核农学报 2012, (8) 1111-1117 DOI: ISSN: 1000-8551 CN: 11-2265/S

植物诱变育种·农业生物技术

转*HBsAg*基因樱桃番茄组培苗叶片总蛋白双向电泳体系的建立

张超[1], 郭斌[1], 祁洋[1], 孙杰[1], 薛柯[1], 戴佳锟[2], 关正君[1], 尉亚辉[1]

1. 西北大学生命科学学院/西部资源生物与现代生物技术教育部重点实验室, 陕西 西安 710069;
2. 陕西省科学院酶工程研究所, 陕西 西安 710600

摘要： 建立转乙肝表面抗原(*HbsAg*)基因樱桃番茄组培苗叶片的蛋白质组双向电泳体系,为转基因番茄的蛋白质组学研究提供技术参数。比较转*HBsAg*基因的樱桃番茄与野生型在不同蛋白裂解液、不同蛋白上样量及不同等电聚焦程序下,叶片总蛋白的双向电泳结果差异。以裂解液Ⅲ制备叶片总蛋白,采用一向13cm、pH3-10L IPG胶条,二向12.5%的SDS-PAGE凝胶,单次上样120μg叶片总蛋白,聚焦程序Ⅲ,银染时,可以得到最理想的双向电泳分析结果。在此操作程序下,野生型樱桃番茄叶片蛋白双向电泳最多可识别699个蛋白点,而转基因型樱桃番茄叶片蛋白双向电泳最多可识别545个蛋白点,其中有368个点匹配,选取丰度百分比差异2倍以上蛋白点25个进行质谱鉴定,16个蛋白点成功鉴定。本研究建立的转*HBsAg*基因番茄植株叶片总蛋白的双向电泳体系可为以番茄试管苗为材料进行的蛋白质组学研究提供技术支持。

关键词： 双向电泳 转基因植物 口服植物疫苗 蛋白组 组培苗

ESTABLISHMENT OF TWO-DIMENSIONAL GEL ELECTROPHORESIS SYSTEM FOR LEAF PROTEINS OF *HBsAg* TRANSGENIC CHERRY TOMATO

ZHANG Chao[1], GUO Bin[1], QI Yang[1], SUN Jie[1], XUE Ke[1], DAI Jia-kun[2], GUAN Zheng-jun[1], WEI Ya-hui[1]

1. Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, School of Life Science, Northwest University, Xi’an, Shaanxi 710069;
2. Enzeme Engineering Insitute of Shaanxi Academy of Sciences, Xi'an, Shaanxi 710600

Abstract: Two-dimensional gel electrophoresis (2-DE) system was established for detection of the leaf proteins of *HBsAg* transgenic cherry tomato, which provided the experiment data for proteomics study of transgenic cherry tomato. This study compared the effects of different protein lysis buffers, protein loading amounts and isoelectric focusing conditions on the 2-DE maps of the transgenic plants. An optimum system was obtained as follows: dissolving total proteins of samples with the lysis buffer Ⅲ, separating the proteins with 13cm pH3-10L IPG strips, loading protein samples of 120μg followed by isoelectric focusing program Ⅲ,and staining the gels with silver staining after 12.5% SDS-PAGE. Under the above conditions, 545 protein spots were identified from the transgenic plants and 699 protein spots from control plants, of which 368 protein spots were matched. A total of 25 protein spots were analyzed by MALDI-TOF MS and 16 proteins were identified by PMF. The 2-DE system for leaf proteins of *HBsAg* transgenic cherry tomato established in this work provides technical base for the studies of tomato proteomics.

Keywords: two-dimensional electrophoresis transgenic plants plant-derived oral vaccine proteomics tissue culture plantlet

收稿日期 2012-01-11 修回日期 2012-04-20 网络版发布日期

DOI:

基金项目:

国家自然科学基金青年基金(No.30900914);国家自然科学基金(No.310001444);陕西省生物技术重点实验室项目(No.08JZ72);西北大学自主创新类项目(No.10YZZ36)

通讯作者: 尉亚辉(1960-),男,陕西凤翔人,博导,教授,研究方向为基因与细胞工程制药。Tel:029-88303484;E-mail:weiyahui@nwu.edu.cn

扩展功能
本文信息
Supporting info
PDF(1473KB)
[HTML全文]
参考文献[PDF]
参考文献
服务与反馈
把本文推荐给朋友
加入我的书架
加入引用管理器
引用本文
Email Alert
文章反馈
浏览反馈信息
本文关键词相关文章
双向电泳
转基因植物
口服植物疫苗
蛋白组
组培苗
本文作者相关文章
张超
郭斌
祁洋
孙杰
薛柯
戴佳锟
关正君
尉亚辉
PubMed
Article by ZHANG Chao
Article by GUO Bin
Article by QI Yang
Article by SUN Jie
Article by XUE Ke
Article by DAI Jia-kun
Article by GUAN Zheng-jun
Article by WEI Ya-hui

作者简介:
作者Email: weiyahui@nwu.edu.cn

参考文献：

[1] Guan Z J, Guo B, Huo Y L, Guan Z P, Wei Y H. Overview of expression of hepatitis B surface antigen in transgenic plants [J]. Vaccine, 2010, 28: 7351-7362
[2] Michel M L. Towards immunotherapy for chronic hepatitis B virus infections [J]. Vaccine, 2002,20:83-88
[3] Schwartz W, Seyffarth T, Baumgarten H, Gerlich W H. Large surface proteins of hepatitis B virus containing the pre-S sequence [J]. J Virol, 1984, 52:396-402
[4] Yang H J. The exploratory study on genetically engineered therapeutic hepatitis B vaccine [D]. Dissertation for Master Degree of Xiamen University, 2002:7-10
[5] Mor T S, Gómez-lim M A, Palmer K E. Perspective: edible vaccines-a concept coming of age [J]. Trends in Microbiology, 1998, 6(11):449-453
[6] Sunil-kumar G B, Ganapthi T R, Revathi C J, Prasad K S N, Bapat V A. Expression of hepatitis B surface antigen in tobacco cell suspension culture [J]. Protein Expres Purif, 2003, 32: 10-17
[7] Mason H S, Lam D K, Arntzen C J.Expression of hepatitis B surface antigen in transgenic plants [J].Proc Natl Acad Sci USA, 1992,89(24): 11745-11749
[8] Qingxian K, Richter L, Yang Y F. Oral immunization with hepatitis B surface antigen expressed in transgenic plants[J].Proc Natl Acad Sci USA, 2001, 98(20): 11539-11544
[9] Richter L J, Thanavala Y, Arntzen C J.Production of hepatitis B surface antigen in transgenic plants for oral immunization[J].Nat Biotechnol, 2000, 18: 1167-1171
[10] Kapusta H. A plant-derived edible vaccine against hepatitis B virus[J].FASEB J,1999,13:1796-1799
[11] Smith M L, Mason H S, Shuler M L. Hepatitis surface (HBsAg) expression in plant cell culture: Kinetics of antigen accumulation in batch culture and its intracellular form [J]. Biotechnol Bioeng, 2002, 80: 812-822
[12] Shulga N Y, Rukavtsova E B, Krymsky M A, Borisova V N, Melnikov V A, Buryanov Y I. Expression and characterization of hepatitis B surface antigen in transgenic potato plants [J]. Biochemistry(Moscow), 2004, 69(10): 1422-1430
[13] Gao Y, Ma Y, Cheng T, Li S W, Zhang J, Xia N S. Oral immunization of animals with transgenic cherry tomatillo expressing HBsAg[J]. World J Gastroentero, 2003, 9(5): 996-1002
[14] 郝浩永,尉亚辉,朱建光,孙 杰,王娅宁,荆二勇,张变玲,薛 柯. 转基因番茄表达口服乙肝疫苗[J].食品科学, 2007, 28(6):201-204
[15] 阮松林,马华升,王世恒,忻 雅,钱丽华,童建新,赵杭苹,王 杰. 植物蛋白质组学研究进展 Ⅱ.蛋白质组技术在植物生物学研究中的应用[J]. 遗传, 2006, 8(12): 1633-1648
[16] Gorg A, Obermaier C, Boguth G, Csordas A, Diaz J J, Madjar J J. Very alkaline immobilized pH gradients for two-dimensional electrophoresis of ribosomal and nuclear proteins [J]. Electrophoresis, 1997, 18: 328-337
[17] 梁 宇,荆玉祥,沈世华. 植物蛋白质组学研究进展[J]. 植物生态学报, 2004, 28(1): 114-125
[18] Kerk D, Bulgrien J, Smith D W, Barsam B, Veretnik S, Gribskov M, David K, Joshua B, Douglas S. The complement of protein phosphatase catalytic subunits encoded in the genome of Arabidopsis [J]. Plant Physiol , 2002, 129: 908- 925
[19] Mumby M C, Walter G. Protein serine / threonine phosphatases: structure, regulation, and function [J]. Physiological Review, 1993, 73: 673-699
[20] O’farrell P H. High resolution two-dimensional electrophoresis of proteins [J]. J Biol Chem, 1975, 250(10): 4007- 4021
[21] 黎 飞,徐秋芳,臧宪朋,赖亿玉,程维舜,徐幼平,蔡新忠. 番茄子叶总蛋白双向电泳体系的建立[J]. 园艺学报, 2010,37(4): 661-668
[22] 张 儒,尉亚辉,刘 科,刘 蕾,朱剑光,郭芝光. 乙肝表面抗原基因植物表达载体的构建[J]. 西北大学学报:自然科学网络版, 2005, 3(4): 1-6
[23] Damerval C, Devienne D, Zivy M, Thiellement H. Technical improvements in two-dimensional electrophoresis increase the level of genetic-variation detected in wheat-seedling proteins[J]. Electrophoresis, 1986,7:52-54
[24] Jacobs D I, van Rijssen M S, van der Heijden R,Verpoorte R. Sequential solubilization of proteins precipitated with trichloroacetic acid in acetone from cultured Catharanthus roseus cells yields 52% more spots after two-dimensional electrophoresis[J]. Proteomics, 2001,1:1345-1350
[25] 陈蕊红,张改生,刘 卫,叶景秀,牛 娜. 小麦花药蛋白质组双向电泳技术体系的优化[J]. 核农学报, 2008, 22 (4):404-409
[26] Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding [J]. Analytical Biochemistry, 1976, 72:248-254
[27] 夏其昌, 曾 嵘. 蛋白质化学与蛋白质组学
[M]. 北京: 科学出版社, 2004: 261-265
[28] 林文芳,陈林娇,彭 浩,朱学艺.不同生态型芦苇叶片蛋白质双向电泳系统的筛选和优化[J].生物化学与物理学进展,2008,35(10):1209-1214

[29] Yoshito K, Hireshi I, Tohru K. Structure and function of signal transducting GTP binding protein [J]. Annu Rev Biochem, 1991, 60:349-400
[30] Williams P M, Barkan A. A chloroplast-localized PPR protein required for plastid ribosome accumulation [J]. Plant J, 2003, 36(5):675-686
[31] Corpillo D, Gardini G, Vaira A M, Basso M, Aime S, Accotto G R, Fasano M. Proteomics as a tool to improve investigation of substantial equivalence in genetically modified organisms:The case of a virus-resistant tomato[J]. Proteomics, 2004, 4:193-200
[32] Rocco M, D'Ambrosio C, Arena S, Faurobert M, Scaloni A, Marra M. Proteomic analysis of tomato fruits from two ecotypes during ripening[J]. Proteomics, 2006, 6:3781-3791
[33] Saravanan R S,Rose J K C. A critical evaluation of sample extraction techniques for enhanced proteomic analysis of recalcitrant plant tissues [J]. Proteomics, 2004, 4:2522-2532
[34] Sheoran I S,Olson D J H,Ross A R S,Sawhney V K. Proteome analysis of embryo and endosperm from germinating tomato seeds[J]. Proteomics, 2005, 5:3752-3764
[35] Casado-Vela J,Selles S,Martinez R B. Proteomic approach to blossom-end rot in tomato fruits (*Lycopersicon esculentum* M.):Antioxidant enzymes and the pentose phosphate pathway[J]. Proteomics, 2005, 5: 2488-2496
[36] Ahsan N, Lee D G, Lee S H, Lee K W, Bahk J D,Lee B H. A proteomic screen and identification of waterlogging-regulated proteins in tomato roots [J]. Plant Soil, 2007,295:37-51
[37] 吴庆丰,王崇英,王新宇,李志孝,王亚馥.山黧豆叶片蛋白质双向电泳技术的建立[J]. 西北植物学报,2006,26(7):1330-1336

本刊中的类似文章

1. 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
2. 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
3. 刘艳萌;张学英;葛会波;.EMS诱发草莓不同组培材料的耐盐性变异[J]. 核农学报, 2008,22(06): 798-802
4. 陈蕊红;张改生;刘卫;叶景秀;牛娜;.小麦花药蛋白质组双向电泳技术体系的优化[J]. 核农学报, 2008,22(04): 404-409
5. 张宏纪;刁艳玲;孙连发;孙岩;刘东军;郭强;兰静;黄景华;杨淑萍;孙光祖;.航天诱变新品种龙辐麦18的选育及其主要特征特性分析[J]. 核农学报, 2008,22(03): 243-247
6. 孙锋, 王云生.盐胁迫下AM菌侵染的棉花幼苗根系蛋白质组分析[J]. 核农学报, 2012,26(1): 170-175
7. 王彩霞,吴殿星,沈圣泉,舒庆尧.转基因及常规水稻幼苗对潮霉素敏感性的研究[J]. 核农学报, 2005,19(03): 168-171
8. 朱海生, 李永平, 林珲, 温庆放.草莓品种的亲缘关系及其不同继代次数的叶片组培苗的RAPD分析[J]. 核农学报, 2010,24(2): 264-268

Copyright by 核农学报