

### 应用RNA沉默技术获取抗黄瓜花叶病毒(CMV)和烟草花叶病毒(TMV)转基因烟草

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### Transgenic Tobacco Plants Resistant to Two Viruses via RNA Silencing

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摘要

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**摘要** RNA沉默是迄今最为有效的抗病毒策略, 利用该策略不但能获得免疫转基因植株, 且所得植株不易与其他病毒基因重组或异源包裹、生物安全性较高。将本实验室已构建的携带有烟草花叶病毒(TMV)部分移动蛋白基因( $\Delta MP$ )和黄瓜花叶病毒(CMV)部分复制酶基因( $\Delta Rep$ )反向重复结构的植物表达载体pBIN438-MP-Rep(i/r), 用农杆菌浸润法转化普通烟草品种K326, 共得196株转化植株, 经卡那霉素筛选和PCR检测发现128株为阳性转基因株; PCR-Southern和RT-PCR分析表明外源基因已整合到烟草基因组并在转录水平上得到表达; ELISA结果显示20.3%的转基因植株对CMV和TMV复合侵染表现免疫性。本结果为利用RNA沉默技术进行植物抗多种病毒育种提供重要数据, 为防治其他多种病毒复合侵染提供借鉴。

**关键词:** RNA沉默 黄瓜花叶病毒 烟草花叶病毒 双病毒抗性

**Abstract:** RNA silencing is a phenomenon of homologous RNA degradation induced by dsRNA, which is an effective strategy to obtain virus resistant plants so far. By means of this strategy, higher bio-security immune transgenic plants were obtained, avoiding recombining and transcapsidating with other virus genes. In this study, the recombinant plant expression vector pBIN438-MP-Rep(i/r) consisting the inverted repeat of TMV- $\Delta MP$  and CMV- $\Delta Rep$  fusion fragment was transformed into tobacco cultivar K326 via *Agrobacterium-mediated*. The transformants were selected in the culture medium with 100 mg L<sup>-1</sup> Kan. One hundred and ninety-six transgenic plants were obtained, One hundred and twenty-eight of which were positive plants, and the resistance to CMV and TMV was tested at the degree of virus. PCR-Southern blot and RT-PCR analysis of the transgenic plants demonstrated that the exogenous DNA was integrated into the tobacco genomic DNA and was expressed in transcriptional level. Resistance assay indicated that about 20.3% transgenic plants were immune to the co-infection with TMV and CMV. This result will provide to crops an important reference for plant anti-viral breeding and for preventing the viral co-infection.

**Keywords:** RNA silencing Cucumber mosaic virus Tobacco mosaic virus Dual-virus resistance

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