

PP-02: Development of a PCR based assay for the detection of *Rigidoporus microporus* (causative organism of white root disease of rubber)

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Introduction: *Rigidoporus microporus*, one of the most harmful fungal pathogens which cause white root disease, affects Para rubber (*Hevea brasiliensis*) plantation, the second largest plantation in Sri Lanka. *R. Microporus*, a member of basidiomycetes division, affects the plants starting from its root to other parts. The damage is caused due to certain enzymes produced by the fungus and this occurs stage by stage. Firstly, a formation of white rhizomorphs grows and firmly adheres to the root's debris. Next, the collar region of trees gets infected and basidiocarps occur. The primary justification for conducting this study is because the roots of plants are initially affected making it difficult to detect the fungus. Hence initial identification of the fungus is important so that the pathogen can be detected at an early stage which helps to halt the progression of the disease. Accordingly, the objective of the study was to develop a polymerase chain reaction (PCR) based assay for the detection of *R. microporus*.

Methods: *R. microporus* isolates' DNA was extracted using two methods and PCR amplification was performed. For PCR amplification, β -tubulin primers were designed using the Bioedit software and Primer3 Input (version 0.4.0). With the chosen primer, temperature, MgCl₂ and dNTPs were optimised. Sensitivity and specificity assay was performed using prior PCR conditions.

Results: The annealing temperature was adjusted to 60.7°C. Optimal PCR conditions were found to be 2.0mM MgCl₂, and 0.15mM dNTPs. Sensitivity and specificity assay showed the lowest detectable template concentration was 0.29ng and the primers were specific for this particular fungus, respectively.

Conclusions: In conclusion, the PCR assay developed in the current study is sensitive and specific for the detection of *R. microporus*.

Keywords: *Hevea brasiliensis*, polymerase chain reaction, primer, *Rigidoporus microporus*, sensitivity