Development of A Sensitive Nested-PCR Assay for the Detection of *Ustilago scitaminea*

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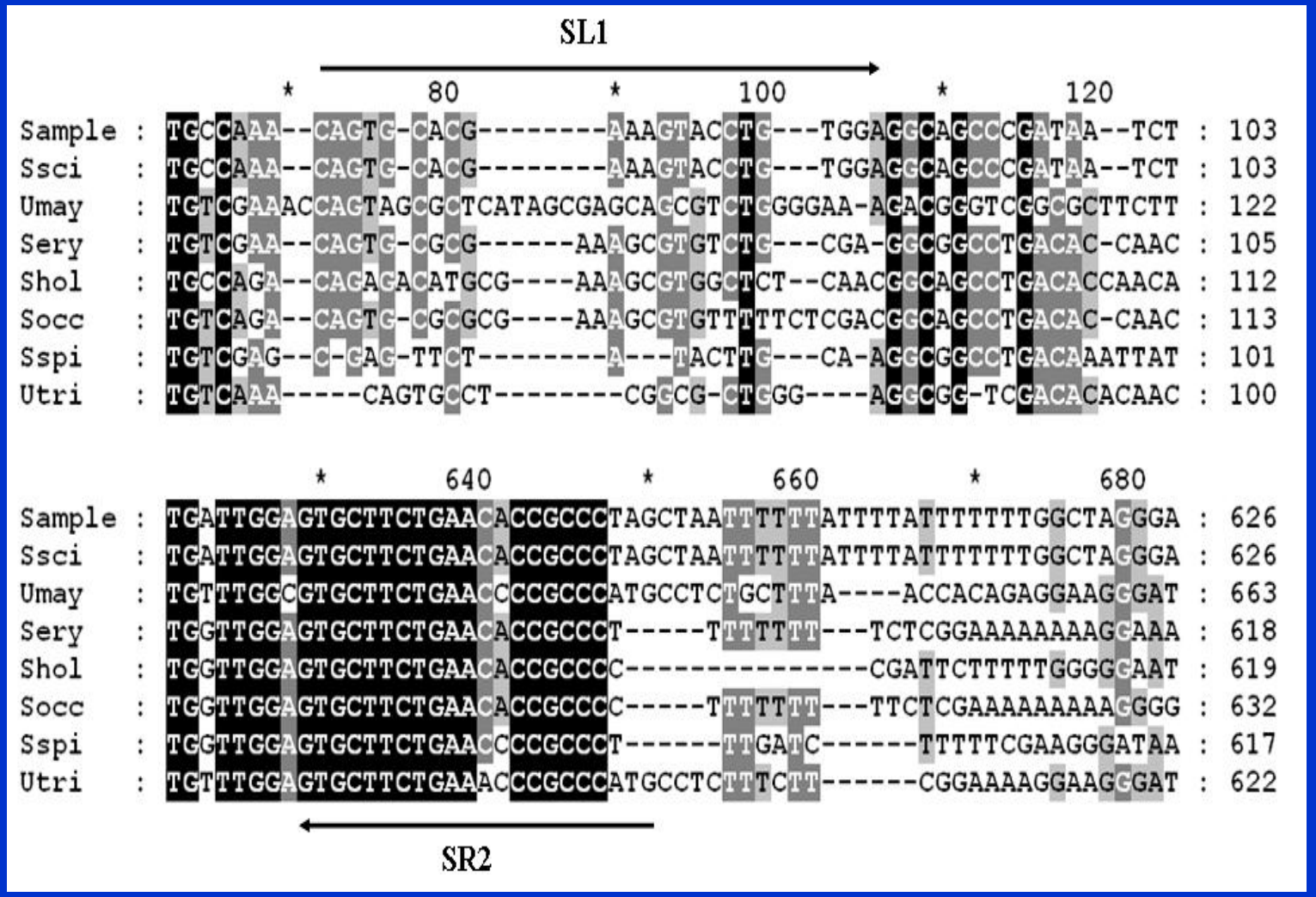
A species-specific polymerase chain reaction (PCR) assay was developed for rapid and accurate detection of *Ustilago scitaminea*, the causal agent of sugarcane smut disease. Based on nucleotide differences in the internal transcribed spacer (ITS) sequences of *U. scitaminea*, a pair of species-specific primers, SL1 (5`-CAGTGCACGAAAGTACCTGTGG-3`) and SR2 (5`-CTAGGGCGGTGTTCAGAAGCAC-3`), was designed by using a panel of fungal and bacterial species as controls.

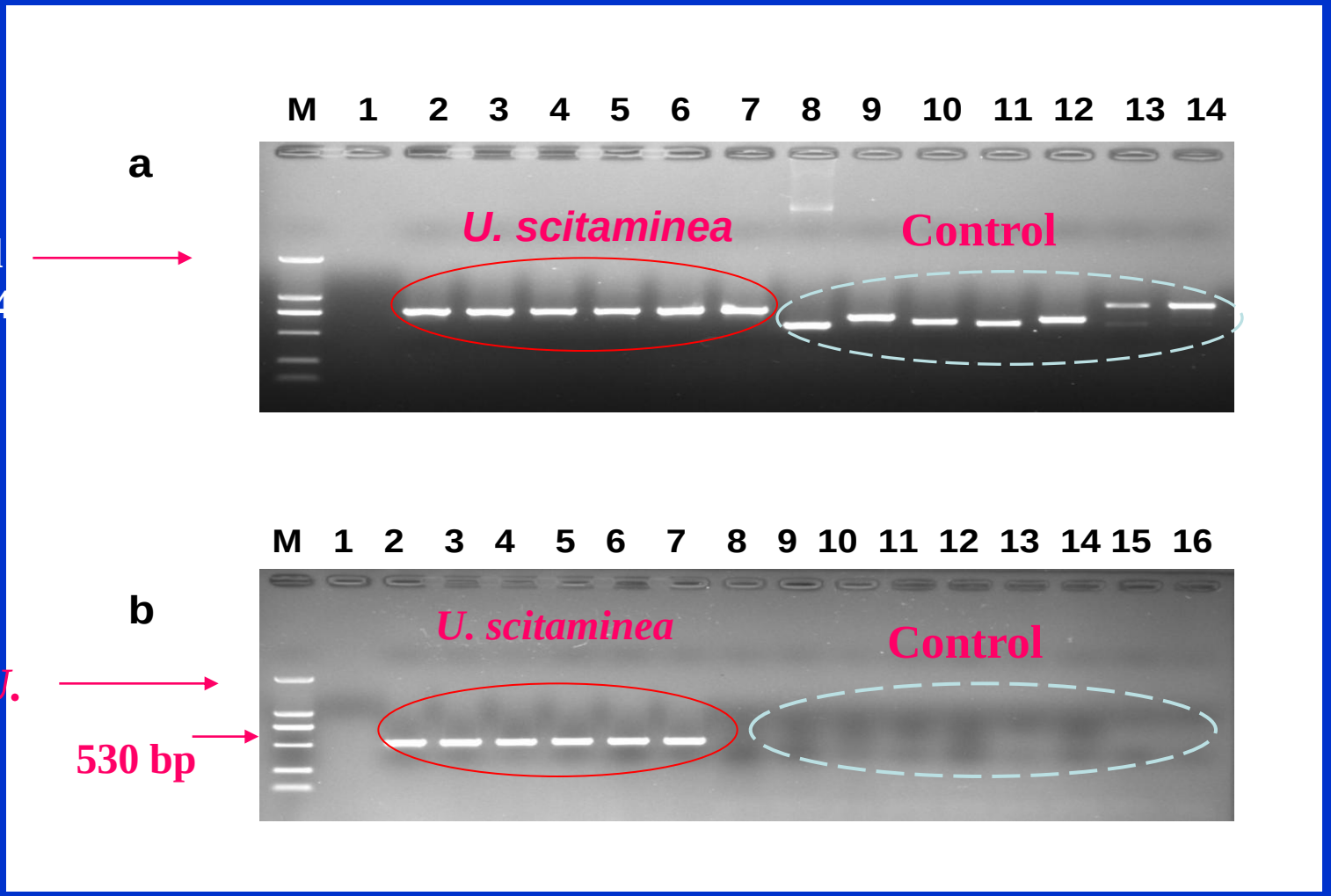
The primers SL1/SR2 specifically amplified a unique PCR product about 530 bp in length from *U. scitaminea* strains with a detecting sensitivity at 200 fg of the fungal genomic DNA in a 25 μl reaction solution. To increase sensitivity, a nested-PCR protocol was further established, which used ITS4/ITS5 as the first-round primers followed by the primer pair SL1/SR2. This protocol increased the detection sensitivity by 10,000-fold compared to the PCR method and could detect the fungal DNA as low as 20 ag.

The nested PCR detected *U. scitaminea* from young sugarcane leaves with no visible smut disease symptoms. The findings from this study provide a sensitive and reliable technique for the early detection of *U. scitaminea*, which would be useful for sugarcane quarantine and production of germ-free seedcanes.



ITS sequence alignment and primers design

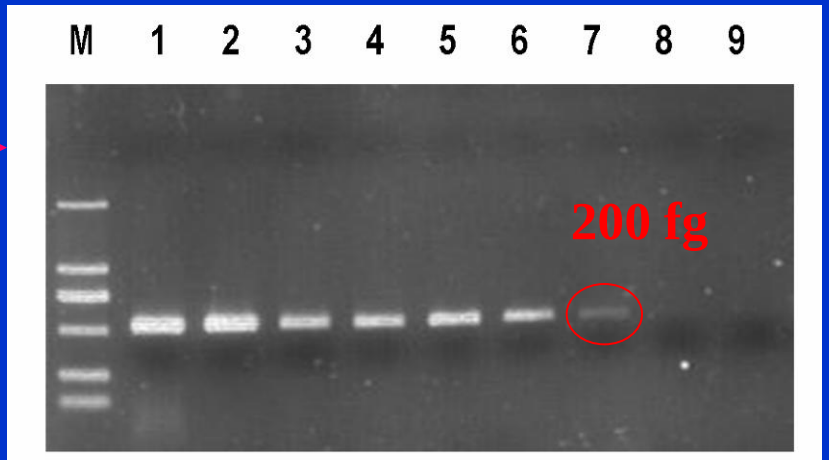
the universal primers ITS4 and ITS5



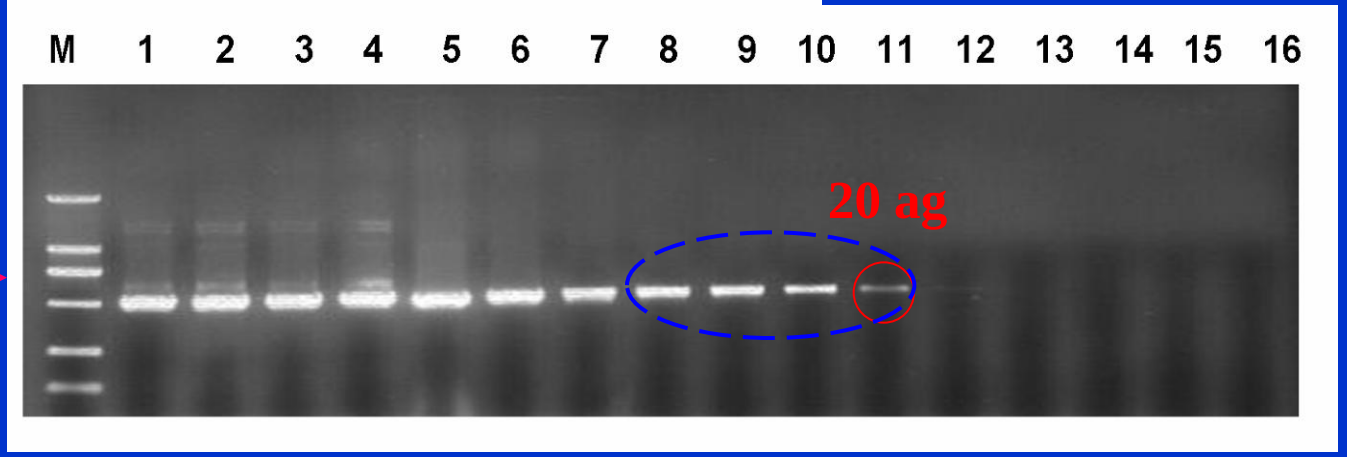
Primer pair SL1/SR2 of *U. scitaminea*

Specificity of the designed primers

PCR

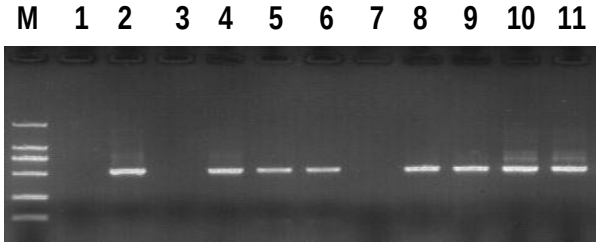


nested-PCR

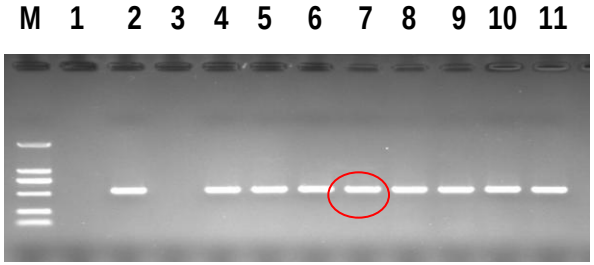


Sensitivity of the PCR detection and the nested-PCR detection

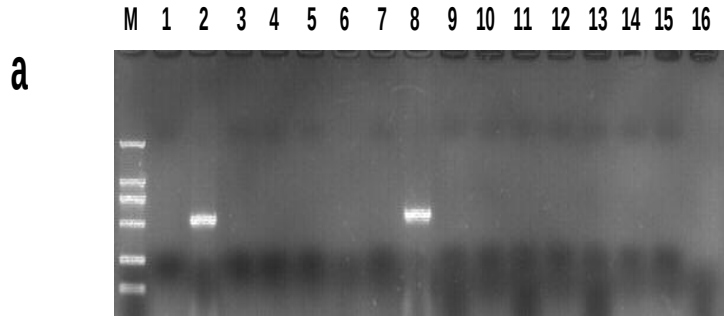
PCR



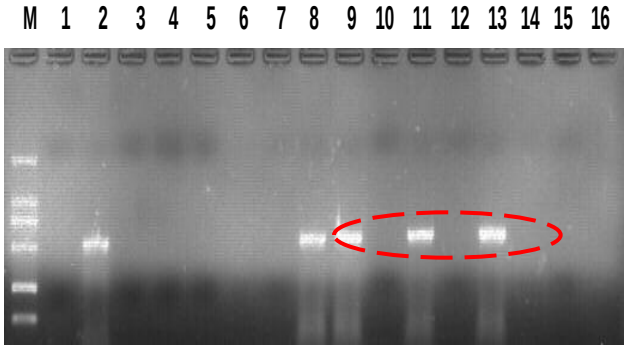
nested-PCR



Detection of the pathogen from the symptomatic sugarcane plants



PCR



nested-PCR

Detection of the pathogen from the asymptomatic sugarcane plants

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Thank you for your attention!