Detection and Confirmation of Phytoplasma Associated with Cucurbit Species in Sri Lanka

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Phytoplasma is an intracellular, pleomorpic, gram positive bacteria with lack of cell wall and important for plant disease in hundreds of economically important plant species in Sri Lanka. In plants, phytoplasmas induce symptoms that suggest interference with plant development. Typical symptoms include: witches' broom of developing tissues, phyllody, virescence, bolting, formation of bunchy fibrous secondary roots, reddening of leaves and stems, generalized yellowing, decline and stunting of plants and phloem necrosis. Some of the Cucurbit species showed the little leaf disease symptoms including dwarfed, thickened and puckered leaves and shortened internodes, which were very close to pjytoplasma symptoms. This study was conducted to identify and confirm phytoplasma disease in selected cucurbit species. The DNA was extracted from disease suspected plants and were subjected to direct PCR with universal primers P1/P2. Then the DNA was again assayed in a nested-PCR. Primers P1/P7 were used in the nested PCR round 1 to amplify desired product of 1.78 kb and primers R16F2N and R16R2 were used for the second PCR to amplify the 1.23 kb size product. The phytoplasma 16SRNA region was sequenced directly with P1/P2 primers and compared by NCBI BLAST analysis. The highest homology obtained for all three crops (bitter gourd, snake gourd and ridge gourd) was Lethal wilt oil palm phytoplasma clone LWP-16S23S-P2P1 16S ribosomal RNA gene. These transcripts for all tested species showed sufficient similarity to phytoplasma query to appear on the list of hits with a very significant E value. Bitter gourd, snake gourd and ridge gourd showed 2e89 , 1e-118 and 2e-107 E values respectively with 82% , 86%. 85% identities respective to each species.

Keywords: NCBI BLAST, Nested PCR, Phytoplasma