Use of PCR amplification to investigate alternate hosts of sugarcane white leaf phytoplasma in Sri Lanka

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A phytoplasma has been reported to be the causal agent of the sugarcane white leaf disease which first appeared in Kantale in 1972. At present, the disease has become more prominent in Sri Lanka. Sugarcane associated weed species, which show the related symptoms of this disease were assumed to play an important role as alternate hosts for the survival of sugarcane white leaf phytoplasma in Sri Lanka (SWLP-SL). This was explored in seven species of symptomatic and non-symptomatic weeds through possible PCR amplification of about a 557 bp fragment of the 16S ribosomal DNA of the phytoplasma using a universal primer pair P1/ P2 followed by similar amplification of 321 bp fragment of the same DNA of SWLP-SL with the specific primers SPP1/SPP2. Template DNA of SWLP-SL was extracted from sugarcane shoots both, severely affected by SWLP-SL and SWLP-SL symptom-masked shoots. DNA of sugarcane plants raised from apical meristem tissues was used as the negative control. Amplified DNA fragments were separated on agarose gel and visualized under UV.

None of the weeds was positive for harboring SWLP-SL. However, symptomatic *Brachiaria sp., Calyptocarpus viatis, Phaseolus lathyroides* and *Commelina benghalensis* were positive for phytoplasmas whereas both symptomatic and non-symptomatic *Borreria hispida, Euphorbia heterophylla* and *Acalypa indica* were not associated with any phytoplasmas. Only the DNA extracted from the shoots severely affected by SWLP-SL and SWLP-SL symptom-masked shoots resulted bands on gel at the 321 bp level showing successful PCR amplification. Detection of even low titers of SWLP-SL in symptom-masked shoots indicates the efficacy and reliability of the method.

The hypothesis of SWLP-SL being harbored in those weeds as alternate hosts can be ruled out with the finding and that would be valuable in the management of the disease.