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Cry2Aa2 和 *PamPAP* 双价表达载体的构建及其对辣椒的遗传转化

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Construction of Binary Expression Vector Containing *Cry2Aa2* and *PamPAP* and Its Transfer to Pepper

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摘要 为了获得既抗虫又抗病毒病，又对人体和环境安全的转基因辣椒（*Capsicum annuum* L．）材料，将启动子CaMV35S、终止子Nos-ter 及缺失无毒型商陆抗病毒蛋白基因*PamPAP* 一起插入到植物表达载体pCAMBIA1301-Cry2Aa2-PMI 的多克隆位点上，构建双价表达载体，采用农杆菌介导法将其转入辣椒中，并用PMI/甘露糖筛选体系对转化植株筛选。经PCR 扩增和Southern 杂交检测，结果表明*Cry2Aa2*和*PamPAP* 已经整合到辣椒基因组中。进一步经RT-PCR 分析，证实*Cry2Aa2* 和*PamPAP* 在T_0 代转基因辣椒植株中共表达。经抗虫性、抗病毒病表型鉴定：T_0 代转基因辣椒植株对黄瓜花叶病毒（CMV）和斜纹夜蛾幼虫的抗性均显著高于非转基因植株。

关键词： 辣椒 　 PMI/甘露糖筛选体系 　 *Cry2Aa2* 　 *PamPAP* 　 遗传转化 　 抗病毒 　 抗虫害

Abstract： The aim of this study was to obtain transgenic pepper plants with double resistances to virus and insect that could be used in developing new pepper variety. The non-virulent defective PAP gene （PamPAP），CaMV35S promoter and Nos terminal were inserted into MCS of pCAMBIA1301- Cry2Aa2-PMI. Cry2Aa2 and PamPAP were transferred into pepper （*Capsicum annuum* L.） mediated by Agrobacterium tumefaciens with PMI/Man selection system. The results of PCR and Southern blot indicated that *Cry2Aa2* and *PamPAP* had been integrated into the pepper genome. RT-PCR analysis further showed that the *Cry2Aa2* and *PamPAP* had been expressed simultaneously. In addition，resistances to virus and insect were assayed，and the results showed that the positive transgenic pepper plants had higher degree of resistances to CMV and Prodenia litura larva than the non-transgenic plants. This suggested that transgenic pepper plants with double resistances to virus and insect could be obtained by transferring *Cry2Aa2* and *PamPAP*.

Keywords： pepper, 　 PMI/Man system, 　 *Cry2Aa2*, 　 *PamPAP*, 　 genetic transformation, 　 resistance to virus, 　 resistance to insect

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