Development of a DNA Probe to Detect the Pathogen of White Leaf Disease of Sugarcane

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Development of a pathogen specific probe is useful for the detection of infections in plant tissues, especially for diseases caused by unculturable pathogens. Sugarcane white leaf disease (SWLD) is a devastating biotic threat to sugarcane industry in Sri Lanka and it is caused by a phytoplasma. The present study was conducted to develop a DNA probe for specific detection of SWLD pathogen in infected plant tissues and to confirm its ability to detect the pathogen by nucleic acid hybridization. Samples were collected from plants showing symptoms and from symptomless plants from Hingurana, Sri Lanka. Three methods were used to optimize the extraction of genomic DNA from sugarcane samples. Using different sets of phytoplasma specific primers (P1/P7, R16F2n/R16R, PC399/P1694 and SPP1/SPP2) PCR amplification was optimized. Standard PCR, nested PCR and gradient PCR were performed to amplify the expected PCR products from different primer pairs. PCR products obtained were sequenced and subjected to homology search. After confirmation of the identity of the PCR products, a probe was prepared by labeling with Digoxygenin. Efficiency of the probe was tested by dot blot hybridization using genomic DNA from healthy and SWLD-infected plants and pathogen specific PCR products as a positive control. Among the tested methods, DNA extraction method developed by Plant Virus Indexing Centre, Homagama resulted in intact genomic DNA from the sugarcane leaf samples. Standard PCR using SPP1/SPP2 primer pair was successful in producing the expected PCR amplicon (321 bp). The sequence of the resulted amplicon gave the highest DNA homology with *Candidatus* Phytoplasma oryzae. Dot blot hybridization and subsequent chemiluminesence detection gave signals for genomic DNA of SWLD-infected plants and for the positive PCR control. Results confirmed the potential use of the developed DNA probe for detection of the causal agent of SWLD in sugarcane.

Keywords: SCWL, Dot blot hybridization, Non-radioactive labeling, Phytoplasma

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