核农学报 2011, 25(5) 892-897 DOI: ISSN: 1000-8551 CN: 11-2265/S

植物诱变育种·农业生物技术

植酸酶根特异表达载体的构建及大豆转化

李桂兰[1], 乔亚科[1], 李明刚[2], 崔姗姗[1], 乔潇[3], 乔坤艳[2]

1. 河北科技师范学院生命科技学院,河北 昌黎 066600;
2. 南开大学 生命科学学院,天津 300071;
3. 上海大学,材料科学与工程学院,上海 200072

摘要:

应用PCR方法分别从胡萝卜基因组中扩增出96bp的外展蛋白信号肽编码序列片段,从拟南芥基因组中扩增出1454bp的 *pyk*10启动子片段,用RT-PCR方法从无花果曲霉(*Aspergillus ficuum* 3.4322)中扩增出 *phy*A 基因,长1347bp。然后,分别克隆到pMD18-T载体。应用已设计的限制酶切位点,通过5个中间载体将3段DNA片段连接构成2.9kb的表达单元Ppyk10-S-phJA(KSA),将KSA片段插入初始载体pC-GENERAL,构建成植酸酶根特异表达载体pPC-KSA。利用农杆菌介导法将无花果曲霉植酸酶基因 *phy*A 转入到栽培大豆品种吉林35中,在大豆转基因植株中正确表达,产生有活性的植酸酶,且能分泌到根外。T_3代植株根系分泌植酸酶活性比未转化植株提高了2~4倍。

关键词: 外展蛋白信号肽 *pyk*10启动子 *phy*A 基因 大豆 转基因 植酸酶 根特异表达

CONSTRUCTION OF THE VECTOR OF PHYTASE GENE SPECIFIC EXPRESSION IN ROOTS AND TRANSFORMATION OF SOYBEAN

LI Gui-lan[1], QIAO Ya-ke[1], LI Ming-gang[2], CUI Shan-shan[1], QIAO Xiao[3], QIAO Kun-yan[2]

1. College of life& Technology, Hebei Normal University of Science & Technology, Changli, Hebei 066600;
2. College of life science, Nankai University,Tianjin 300071;
3. School of materials science and engineering, Shanghai Uniwersity, Shanghai 200072

Abstract:

A 96bp sequence encoding the extension signal peptide was cloned by PCR from carrot(Daucus、carota L var. sativus H offm) genomic DNA. It was identical to the sequence reported (GenBank accession No: XO2873). A promoter fragment of 1454bp long of *Pyk*10 gene was cloned by PCR from *Arabidopsis thaliana* genomic DNA. A cDNA of *phy*A gene was cloned from *Aspergillus ficuum* 3.4322 by RT-PCR, which comprised of 1347bp without signal peptide coding sequence. An expression cassette P*pyk*10-*s*-*phy*A (KSA), comprising of the *pyk*10 promoter fragment, the carrot extension signal peptide coding sequence and the cDNA of *phy*A gene, was successfully constructed through five transitional plasmids. The expression plasmid of pPC-KSA was constructed finally by inserting KSA fragment into original vector pC-GENERAL.The *phy*A gene from *Aspergillus ficuum* was successfully transfermed into soybean (*Glycine max* L. Merr. c.v. Jilin 35) via *Agrobacterium tumefaciens*-mediated method, which was able to be expressed in the recipient soybean cultivar. The introduction of fungal *phy*tase from *Aspergillus ficuum* resulted in about 2~4-fold increase of *phy*tase activity secreted from roots in the T_3generation transgenic plants compared to the non-transgenic control.

Keywords: carrot extension signal peptide coding sequence *Pyk*10promoter phyA *Glycine max* L. Merr transgene phytase specific expression in root

收稿日期 2010-12-22 **修回日期** 2011-04-24 **网络版发布日期**

DOI:

基金项目:

国家科技部转基因生物新品种培育重大科技专项(2009ZX08004-004B, 2009ZX08004-001B), 河北省自然科学基金 (C2009000868)

通讯作者:

作者简介: 李桂兰(1963-),女,河北肃亭人,博士,教授,研究方向为植物基因工程。E-mail:lgl63@126.com

作者Email:

参考文献：

[1] Nelson T S. The utilization of phytase phosphorus by poulty -a review ［J］. Poult Sci, 1967, 46: 862-871

[2] 任学良,舒庆尧.低植酸作物的研究进展及展望［J］.核农学报,2004,18(6):438-442

[3] Rudy J, Wodzinski, Ullah A H J. Phytase［J］. Advances in applied.microbiology, 1996, 42: 263-302

[4] Pedersen N E J.On phytin phosphorus in soil ［J］. Plant Soil, 1953, 4: 252-256

[5] Saxena S N. Phytase activity of plant roots ［J］. J Exp Bot, 1964, 15: 654-658

[6] Tarafdar J C, Claassen N. Orgaic phosphorous compounds as a phosphorous source for higher plants through the activity of phosphatases produced by plant roots and icroorganisms ［J］.Bio Fertil Soils, 1988, 5: 308-312

[7] Li M G,Tadano T. Comparison of characteristics of acid phosphatases secreted from roots of lupin and tomato［J］.Soil Sci Plant Nutr, 1996, 42: 753-763

[8] Li M G,Osaki M. Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions ［J］. Plant and Soil, 1997, 1:179-190

[9] Verwoerd T C, Van Paridon P A, Van Ooyen A J, van Lent J.W, Hoekema A, Pen J. Stable accumulation of aspergillus nigerPhytase in transgenic tobacco leaves ［J］.Plant physiology, 1995, 109: 1199-1205

[10] Richardson A E, Hadobas, P A, Hayes J E., Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate ［J］. The Plant Journal, 2001, 25 (6): 641-649

[11] Bartling D. Cloning and expression of an Arabidopsis nitrilase which can convert indole-3-acetonitrile to the plant hormone indol-3-acetic［J］. Eur J Biochem, 1992, 205: 417-424.

[12] Nitz I, Berkefeld H, Piotr S, Puzio, Florian M W.Grundler, Pyk 10, a seeding and root specific gene and promoter from Arabidopsis thaliana ［J］. Plant Science, 2001, 161: 337-346

[13] Li G L, Zhu J H, Sun, J, Wu Z W, Chen J, Yan J, Wang L F, Chen G, Jiang H Z, Li M.G. Cloning of the phytase gene phyA from Aspergillus ficuum 3.4322 and its expression in yeast ［J］. Fungal Diversity, 2003, 13:85-93

[14] 李桂兰,王文颇,臧少先,乔亚科,杨少辉,薄 涛,李明刚.拟南芥黑介子酶基因根特特异性表达启动子的克隆［J］. 河北农业大学学报,2005,28(1):49-52

[15] 王冠林,方宏筠.植物基因工程原理与技术［M］. 北京:科学出版社,1998:221-227

[16] Chen J, Varner J E. An extracellular matrix protein in plants: Characterization of a gnomic clone for carrot extension［J］.EMBO M J, 1985, 4(9): 2145-2151

[17] 李桂兰,乔亚科,杨少辉,靳朝霞,李明刚.农杆菌介导大豆子叶节遗传转化的研究［J］.作物学报,2005,31 (2):170-176

[18] Ames B N. Assay of inorganic phosphate, total phosphate and phosphatase ［J］. Methods enzymol,1966, 8:115-118

[19] Ullah A H, Sethumadhavan K, Mullaney E J, Ziegelhoffer T, Austin-Phillips S. Clond and expressed fungal phyA gene in alfalfa produces a stable phytase［J］. Biochem Biophys Res Commun,2002, 290 (4): 1343-1348

[20] Ullah A H, Sethumadhavan K, Mullaney E J, Ziegelhoffer T, Austin-Phillips S. Fungal phyA gene expressed in potato leaves produces active and stable phytase［J］. Biochem Biophys Res Commun,2003, 306(2): 603-610

[21] Winch-Pedersen H, Olesen A, Rasmussen S K, et al. Generation of transgenic wheat(Triticum aestirnun L.)for constitutive accumulation of an Aspergdlus phytase［J］. Molecular Breeding, 2000, 6: 195-206

[22] Pen J, Verwoerd T C, van-Paridon P A, et al. Phytase-containing transgenic seeds as a novel feed additive for improved phosphorus utilization ［J］. Bio/Technology, 1993, 11:811-814

[23] Ponstein A S, Bade J B, Verwoerd T C, et al. Stable expression of Phytase (phyA)in canola (Brassica napes)seeds: towards a commercial product e［J］. Molecular Breeding, 2002, 10: 31-44

本刊中的类似文章

1． 傅旭军, 朱申龙, 袁凤杰, 朱丹华, 董德坤, 汪自强.浙江省和其他省份大豆种质资源的遗传多样性分析[J]. 核农学报, 2009,23(5): 747-751

2． 魏冰, 李云, 杜宁霞, 刘欣.毛白杨杂种外源基因稳定性及对土壤微生物的影响[J]. 核农学报, 2009,23(6): 1054-1059

3．郝再彬,吴东岚.矮秆大豆突变体的获得[J]. 核农学报, 2004,18(03): 204-206

4．闫洪睿,张雷,鹿文成,梁吉利,刘英华,刘发.黑龙江省高纬寒地大豆辐照诱变效应研究[J]. 核农学报, 2003,17(06): 430-433

5．焦浈,秦广雍,曹刚强,霍裕平.离子注入在生命科学中的应用研究[J]. 核农学报, 2003,17(05): 354-357

6．叶庆富.同位素示踪技术与转基因植物生态风险性评价研究[J]. 核农学报, 2003,17(04): 313-318

7．王连铮,王岚,赵荣娟,裴颜龙,傅玉清,颜清上,李强.辐射和生物技术相结合进行大豆育种(英文)[J]. 核农学报, 2001,15(05): 274-281

8．刘智宏,胡张华,郎春秀,黄锐之,陈锦清.大豆幼嫩子叶离体培养及辐照处理配合基因枪遗传转化研究[J]. 核农学报, 2001,15(05): 282-285

9． 郭慧琴, 任卫波, 徐柱, 王蜜, 陈立波.紫花苜蓿转基因研究进展[J]. 核农学报, 2010,24(1): 55-61

10．吴丽芳,余增亮.离子注入法获得大豆—小麦分子远缘杂种及后代的变异分析[J]. 核农学报, 2000,14(04): 206-211

11．严建民.作物转Bt抗虫基因育种研究进展[J]. 核农学报, 2000,14(03): 184-188

12．王培英,许德春,郭玉虹,孟丽芬,赵晓南.人工诱变改良大豆品质的研究[J]. 核农学报, 2000,14(01): 21-23

13．卢一凡,邓继先,肖成祖,马清钧.人粒细胞集落刺激因子(G-CSF)转基因小鼠的建立[J]. 核农学报, 1999,13(02): 0-0

14．郭玉虹,王培英,王连铮,钟立梓,许德春,孟丽芬,宋凤娟,闫晓东,许桂芳.利用突变系育成高蛋白大豆新品种黑农41[J]. 核农学报, 1999,13(01): 0-0

15．李海先,李新民.应用~(15)N稀释法筛选高固氮能力的大豆品种[J]. 核农学报, 1998,12(05): 0-0