

## **Detection and Quantification of Sugarcane White Leaf Disease Causing Phytoplasma in Fertilizer-treated Sugarcane Plants**

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Sugarcane white leaf disease (SWLD) is a destructive phytoplasmal infection prevalent in sugarcane plantations in Sri Lanka. Application of heavy doses of fertilizer (N, P and K) masks the symptoms of SWLD and such plants are sold by farmers as disease-free planting material due to the absence of visual symptoms. The present study was conducted to detect and quantify the causal pathogen of SWLD in selected fertilizer-treated sugarcane varieties, maintained at Sugarcane Research Institute, Sri Lanka. Samples were collected after application of fertilizer, three times, at monthly intervals. Based on the intensity of symptoms developed at the time of initial sample collection, two groups of plants were identified, namely having severe SWLD symptoms (SS) and showing mild symptoms (MS). Each group contained five varieties. Genomic DNA was extracted from leaves of all plants from groups SS and MS, and PCR amplification was done using SWLD phytoplasma specific primers (SPP1 and SPP2). Expected amplicon (321 bp) was resulted in all samples of SS and MS groups. DNA was sequenced and subjected to homology search. qPCR was performed for quantification of the copy number of SCWL phytoplasma/sample by absolute quantification using standard curve method. The copy number/sample in group SS significantly differed ( $p < 0.05$ ) among the varieties. However, in group MS, copy number of the pathogen/sample varied significantly ( $p < 0.05$ ) due to the interaction effect (variety x time). DNA homology search revealed the presence of sugarcane white leaf (SCWL) phytoplasma and sugarcane grassy shoot phytoplasma (SCGS) in the plants of SS and MS groups, respectively. Findings confirmed the presence of SCWL and SCGS phytoplasma in plants treated with high doses of fertilizer in varying quantities.

**Keywords:** Absolute quantification, Copy number, Standard curve, Sugarcane grassy shoot, qPCR

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