OPTIMISATION OF PCR PROTOCOL FOR DETECTION OF SUGARCANE LEAF SCALD BACTERIA AND IDENTIFICATION OF THE LEAF SCALD DISEASES BACTERIAL STRAIN IN SRI LANKA

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Xanthomonas albilineans is the gram-negative bacteria caused for sugarcane leaf scald disease, which is one of the important sugarcane diseases in Sri Lanka. Detection of the disease is being done based on distinct symptoms of the leaf scald disease. Though it is accurate, latent infection of the disease cannot be detected. Therefore, the objective of this study was to optimise a PCR protocol for detection of leaf scald bacteria and identification of bacterial strain in Sri Lanka. Two gradient PCR were performed to determine the optimum annealing temperature of the XaF/XaR and L1/Ala4 primers which are the commonly used for detection of *Xanthomonas albilineans*. Only the primer pair L1/Ala4 was amplified during gradient PCR and optimum band intensity was given at 59.5 ºC. Genomic DNA from the 10 visually-positive and 10 visually-negative plants derived from leaf scaled infected seedcane were tested for L1/Ala4 primer pair. PCR master mixture was prepared by adding each PCR ingredient with standers concentration instead of commercially available master mixtures. PCR products were sequenced and NCBI-BLAST analysis was done for confirmation of the organism and its strain. The sequence was uploaded into GenBank available under MN 460365 accession number. All visually positive samples were positive for the PCR test and 9 visually negative samples were positive for the PCR. The Sequenced results confirmed the size of the amplicon is 366 bp. The sequence was 100% identical with *Xanthomonas albilineans* 16S-23S ribosomal RNA intergenic spacer region of Xa-FJ1 under MH709164 GenBank accession number. Top 18 BLAST hits were 100% identical with the query sequence and top 13 subject sequences were reported in different provinces of China. The results concluded the primer pair L1/Ala4 can successfully used for molecular detection of sugarcane leaf scald bacteria at symptomatic and latent stages by PCR program with standard PCR ingredients. The Sri Lankan strain of *Xanthomonas albilineans* is almost identical for the Chinese strain of *Xanthomonas albilineans* which were reported in different provinces of China.

Keywords: *GenBank, Leaf Scald Disease, PCR protocol, Sugarcane, Xanthomonas albilineans*