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烟草赤星病菌ISSR PCR反应体系的优化

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Optimization of ISSR-PCR Reaction System for *Alternaria alternata* in Tobacco

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摘要

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摘要 采用改良的CTAB法提取烟草赤星病菌丝基因组DNA, 通过正交与单因素变量设计试验, 对烟草赤星病菌ISSR PCR反应体系的各个影响因素进行优化, 建立了适合烟草赤星病菌ISSR分子标记的最佳反应体系, 即在25 μ L反应体系中包含10×buffer 2.5 μ L, 模板20ng,Mg²⁺浓度2 .0mmol/L, 引物浓度0.8 μ mol/L , dNTPs浓度0.2 mmol/L , Taq DNA酶 0.75U。利用所建立的体系对采自云南昆明、德宏、楚雄、曲靖、玉溪等地8份材料进行检验, 其结果表明优化后的体系适合烟草赤星病菌的ISSR PCR反应。

关键词: 烟草赤星病 ISSR PCR 正交体系优化

Abstract: The genomic DNA was extracted from *Alternaria alternata* mycelium infecting Tobacco using modified CTAB method. Using the genomic DNA of *A. alternata*, the factors influencing ISSR PCR system were optimized with the orthogonal and single factor variable design. The results show that the system, which contains 2.5 μ L 10 × buffer, 20ng template, 2.0mmol/L Mg²⁺, 0.8 μ mol/L primer, 0.2mmol/L dNTPs, 0.75U Taq DNA polymerase in 25 μ L reaction is suitable for *A. alternata* ISSR molecular marker analysis. DNA of eight samples collected from Kunming, Dehong, Chuxiong, Qujing, Yuxi and other areas were used in ISSR-PCR application with this system and results indicate it is suitable for this reaction system.

Keywords: *Alternaria alternata* ISSR PCR optimization of orthogonal

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