香榧树根系病原真菌多重荧光定量 PCR 检测方法的建立及初步应用

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摘 要：由尖孢镰刀菌（*Fusarium oxysporum*）、立枯丝核菌（*Rhizoctonia solani*）、齐整小核菌(*Sclerotium rolfsii*)等土传病原菌引起香榧根腐病是香榧（*Torreya grandis* *'Merrillii'*）的主要病害之一，目前常见的土壤病原菌检测法有平板培养法，但该方法存在检测周期长，无法做到菌株定量等缺点。因此本研究了建立对这三种土壤习居病原菌的快速检测及香榧病害预防的核酸检测体系，本研究基于上述三种病原菌的基因序列保守区设计三组引物和 TaqMan 探针，建立三重荧光定量 PCR 检测方法。用三种病原菌基因序列构建的标准品质粒进行灵敏度和重复性检验，并使用其他土壤习居真菌基因组 DNA 进行特异性验证。结果显示，三重实时荧光定量 PCR 扩增效率均可达到 92.5%以上，特异性和重复性良好，检测的浓度范围在 1.5×10^3 ~1.5×10^9 copies/，且标准曲线均具有良好的线性，相关系数均在 0.99 以上 。经过对接种的一年生榧树根际土壤中病原菌检测以及田间健康香榧和患根腐病的香榧根际土壤检测，结果表明该多重实时荧光定量 PCR 可快速有效检测土壤中的目的病原菌含量。

关 键 词：香榧；根腐病；土传病害；三重实时荧光定量 PCR

Establishment and Preliminary Application of Multiple Fluorescence Quantitative PCR Detection Method for Pathogenic Fungi in the Roots of *Torreya grandis*

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Abstract: The root rot of Torreya grandis 'Merrillii' caused by soil-borne pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, etc. One of the diseases, the common soil pathogen detection method at present is the plate culture method, but this method has the shortcomings of long detection cycle and inability to quantify the strain. Therefore, this study established a nucleic acid detection system for the rapid detection of these three soil-habiting pathogens and the prevention of *Torreya* serrata disease. In this study, three sets of primers and TaqMan probes were designed based on the conserved regions of the gene sequences of these three pathogens to establish triple fluorescence quantification. PCR detection method. The sensitivity and reproducibility of standard grains constructed with the gene sequences of the three pathogens were tested, and the genomic DNA of other soil-habiting fungi was used for specific verification. The results showed that the amplification efficiency of triple real-time fluorescence quantitative PCR can reach more than 92.5%, with good specificity and reproducibility. The detection concentration range is 1.5×103 ~1.5×109 copies/, and the standard curves have good linearity and correlation. The coefficients are all above 0.99. After detecting the pathogenic bacteria in the rhizosphere soil of the inoculated annual Torreya, and the field healthy torreya and the root rot-affected rhizosphere soil of the torreya, the results showed that the multiple real-time fluorescence quantitative PCR can quickly and effectively detect the content of the target pathogen in the soil.

Key words: *Torreya grandis*; root rot; soil-borne diseases; triple real-time fluorescence quantitative PCR