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研究论文

# 转外源法呢基焦磷酸合酶基因烟草抗赤星病研究

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摘要 将已克隆的薄荷（Mentha spicata L.）法呢基焦磷酸合酶（farnesyl diphosphate synthase, fps）的cDNA插入载体，构建CaMV35S启动子驱动下的植物表达载体pBinARfps。用捕获该质粒的根癌农杆菌菌株LBA4404与烟草叶片外植体共培养，并在附加30 mg/L Kan的MS＋0.1 mg/L IAA＋1.5 mg/L BA培养基上诱导植株分化，再生芽在附加30 mg/L Kan的MS培养基上生根，再生植株。Kan阳性植株经PCR-Southern检测筛选，得到5株PCR阳性植株（K-4，K-6，K-17，K-19，K-35），证明外源fps基因在烟草基因组中的整合；Northern blot检测证明外源fps基因在转录水平进行了表达；离体叶片接种实验表明，转基因植株（T1代）对赤星病抗性明显提高。这表明fps基因在植物抗病基因工程中具有潜在应用价值。

关键词 烟草 法呢基焦磷酸合酶基因 基因转导 赤星病

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## Expression of Foreign Farnesyl Diphosphate Synthase Gene in Transgenic Tobacco Enhances Disease Resistance to Alternaria alternata *in vitro*

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**Abstract** Farnesyl diphosphate synthase catalyzes two consecutive condensations of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DIMP) and the resultant geranyl diphosphate (GDP) to produce farnesyl diphosphate (FPP). Since FPP is the starting point of different branches of the pathway leading to the synthesis of large variety of isoprenoid end products, it is considered to play a key regulatory role of isoprenoid biosynthesis pathway. Previous reports proved that FPS regulated the sesquiterpenes biosynthesis in plants. To investigate the contribution of FPS to plant disease resistance, fps gene of Mentha spicata was transformed into and expressed in tobacco to observe its antifungal activity variation. The gene was cloned and inserted into binary vector under CaMV35S promoter to construct the plant constitutive expression vector pBinARfps. The leaf discs of tobacco (Nicotinan tabacium L. cv. K326) were transformed with fps gene via Agrobacterium-mediated transformation. Shoots were regenerated on MS medium supplemented with 30 mg/L Kan, 0.1 mg/L IAA and 0.5 mg/L BA and rooted on MS medium without hormone. PCR-Southern analysis proved foreign fps gene integration in 5 Kanamycin resistance plantlets (K-, K-6; K-17, K-19, K-35). Northern-blot indicated the foreign fps gene in transgenic plantlet was expressed at the transcriptional level. Disease challenge test of the detached leaves of transgenic plantlet by inoculation of Alternaria alternata showed that the resistance was dramatically enhanced compared with that of non-transgenic plants. The result implicated the potential application of fps gene in plant disease-resistance engineering.Farnesyl diphosphate synthase catalyzes two consecutive condensations of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DIMP) and the resultant geranyl diphosphate (GDP) to produce farnesyl diphosphate (FPP). Since FPP is the starting point of different branches of the pathway leading to the synthesis of large variety of isoprenoid end products, it is considered to play a key regulatory role of isoprenoid biosynthesis pathway. Previous reports proved that FPS regulated the sesquiterpenes biosynthesis in plants. To investigate the contribution of FPS to plant disease resistance, fps gene of Mentha spicata was transformed into and expressed in tobacco to observe its antifungal activity variation. The gene was cloned and inserted into binary vector under CaMV35S promoter to construct the plant constitutive expression vector pBinARfps. The leaf discs of tobacco (Nicotinan tabacium L. cv. K326) were transformed with fps gene via Agrobacterium-mediated transformation. Shoots were regenerated on MS medium supplemented with 30 mg/L Kan, 0.1 mg/L IAA and 0.5 mg/L BA and rooted on MS medium without hormone. PCR-Southern analysis proved foreign fps gene integration in 5 Kanamycin resistance plantlets (K-, K-6; K-17, K-19, K-35). Northern-blot indicated the foreign fps gene in transgenic plantlet was expressed at the transcriptional level. Disease challenge test of the detached leaves of transgenic plantlet by inoculation of Alternaria alternata showed that the resistance was dramatically enhanced compared with that of non-transgenic plants. The result implicated the potential application of fps gene in plant disease-resistance engineering.

**Key words** Nicotinan tabacium Farnesyl diphosphate synthase Gene transformation Alternaria alter

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