

分泌型Cecropin B 抗菌肽基因转化血橙提高其抗溃疡病水平

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Secreted Expression of Cecropin B Gene Enhances Resistance to *Xanthomonas axonopodis* pv. *citri* in Transgenic *Citrus sinensis* 'Tarocco'

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摘要 为了利用Cecropin B (CB) 抗菌肽基因提高柑橘对溃疡病的抗性, 人工合成了两个含有信号肽的Cecropin B 抗菌肽基因PR1aCB 和AATCB。洋葱表皮瞬时表达分析表明, 与非分泌型CB 抗菌肽相比, PR1aCB 和AATCB 抗菌肽在细胞间隙中优势积累。进一步构建了CaMV 35S 调控 PR1aCB 和AATCB 基因的植物表达载体, 转化塔罗科血橙 (*Citrus sinensis* Osbeck) 上胚轴, 获得转基因植株。GUS 组织化学染色、PCR 和Southern blot 分析表明外源基因已成功整合入柑橘基因组。Real-time PCR 定量分析表明, 抗菌肽基因在转基因植株中成功表达。柑橘叶片离体抗性分析表明, 转PR1aCB 和AATCB 基因植株的抗性显著强于非转基因植株, 其抗性水平与高抗品种‘南丰蜜橘’ (*C. succosa* Hort. Ex Tan) 和‘四季橘’ (*C. madurensis*) 相当。

关键词: 柑橘 信号肽 抗菌肽 柑橘溃疡病 遗传转化 抗性

Abstract: A Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Xac) is a very destructive disease, which affects the citrus industry in most citrus-producing areas of the world. Here, we reported the production of transgenic Tarocco blood orange (*C. sinensis* Osbeck) containing the synthetic antibacterial peptide genes and the evaluation of transgenic plants for resistance to Xac. Two new Cecropin B (CB) antibacterial peptide genes PR1aCB and AATCB with signal peptide (SP) sequence were synthesized by PCR. Transcription and expression showed that fluorescence accumulation was observed predominantly in intercellular space surrounding the onion epidermal cells transformed with PR1aCB : rfp and AATCB : rfp genes compared with CB : rfp without SP, indicating the SPs could direct protein secretion to the apoplast. PR1aCB and AATCB gene cassettes driven by CaMV 35S were introduced into Tarocco blood orange by *Agrobacterium*-mediated transformation. Integration of transgenes into citrus genome was confirmed by GUS histochemical staining, PCR and Southern blot. Transcriptions of PR1aCB and AATCB genes were detected by Real-time quantitative PCR in transgenic plants. Transgenic leaves were in vitro inoculated with suspension of Xac. Compared to control (nontransgenic plants and pGN transgenic plants without antibacterial gene), ten transgenic lines showed significant increase in resistance to citrus canker. The levels of the resistance of the transgenic lines were equivalent to that of the highly resistant varieties: Nanfeng Miju (*C. succosa* Hort. Ex Tan) and Calamondin (*C. madurensis*).

Keywords: Citrus, signal peptide, antibacterial peptide, citrus canker, genetic transformation, resistance

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