & Developmental Biology-Plant, 1991, 27(4): 175-182
- [3] Nathalie D, Bernard P, Frederic G, et al. Generation of fertile transplastomic soybean [J]. Plant Molecular Biology, 2004, 55(4): 479-489
- [4] Zhang Z, Xing A, Staswick P, et al. The use of glufosinate as a selective agent in Agrobacterium-mediated transformation of soybean [J]. Plant Cell Tissue Organ Culture, 1999, 56(1): 37-46
- [5] Clemente T, La Valle B J, Howe A R, et al. Progeny analysis of glyphosate selected transgenic soybeans derived from Agrobacterium-mediated transformation [J]. Crop Science, 2000, 40(3): 797-803
- [6] Olhoft P M, Somers D A. L-Cysteine increases Agrobacterium-mediated T-DNA delivery into soybean cotyledonary-node cells [J]. Plant Cell Reports, 2001, 20(8): 706-711

- [7] Olhofs P M, Lin K, Galbraith J, et al. The role of thiol compounds in increasing Agrobacterium-mediated transformation of soybean cotyledonary-node cells [J]. *Plant Cell Reports*, 2001, 20(8): 731-737
- [8] Paz M, Shou H, Guo Z, et al. Assessment of conditions affecting Agrobacterium-mediated soybean transformation using the cotyledonary node explants [J]. *Euphytica*, 2004, 136(2): 167-179
- [9] 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
- [10] Tae-Seok Ko, Sangman Lee, Sergei Krasnyanski, et al. Two critical factors are required for efficient transformation of multiple soybean cultivars: Agrobacterium strain and orientation of immature cotyledonary explant [J]. *Theoretical and Applied Genetics*, 2003, 107(3): 439-447
- [11] Santarém E R, Trick H N, Essig J S, et al. Sonication-assisted Agrobacterium-mediated transformation of soybean immature cotyledons: Optimization of transient expression [J]. *Plant Cell Reports*, 1998, 17(10): 752-759
- [12] Hong H P, Zhang H, Olhofs P, et al. Organogenic callus as the target for plant regeneration and transformation via Agrobacterium in soybean (*Glycine max*(L) Merr) [J]. *In Vitro Cellular & Developmental Biology -Plant*, 2007, 43(6): 558-568
- [13] Wang G L, Xu Y N. Hypocotyl-based Agrobacterium-mediated transformation of soybean (*Glycine max*) and application for RNA interference [J]. *Plant Cell Reports*, 2008, 27(7): 1177-1184
- [14] Cao D, Hou W S, Song S K, et al. Assessment of conditions affecting Agrobacterium rhizogenes-mediated transformation of soybean [J]. *Plant Cell Tissue Organ Culture*, 2009, 96(1): 45-52
- [15] Hinchee M A W, Connor-Ward D V, Newell C A, et al. Production of transgenic soybean plants using Agrobacterium-mediated DNA transfer [J]. *Nature Biotechnology*, 1988, 6(8): 915-922
- [16] Olhofs P M, Fligel 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
- [17] Meurer C A, Dinkins R D, Collins G B. Factors affecting soybean cotyledonary node transformation [J]. *Plant Cell Reports*, 1998, 18(3-4): 180-186
- [18] 武小霞, 李静, 刘伟婷, 等. 大豆农杆菌子叶节转化菌株适宜生长期及浸染浓度的研究 [J]. *东北农业大学学报*, 2010, 41(1): 1-6 (Wu X X, Li J, Liu W T, et al. Optimization study on strain cultivation period and infectious concentration on soybean cotyledonary node via Agrobacterium-mediated transformation system [J]. *Journal of Northeast Agricultural University*, 2010, 41(1): 1-6).
- [19] 刘海坤, 卫志明. 一种大豆成熟种子的消毒的方法 [J]. *植物生理通讯*, 2002, 38(3): 260-261 (Liu H K, Wei Z M. A method for sterilizing mature seeds of soybean [J]. *Plant Physiology Journal*, 2002, 38(3): 260-261).
- [20] Jefferson R A, Kavanagh T A, Bevan M W. GUS fusions: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants [J]. *The European Molecular Biology Organization Journal*, 1987, 6(13): 3901-3907.
- [21] Song Z Y, Tian J L, Fu W Z, et al. Screening Chinese soybean genotypes for Agrobacterium-mediated genetic transformation suitability [J]. *Journal of Zhejiang University-Sci B (Biomed & Biotechnology)*, 2013, 14(4): 289-298. [22] 高乐, 翟锐, 丁雪妮, 等. 大豆花叶病毒 CP 基因 RNAi 载体的构建及大豆遗传转化 [J]. *植物保护学报*, 2014, 41(4): 453-460. (Gao L, Zhai R, Ding X N, et al. RNAi vector construction of soybean mosaic virus CP gene and soybean transformation [J]. *Acta Phytophylacica Sinica*, 2014, 41(4): 453-460)
- [23] 薛仁镐. 农杆菌介导大豆萌动种子遗传转化的影响因素研究 [J]. *安徽农业科学*, 2008, 36(7): 2666-2667. (Xue R G. Study on the factors affecting the genetic transformation efficiency of soybean germinating seeds mediated by Agrobacterium tumefaciens [J]. *Journal of Anhui Agricultural Science*, 2008, 36(7): 2666-2667)

相似文献/References:

- [1] 刘章雄, 李卫东, 孙石, 等. 1983~2010年北京大豆育成品种的亲本地理来源及其遗传贡献[J]. (article.aspx?type=view&id=201301001) *大豆科学*, 2013, 32(01): 1. [doi:10.3969/j.issn.1000-9841.2013.01.002]
- LIU Zhang-xiong, LI Wei-dong, SUN Shi, et al. Geographical Sources of Germplasm and Their Nuclear Contribution to Soybean Cultivars Released during 1983 to 2010 in Beijing [J]. *Soybean Science*, 2013, 32(05): 1. [doi:10.3969/j.issn.1000-9841.2013.01.002]
- [2] 李彩云, 余永亮, 杨红旗, 等. 大豆脂质转运蛋白基因GmLTP3的特征分析[J]. (article.aspx?type=view&id=201301002) *大豆科学*, 2013, 32(01): 8. [doi:10.3969/j.issn.1000-9841.2013.01.003]
- LI Cai-yun, YU Yong-liang, YANG Hong-qi, et al. Characteristics of a Lipid-transfer Protein Gene GmLTP3 in *Glycine max* [J]. *Soybean Science*, 2013, 32(05): 8. [doi:10.3969/j.issn.1000-9841.2013.01.003]
- [3] 王明霞, 崔晓霞, 薛晨晨, 等. 大豆耐盐基因GmHAL3a的克隆及RNAi载体的构建[J]. (article.aspx?type=view&id=201301003) *大豆科学*, 2013, 32(01): 12. [doi:10.3969/j.issn.1000-9841.2013.01.004]
- WANG Ming-xia, CUI Xiao-xia, XUE Chen-chen, et al. Cloning of Halotolerance 3 Gene and Construction of Its RNAi Vector in Soybean (*Glycine max*) [J]. *Soybean Science*, 2013, 32(05): 12. [doi:10.3969/j.issn.1000-9841.2013.01.004]
- [4] 张春宝, 李玉秋, 彭宝, 等. 线粒体ISSR与SCAR标记鉴定大豆细胞质雄性不育系与保持系[J]. (article.aspx?type=view&id=201301005) *大豆科学*, 2013, 32(01): 19. [doi:10.3969/j.issn.1000-9841.2013.01.005]
- ZHANG Chun-bao, LI Yu-qiu, PENG Bao, et al. Identification of Soybean Cytoplasmic Male Sterile Line and Maintainer Line with Mitochondrial ISSR and SCAR Markers [J]. *Soybean Science*, 2013, 32(05): 19. [doi:10.3969/j.issn.1000-9841.2013.01.005]
- [5] 卢清瑶, 赵琳, 李冬梅, 等. RAV基因对拟南芥和大豆不定芽再生的影响[J]. (article.aspx?type=view&id=201301006) *大豆科学*, 2013, 32(01): 23. [doi:10.3969/j.issn.1000-9841.2013.01.006]
- LU Qing-yao, ZHAO Lin, LI Dong-mei, et al. Effects of RAV gene on Shoot Regeneration of Arabidopsis and Soybean [J]. *Soybean Science*, 2013, 32(05): 23. [doi:10.3969/j.issn.1000-9841.2013.01.006]
- [6] 杜景红, 刘丽君. 大豆fad3c基因沉默载体的构建[J]. (article.aspx?type=view&id=201301007) *大豆科学*, 2013, 32(01): 28. [doi:10.3969/j.issn.1000-9841.2013.01.007]
- DU Jing-hong, LIU Li-jun. Construction of fad3c Gene Silencing Vector in Soybean [J]. *Soybean Science*, 2013, 32(05): 28. [doi:10.3969/j.issn.1000-9841.2013.01.007]
- [7] 张力伟, 樊颖伦, 牛腾飞, 等. 大豆“冀黄13”突变体筛选及突变体库的建立[J]. (article.aspx?type=view&id=201301008) *大豆科学*, 2013, 32(01): 33. [doi:10.3969/j.issn.1000-9841.2013.01.008]
- ZHANG Li-wei, FAN Ying-lun, NIU Teng-fei, et al. Screening of Mutants and Construction of Mutant Population for Soybean Cultivar "Jihuang13" [J]. *Soybean Science*, 2013, 32(05): 33. [doi:10.3969/j.issn.1000-9841.2013.01.008]
- [8] 盖江南, 张彬彬, 吴瑶, 等. 大豆不定胚悬浮培养基因型筛选及基因枪遗传转化的研究[J]. (article.aspx?type=view&id=201301009) *大豆科学*, 2013, 32(01): 38. [doi:10.3969/j.issn.1000-9841.2013.01.009]
- GAI Jiang-nan, ZHANG Bin-bin, WU Yao, et al. Screening of Soybean Genotypes Suitable for Suspension Culture with Adventitious Embryos and Genetic Transformation by Particle Bombardment [J]. *Soybean Science*, 2013, 32(05): 38. [doi:10.3969/j.issn.1000-9841.2013.01.009]
- [9] 王鹏飞, 刘丽君, 唐晓飞, 等. 适于体细胞胚发生的大豆基因型筛选[J]. (article.aspx?type=view&id=201301010) *大豆科学*, 2013, 32(01): 43. [doi:10.3969/j.issn.1000-9841.2013.01.010]
- WANG Peng-fei, LIU Li-jun, TANG Xiao-fei, et al. Screening of Soybean Genotypes Suitable for Somatic Embryogenesis [J]. *Soybean Science*, 2013, 32(05): 43. [doi:10.3969/j.issn.1000-9841.2013.01.010]
- [10] 刘德兴, 年海, 杨存义, 等. 耐酸铝大豆品种资源的筛选与鉴定[J]. (article.aspx?type=view&id=201301011) *大豆科学*, 2013, 32(01): 46. [doi:10.3969/j.issn.1000-9841.2013.01.011]
- LIU De-xing, NIAN Hai, YANG Cun-yi, et al. Screening and Identifying Soybean Germplasm Tolerant to Acid Aluminum [J]. *Soybean Science*, 2013, 32(05): 46. [doi:10.3969/j.issn.1000-9841.2013.01.011]
- [11] 郝荣华, 邵群, 杨素欣, 等. 根瘤农杆菌介导的大豆子叶节转化体系的优化[J]. (article.aspx?type=view&id=201202002) *大*

豆科学, 2012, 31 (02):167. [doi:10.3969/j.issn.1000-9841.2012.02.002]

HAO Rong-hua, SHAO Qun, YANG Su-xin, et al. Optimization of Agrobacterium-mediated Soybean Transformation using the Cotyledonary Node[J]. Soybean Science, 2012, 31 (05):167. [doi:10.3969/j.issn.1000-9841.2012.02.002]

[12] 郑丽红, 季静, 王罡, 等. 适于子叶节和胚尖再生体系的大豆基因型筛选[J]. (article.aspx?type=view&id=201202011) 大豆科学, 2012, 31 (02):212. [doi:10.3969/j.issn.1000-9841.2012.02.011]

ZHENG Li-hong, JI Jing, WANG Gang, et al. Selection of Suitable Soybean Genotype Based on Cotyledon Node and Embryonic Tip Regeneration Systems[J]. Soybean Science, 2012, 31 (05):212. [doi:10.3969/j.issn.1000-9841.2012.02.011]

[13] 王伟, 王罡, 季静, 等. 大豆胚尖再生体系的优化及与子叶节再生体系的比较[J]. (article.aspx?type=view&id=201203004) 大豆科学, 2012, 31 (03):353. [doi:10.3969/j.issn.1000-9841.2012.03.004]

WANG Wei, WANG Gang, JI Jing, et al. Optimization of Embryonic Tip Regeneration System and Comparison with Cotyledonary Node Regeneration System in Soybean[J]. Soybean Science, 2012, 31 (05):353. [doi:10.3969/j.issn.1000-9841.2012.03.004]

[14] 姚丙辰, 沈艳茹, 韩雪, 等. 大豆子叶节和胚尖再生体系的比较及大豆SR1基因的遗传转化[J]. (article.aspx?type=view&id=201203006) 大豆科学, 2012, 31 (03):364. [doi:10.3969/j.issn.1000-9841.2012.03.006]

YAO Bing-chen, SHEN Yan-ru, HAN Xue, et al. Comparison with Cotyledonary Node and Embryonic Tip Regeneration System in Soybean[Glycine max(L.)Merrill] and Genetic Transformation of SR1 [J]. Soybean Science, 2012, 31 (05):364. [doi:10.3969/j.issn.1000-9841.2012.03.006]

[15] 陈秀华, 柏锡, 潘欣, 等. 基因大豆的培育及抗虫性检测[J]. (article.aspx?type=view&id=200906003) 大豆科学, 2009, 28 (06):959. [doi:10.11861/j.issn.1000-9841.2009.06.959]

CHEN Xiu-hua, BAI Xi, PAN Xin, et al. Cultivation of cry IIem Gene Transformed Soybean and Insect Resistant Assay [J]. Soybean Science, 2009, 28 (05):959. [doi:10.11861/j.issn.1000-9841.2009.06.959]

[16] 赖冰冰, 韩阳, 李春风, 等. 大豆子叶节植株再生体系的研究[J]. (article.aspx?type=view&id=201102028) 大豆科学, 2011, 30 (02):303. [doi:10.11861/j.issn.1000-9841.2011.02.0303]

LAI Bing-bing, HAN Yang, LI Chun-feng, et al. Research on Cotyledonary Nodes Regeneration System of Soybean [J]. Soybean Science, 2011, 30 (05):303. [doi:10.11861/j.issn.1000-9841.2011.02.0303]

[17] 赵晓雯, 吴芳芳, 狄少康, 等. 农杆菌介导的大豆子叶节遗传转化技术流程及操作要点[J]. (article.aspx?type=view&id=201103003) 大豆科学, 2011, 30 (03):362. [doi:10.11861/j.issn.1000-9841.2011.03.0362]

ZHAO Xiao-wen, WU Fang-fang, DI Shao-kang, et al. Technique flow and Key Operation Points of Agrobacterium-mediated Genetic Transformation of Soybean Cotyledonary Node[J]. Soybean Science, 2011, 30 (05):362. [doi:10.11861/j.issn.1000-9841.2011.03.0362]

[18] 朱红林, 沙爱华, 符秀梅, 等. 转录调控基因GmLEC1转化大豆及转化方法的比较[J]. (article.aspx?type=view&id=201001002) 大豆科学, 2010, 29 (01):7. [doi:10.11861/j.issn.1000-9841.2010.01.0007]

ZHU Hong-lin, SHA Ai-hua, FU Xiu-mei, et al. Cloning and Transformation Study of Transcription Factor GmLEC1 in Soybean[J]. Soybean Science, 2010, 29 (05):7. [doi:10.11861/j.issn.1000-9841.2010.01.0007]

[19] 杜升伟, 等. 大豆转化体系的优化和Dof 4基因转入大豆的研究[J]. (article.aspx?type=view&id=201003009) 大豆科学, 2010, 29 (03):398. [doi:10.11861/j.issn.1000-9841.2010.03.0398]

DU Sheng-wei, LIU Ye-li, et al. Optimization of Soybean Transformation System and Transferring Dof 4 Gene into Soybean[J]. Soybean Science, 2010, 29 (05):398. [doi:10.11861/j.issn.1000-9841.2010.03.0398]

[20] 寇坤, 刘丽君, 曲姗姗, 等. 大豆新品系黑农56子叶节再生体系的优化[J]. (article.aspx?type=view&id=200903007) 大豆科学, 2009, 28 (03):400. [doi:10.11861/j.issn.1000-9841.2009.03.0400]

KOU Kun, LIU Li-jun, QU Shan-shan, et al. Improvement of Regeneration System in New Soybean Line Heinong 56 [J]. Soybean Science, 2009, 28 (05):400. [doi:10.11861/j.issn.1000-9841.2009.03.0400]

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