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DETECTION, CONFIRMATION AND MOLECULAR CHARACTERIZATION OF PHYTOPLASMA DISEASES AND STRAINS IN FIVE DIFFERENT CROP COMMODITIES

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Phytoplasma is an intracellular, pleomorphic, gram positive bacteria with lack of cell wall and important for plant disease in hundreds of economic plant species in Sri Lanka. Several symptoms such as witches' broom, phyllody, virescence, bolting, formation of bunchy fibrous secondary roots, yellowing/ reddening, stunting and phloem necrosis have been observed in cultivated crop commodities as well as weeds which collected from different areas in Sri Lanka. Addition to that, seed samples were collected and let them to germinate in inside the laboratory to detect seed bone transmission of phytoplasma. DNA was extracted by using Phenol extraction method for direct Polymerase Chain Reaction (PCR). Symptomatic and asymptomatic plants were detected for phytoplasma through the amplification of a typical genomic fragment of 557 bp by using universal primer P1/ P7. Out of 250 plants 110 gave positive results since Nested PCR technique was practiced for further confirmation. Samples which gave positive results were confirmed Nested PCR, for 1st PCR round R16mF2/ R16Mr1 primers were used and amplification size was 1.4 kb. Second PCR 1.2 kb fragment was taken by using R16F2n/ R16R2 primers. 31 samples were subjected to gene sequenced. Through Nested PCR out of 110 samples 28 samples were gave positive results and they were confirmed as phytoplasma and they were submitted to similarity search (BLAST) in the NCBI database which confirmed that a phytoplasma belonging to the 16Sr " Candidatus Phytoplasma" group. Phylogenetic tree was performed by using sequenced phytoplasma samples 28 positive included samples symptomatic samples and asyptomatic plant samples. 10 samples were observed as seed bone transmission of phytoplasma.

Keywords: Phytoplasma, Witch's broom, Phyllody, Virescence, Polymerase Chain Reaction, Nested PCR