棉花学报
Cotton Science
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棉花学报 » 2013, Vol. 25 » Issue (1) :45-50 DOI: 1002-7807（2013）01-0045-06

双抗夹心酶免疫法检测转*Bt*基因抗虫棉种子的研究

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Determination of Bt Protein in Transgenic Cotton Seeds by Double Antibody Sandwich Enzyme-linked Immunosorbent Assay

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摘要 应用中国农业大学化控中心筛选的抗Bt Cry1Ac蛋白鼠单克隆抗体和兔多克隆抗体，建立了Bt Cry1Ac蛋白的双抗夹心酶联免疫检测法（Sandwich ELISA）。该方法的检测范围为0.78~50.0 ng · g^{-1}，线性回归方程y=0.6634x-1.7387，决定系数为0.992。该方法与国外商业化试剂盒检测结果完全一致，可用于转基因抗虫棉Bt毒蛋白定性和定量检测。采用所建立的方法对亲本之一为非转基因抗虫棉的杂交F_1、F_2种子进行检测，结果F_1全部为阳性，F_2阴性和阳性数量比为1∶3，符合性状分离定律；此外，模拟检测种子的偶然基因改造成分混杂（Adventitious Presence，AP）检测结果为：对于Bt毒蛋白含量在140 ng以上的单粒种子，最低检测比例为1∶110。

关键词： 转Bt抗虫棉 双抗夹心酶免疫法 种子纯度 偶然基因改造成分混杂

Abstract： Bt Cry1Ac protein sandwich enzyme-linked immunosorbent assay was developed with anti-Bt Cry1Ac protein mouse monoclonal antibody (mAb) and rabbit polyclonal antibody produced by Chemical Regulation Research Center (CRRC) of China Agricultural University (CAU). The linear range of the method was 0.78-50.0 ng · mL^{-1}. The linear equation was y = 0.6634x-1.7387, and the determinative coefficient was 0.992. The specificity obtained with the established assay was confirmed and verified by the commercial Bt Cry1Ac protein kit and could be qualitatively and quantitatively used to detect the Bt Cry1Ac protein. By the assay, identification of hybrid F_1 and F_2 with either of the parents is non-transgenic Bt cotton showed that, all the seeds of F_1 were positive and the ratio of seeds positive to negative of F_2 was 3∶1. The assay was also used to imitate AP detection and the limit of AP was 1∶110 for single seed which contained more than 140 ng of Bt Cry1Ac protein.

Keywords： transgenic *Bt* cotton sandwich ELISA seed purity adventitious presence

Received 2012-04-19;

Fund:

转基因生物新品种培育重大专项（2009ZX08012-017B）；国家自然科学基金（31271649）

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引用本文:

孙 硕, 张 坤, 谭桂玉, 曹 振, 南铁贵, 王保民.双抗夹心酶免疫法检测转*Bt*基因抗虫棉种子的研究[J] 棉花学报, 2013,V25(1): 45-50

SUN Shuo, ZHANG Kun, TAN Gui-Yu, CAO Zhen, NAN Tie-Gui , WANG Bao-Min.Determination of Bt Protein in Transgenic Cotton Seeds by Double Antibody Sandwich Enzyme-linked Immunosorbent Assay[J] Cotton Science, 2013,V25(1): 45-50

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http://journal.cricaas.com.cn:8082/mhxb/CN/1002-7807（2013）01-0045-06 或 http://journal.cricaas.com.cn:8082/mhxb/CN/Y2013/V25/I1/45