

## **Development of a DNA Probe for Detection of White Leaf Disease in Sugarcane**

**Nawarathna H.M.O.N., Kumara U.M.A.<sup>1</sup>, Thushari A.N.W.S.<sup>2</sup> and De Costa D.M.\***

Department of Agricultural Biology,  
Faculty of Agriculture, University of Peradeniya, Peradeniya

Sugarcane white leaf disease (SCWL) caused by phytoplasma is a serious biotic threat to the sugarcane cultivation in Sri Lanka. Phytoplasmal diseases can be most effectively controlled by preventive methods. Therefore, a molecular tool would be effective for early detection of the causal agent. The present study was conducted to develop a DNA probe specific to SCWL pathogen and validate it for early detection of the pathogen. The study also focused on detecting the SCWL pathogen in planting material subjected to several physical and chemical treatments. For the development of a non-radioactively-labelled DNA probe, genomic DNA was extracted from leaves and cane setts of the plants showing typical SCWL symptoms. PCR amplification was performed using SPP1 and SPP2 primers. The expected PCR product (321 bp) was labelled using Digoxigenin, sequenced, subjected to homology search and identified the pathogen as sugarcane white leaf phytoplasma. Genomic DNA extracted from cane setts of plants, subjected to 20 different treatments were used to prepare dot blots (3 µg of DNA/dot) and hybridized with the prepared probe. Signal detection was done colourimetrically and intensity was quantified by UN-SCAN-IT software. The probe successfully detected the targeted DNA sequence of the pathogen when genomic DNA is at a concentration of 3 µg and PCR product has a concentration of 1 µg of DNA. Presence of the pathogen in all samples was reconfirmed by amplification using SPP1 and SPP2 primers. Hybridization and PCR results revealed that genomic DNA of tissue cultured plants and cane setts treated with tetracycline and salicylic acid at concentrations of 500, 750 and 1000 ppm did not contain the pathogen. However, the probe detected the pathogen in hot water treated cane setts, immediately after the treatment, three months after treatment and in plants maintained in insect-proof houses, after hot water treatment.

**Keywords:** Digoxigenin, PCR amplification, Salicylic acid, SCWL, Tetracycline

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<sup>1</sup>Department of Agricultural Technology, Faculty of Technology, University of Colombo, Colombo

<sup>2</sup>Division of Crop Protection, Sugarcane Research Institute, Udawalawa

\* devikadecosta@gmail.com