

Development of a Fast and Reliable Molecular-Based Detection Method for Sugarcane White Leaf Disease (WLD) Phytoplasma

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ABSTRACT

White leaf disease (WLD) is one of the devastating diseases caused by phytoplasma in sugarcane plantations in Sri Lanka. A fast and reliable method for detecting the phytoplasma is essential to prevent the spread of the disease through infected seed material. Currently, the Sugarcane Research Institute, Sri Lanka has been using the phytoplasma-specific universal primers with nested PCR reactions for detecting the WLD phytoplasma. This paper presents a protocol developed for detecting the WLD phytoplasma in sugarcane by running a single PCR programme only, with high level of reliability. The PCR reaction was performed using the DNA extracted by CTAB method and SPP1 forward and SPP2 reverse primers. The primer annealing temperature of 53 °C, which was determined by the gradient PCR programme, was used. The PCR products were analysed in 2% Agarose gel electrophoresis and then stained in Ethidium Bromide before their sequencing. The similarity of the query sequence with subject sequences was examined by performing the NCBI nucleotide BLAST analysis. One hundred sugarcane plants that were visually-positive or negative for WLD, obtained from infected planting material, were subjected to the above-mentioned procedure to identify its reliability in detecting the WLD phytoplasma. According to the PCR results, the amplified bands were clearly visible in 2% Agarose gel and the sequenced results confirmed that the size of the PCR product is 287 bp. The nucleotide BLAST results confirmed that the query sequence was 94% identical with the subject sequence, 16S rRNA gene of the sugarcane white leaf disease phytoplasma strain of the Genbank accession number KT270948.1 with the lowest E-value. Out of the 100 plants tested, 96 were proven positive for WLD by this method and 32 plants were detected positive in visual detection method. Thus, this method can be accepted as a fast and reliable molecular method for detecting WLD phytoplasma.

Key words: PCR, Phytoplasma, Sri Lanka, Sugarcane, Sequencing, White Leaf Disease