Diseases Caused by Bacteria and Phytoplasmas

First Report of 16SrII Group (Peanut Witches' Broom) Phytoplasmas Associated with the *Leucas aspera* Phyllody in India

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Leucas aspera (Wild.) L. (family: Lamiaceae) is a commonly found weed throughout India, known for its pharmacological properties. Its white flowers and leaves are used in many Ayurvedic formulations for the treatment of chronic rheumatism, psoriasis, snake bites, and skin eruptions (Prajapathi et al. 2010). During a survey of commercial flower crop fields in May 2018, a few *L. aspera* plants, growing as unwanted weeds in the fields and surrounding agricultural wastelands with the symptoms of phyllody, virescence, and little leaves, were observed in the Emmekoppalu (12.2106, 76.2511; $n = 1/26$ plants) and Beerihundi (12.1630, 76.3225; $n = 2/59$ plants) localities of Mysuru district, and Srirangapatna in Mandya district (12.2541, 76.411; $1/67$ plants), Karnataka, India. The disease incidence in the surveyed localities was less than 4%. The total genomic DNA was extracted from the leaf midrib tissues of three representative symptomatic and two asymptomatic samples using the CTAB method. The phytoplasma 16S rRNA gene was amplified in nested PCR assay by P1/P7 followed by R16F2n/R16R2 primers using long amplification (LA) Taq polymerase (Takara, Japan). Additionally, the PCR assays were performed for the amplification of phytoplasma *secA* gene using the primers SecAfor1/SecArev3 and SecAfor2/SecArev3 (Hodgetts et al. 2008). The DNA templates from all the symptomatic samples generated amplicons of

approximately 1.25 kb (16S rRNA gene) and 480 bp (*secA* gene), revealing the association of phytoplasma strains. No amplifications were observed for the asymptomatic *L. aspera* samples. The obtained 16S rRNA gene sequences (MN223676, MT807111, and MZ093053) showed 97.96, 98.37, and 98.18% sequence identity, respectively, with the '*Candidatus* Phytoplasma aurantifolia' strain WBDL (U15442) using the EzBioCloud database. NCBI-BLAST analysis revealed maximum identity to various peanut witches' broom (PWB) phytoplasma strains. The virtual RFLP tool, iPhyClassifier, delineated the *Leucas* phyllody phytoplasma strains (MN223676, MT807111, and MZ093053) to group 16SrII (PWB group) subgroup D with the similarity coefficient 1.0 (Zhao et al. 2009). The obtained *secA* gene sequences (MZ151944, MZ151945, and MZ151946) were 98.15 to 100% similar to the strain sequences of PWB phytoplasma strains. Further, the clustering pattern in the phylogenetic trees (16S rRNA and *secA* genes) constructed using MEGA 7 confirmed that the *Leucas* phyllody phytoplasma sequences were closely related to PWB strains. To the best of our knowledge, this is the first report on the association of 16SrII-D subgroup phytoplasma with the phyllody disease of *L. aspera*. In India, many weeds and wild plants serve as alternative hosts of PWB phytoplasmas and aid in the emergence of related diseases in economically important crops (Thorat et al. 2016, 2017). The close genetic association of phytoplasma strains found in *L. aspera* and many other crops indicates the presence of common insect vector(s) transmitting these phytoplasmas (Yadav et al. 2015). This report is an addition to the catalog of the weed species harboring phytoplasma strains associated with economically important crop plants (Rao et al. 2017). The screening of phytoplasma strains in weeds, alternate hosts, and known/unknown insect vectors is therefore essential to develop management strategies and effective management of phytophagous insect vectors feeding on both weeds and crop plants.

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