

Rapid and sensitive detection of sugarcane white leaf and sugarcane grassy shoot diseases by Loop mediated isothermal amplification (LAMP) assay

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Phytoplasma born sugarcane white leaf (SCWL) and sugarcane grassy shoot (SCGS) diseases have a major impact on the yield of sugarcane (*Saccharum officinarum* L.), a high potential cash crop in Sri Lanka. Sugarcane plants with phytoplasma disease symptoms were first reported in Sri Lanka in 1990. These phylogenetically closely related phytoplasma strains are categorized under 16SrXI group. Accurate disease diagnosis and proper disease management is crucial in increasing the yield of this crop.

Loop mediated isothermal amplification (LAMP) is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for early detection and identification of microbial diseases and has been adopted for many phytoplasma diseases. Here we report the study undertaken to establish a rapid and sensitive diagnostic tool for detection of SCWL and/or SCGS diseases by LAMP assay.

Partial nucleotide sequences of leucyl tRNA gene of both SCWL and SCGS diseases generated by nested PCR products, revealed 100% identity between these two phytoplasmas and the consensus sequence was used accordingly to design the LAMP primers; Sugar F3, Sugar B3, Sugar FIP, Sugar BIP using Optigene LAMP designer (Optigene, UK). On agarose gel analysis, the LAMP amplicons produced a ladder like pattern in contrast to a single band as observed in PCR. All symptom positive samples scored positive in agarose gel electrophoresis. The LAMP assay was sensitive enough to detect up to a minimum level of 125 ng of total template DNA by agarose gel electrophoresis. Comparison between conventional thermocycler and the thermostatic water bath methods for LAMP showed similar results on 2% agarose gels suggesting that water bath can be used for field level detection.

This study revealed that the established, single step isothermal DNA amplification assay has potential to be used as an accurate and rapid diagnostic assay for SCWL and SCGS diseases in laboratories with low resource settings.

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