

原核表达的PRSV HC-Pro 基因同源dsRNA 诱导番木瓜抗性的研究

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Studies of Bacterially Expressed dsRNAs Targeting PRSV HC-Pro Gene Inducing Resistance to PRSV

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摘要 利用番木瓜环斑病毒 (PRSV) HC-Pro 基因3' 端824 bp 区段, 通过OZ-LIC 法构建了含有PDK 内含子的发夹RNA 编码结构, 并选用pSP73 和M-Jm109LacY 分别作为宿主载体和宿主菌, 构建了高效的同源dsRNA 原核表达工程菌M-Jm109LacY/pSP73-RNAi-H824, 经IPTG 诱导表达的dsRNA 不被DNase I 和RNase A 降解, 稳定性较好。采用喷洒dsRNA 粗制品的方式对番木瓜植株进行保护性处理和 therapeutic 处理, 症状观察及 ELISA、Real-time RT-PCR 分析结果表明, 保护性处理能有效诱发对番木瓜环斑病毒的抗性 (发病率低, 发病时间晚); 治疗性处理 (植株已接种PRSV 25 d) 能在处理初期引起番木瓜环斑病毒积累量发生短暂降低。

关键词: 番木瓜环斑病毒 HC-Pro dsRNA 原核表达 RNA 沉默

Abstract: A Hairpin RNA coding structure of the 824 bp region (from 2 274 to 3 097) of PRSV HC-Pro gene was constructed by the OZ-LIC method. After inserted into the plasmid pSP73, the structure was transformed into M-Jm109LacY of the RNase III-deficient strain and induced with IPTG. The recombinant E. coli strain could express H824-dsRNA that remained stable in the presence of DNase I enzyme and RNase A enzyme. We carry out a protective resistance assay and a therapeutic resistance assay. In the protective resistance assay, dsRNA was used to spray onto the plant surface before inoculation of papaya leaves with PRSV. Protective treatment experimental results showed dsRNA derived from the functional gene of PRSV could protect papaya plants from virus infection. ELISA analysis and Real-time RT-PCR results confirmed that the virus accumulation could be inhibited by dsRNA. In the therapeutic resistance assay, dsRNA was used to spray onto the plant surfaces 25 days after inoculation of papaya leaves with PRSV. ELISA analysis and Real-time RT-PCR results showed that the virus accumulation declined slightly after spraying dsRNA for 3 days.

Keywords: Papaya ringspot virus (PRSV), HC-Pro, dsRNA, prokaryotic expression system, RNA silencing

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