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DETECTION AND CONFIRMATION OF PHYTOPLASMA DISEASE IN *Manilkara zapota* BY USING MOLECULAR TECHNOLOGY

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ABSTRACT

Phytoplasma disease is caused by plant pathogenic Phytoplasmas which are cell wall less bacteria that causes devastating losses in yield and quality of crop products in Sri Lanka. Detection and confirmation of phytoplasma diseases in Sapota (*Manilkara zapota*) infected crop by using molecular technology required to gain rapid accurate results in identification to compete with increment of virulence of the pathogens. Sapota is one of the underutilized fruit crops grown in Sri Lanka which belongs to the family Sapotaceae and have high economic value as a fruit crop. This study was conducted as a molecular approach for phytoplasma detection, identification and confirmation in *Manilkara zapota.* The Polymerase Chain Reaction based method was used with universal primers (P1, P7) for 16S rRNA gene to detect phytoplasma in sapota and the amplified DNA fragments in 557 bp were visualized on 2% agarose gel. Further confirmation was done by using DNA sequencing. The highest homology 83 % obtained was that for Aster yellow witches' broom phytoplasma AYWB, complete genome with $2e^{-60}$ *e* value. For the accurate detection of phytoplasma caused symptoms in Sapota, oligonucleotide primers were designed, using sequenced phytoplasma DNA. Those designed primers were characterized, optimized and primer specificity was analysed. Primers Mx for Sapota is forward -5'-GCCAGGCAGTCCACTTATCA-3' and reverse 5'-GTGCACGCCCTAAACGAATC-3'. The length of the primer was 20 bases and detectable band in gel profile was 880 bp with three unstable hairpin loops. Primer Mx best annealing temperature was 45 °C and showed 90% specificity. Mx primers can be used for specific, sensitive detection of phytoplasma infect to Sapota (*Manilkara zapota*) plant species.

Keywords: Phytoplasma, PCR, Primers, Gene Sequencing, Template DNA

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