DETECTION AND CONFIRMATION OF PHYTOPLASMA DISEASE IN DIFFERENT PLANT SPECIES BY USING MOLECULAR TECHNOLOGY

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ABSTRACT

Phytoplasma disease is caused by plant pathogenic cell wall less bacteria that causes devastating losses in yield and quality of agricultural production in Sri Lanka. Effective control is required to minimize the spread of the disease through identification of the organism. Detection and confirmation of phytoplasma diseases in infected plant species by using molecular technology required to gain rapid accurate results in identification to compete with increment of virulence of the pathogens. However there are least number of research conducted on phytoplasma diseases in Sri Lankan context. Hence, this study was conducted as a molecular approach for phytoplasma detection, identification and confirmation. The Polymerase Chain Reaction based method was used with universal primers for 16S rRNA gene to detect phytoplasma in fifty different suspected plant species and the amplified DNA fragments in 557 bp were visualized on 2% agarose gel. Thirty-six plant species gave positive results with producing DNA fragment in 557 bp size. For accurate detection of phytoplasma caused symptoms in Sapota (*Manilkara zapota*) and Petunia (*Petunia sp.*) two oligonucleotide primers were designed, from sequenced phytoplasma DNA extracted from Sapota and Petunia infected plants by using NCBI, ClustalW2 and BLAST. Those designed primer were characterized, optimized and primer specificity was analyzed. Primers Mx for Sapota is forward -5'- GCCAGGCAG TCCACTTATCA-3' and reverse -5'- GTGCACGCCCTAAACGAATC-3'. The length of the primer was 20 bases and detectable band in gel profile was 88 bp with three unstable hairpin loops. Primer Mx best annealing temperature was 50 °C and showed 90% specificity. Primers Px for petunia is forward -5'-CGGCTTGGCTACCCTTTGTA-3' and reverse -5'- TACCTGGCCTTGACATGCT-3. The length of the primer was 20 bases and detectable band in gel profile was 288 bp with eight unstable hairpin loops. Primer Mx best annealing temperature was 45 °C and showed 30% specificity. Mx and Px primers can be used for specific, sensitive detection of phytoplasma infected to Sapota (*Manilkara zapota*) and Petunia (*Petunia sp.*) plant species.

Key words: Phytoplasma, PCR, Primers, Gene Sequencing, Template DNA