



### 土壤大丽轮枝菌微菌核的快速定量检测

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#### Rapid detection and quantification of *Verticillium dahliae* microsclerotia in soil

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**摘要** 微菌核是大丽轮枝菌在土壤中的主要存活结构和黄萎病的初侵染来源。对土壤中大丽轮枝菌微菌核进行定量是黄萎病监测和预警的基础。本研究以大丽轮枝菌Internal Transcribed Spacer (ITS)区特异性引物对P1/P2扩增产物的重组质粒为标准品,构建SYBR Green I实时荧光定量PCR反应的标准曲线,结合土样水筛法建立了土壤大丽轮枝菌微菌核定量检测体系。同时,建立了土壤中微菌核数量与棉花黄萎病发病率的关系模型。结果表明,实时定量PCR检测灵敏度比常规PCR高10倍,检测下限为1个微菌核/克土,在 $5.54 \times 10^2 \sim 5.54 \times 10^7$ copies范围内,DNA拷贝数的对数值与Ct值具有良好的线性关系。建立的土壤中微菌核个数 $n$ 与Ct值之间的关系为 $n = e^{7.3 - Ct/3.905}$ 。温室人工接种微菌核数量与棉花黄萎病发病率间的线性关系为 $y = 2.710n + 0.251$ 。

**关键词:** 大丽轮枝菌 微菌核 水筛法 实时荧光定量PCR 棉花黄萎病

**Abstract:** Microsclerotia are the main survival structure of *Verticillium dahliae* and primary causal inoculum agent of cotton in soil. The quantification of *V. dahliae* microsclerotia is the base for monitoring and prediction of Verticillium wilt. The standard curve of SYBR Green I real-time PCR (RT-PCR) detection assay for quantification of *V. dahliae* was successfully established using recombinant plasmid by the specific primer pair P1/P2 from Internal Transcribed Spacer (ITS). The quantification system of microsclerotia was developed using SYBR Green I RT-PCR based on wet-sieving extraction of microsclerotia. The model of microsclerotium number per gram soil and incidence of cotton Verticillium wilt was estimated using this system. The results showed that a linear relationship was observed between the log copies of recombinant plasmids DNA and cycle threshold (Ct) values of RT-PCR over a range of  $5.54 \times 10^2$  to  $5.54 \times 10^7$  copies. The detecting sensitivity for the SYBR Green I RT-PCR detection assay was 10-fold higher than that of the conventional PCR. The detection limit was 1 microsclerotium per gram soil. The relationship between Ct values and the number of microsclerotia was  $n = e^{7.3 - Ct/3.905}$ . The results of artificial inoculation in greenhouse indicated that there was a significantly positive correlation between the microsclerotium number of *V. dahliae* and the incidence of disease,  $y = 2.710n + 0.251$ .

**Key words:** *Verticillium dahliae* microsclerotia wet-sieving SYBR Green I Real-time PCR Verticillium wilt of cotton

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