Diseases Caused by Bacteria and Phytoplasmas

First Report of ‘*Candidatus Phytoplasma trifolii*’ Associated with Little Leaf and Floral Virescence Disease in *Solanum violaceum* from the North East Region of India

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Solanum violaceum Ortega (Indian nightshade) is a medicinal plant belonging to the family Solanaceae. It grows naturally as a weed in agricultural fields, forest edges, roadsides, and fallow lands throughout the North East Region of India. It is mostly consumed as a vegetable by the native people of the region because of its promising therapeutic effects (Islam and Islam 2018). In November 2019, typical phytoplasma-suspected symptoms such as little leaf, yellowing and floral virescence were observed on *S. violaceum* plants in Kaliabor, Nagaon district of Assam, India (26.3220°N, 92.5540°E), with about 8% incidence based on visual observations. To investigate the possibility of a phytoplasma association with the symptoms, total DNA was isolated from collected leaf samples (six symptomatic and nine asymptomatic) by following the CTAB protocol (Kollar et al. 1990). The DNAs isolated were assayed for the presence of phytoplasma using polymerase chain reaction (PCR) assays performed with the P1/P6 primer pair for the 16S rRNA gene (Deng and Hiruki 1990) and SecAfor1/SecArev3 for the *secA* gene (Hodgetts et al. 2008). The direct PCR targeting the 16S rRNA gene and *secA* gene amplified a product of about 1.5 kb and 840 bp, respectively, from all the symptomatic plant samples but not from any of the asymptomatic plant samples. All amplicons were double strand sequenced and corresponding high-quality sequences were deposited in GenBank with accession

numbers MW261863 for the 16S rRNA gene and MW885174 for the *secA* gene with sequence lengths of 1,406 and 532 bp, respectively. Pairwise sequence comparison of the 16S rRNA gene sequence of the *S. violaceum* phytoplasma isolate revealed 100% sequence identity with the strain ‘*Candidatus Phytoplasma trifolii*’ (accession no. EF186820) and *secA* gene showed up to 95% sequence identity with the same organism (accession no. KX784498). Further analyses of the 16S rRNA and *secA* genes based on a phylogenetic tree and the iPhyClassifier-based virtual RFLP analysis of 16S rRNA gene revealed that the phytoplasma associated with little leaf and floral virescence of *S. violaceum* belongs to the 16SrIV-D subgroup with a similarity coefficient of 1.0. The 16S rRNA and *secA* gene sequence comparison confirmed the close association of the phytoplasma strain associated with *S. violaceum* with 16SrVI-D subgroup phytoplasma. The 16SrVI-D subgroup of phytoplasma has been reported to be associated with many horticultural and other agricultural crops in India (Rao 2021). Recently, the 16SrVI-D strain was reported in eggplant from Assam, India (accession no. MW261866), showing up to 100% sequence identity with *S. violaceum* strain (accession no. MW261863) (Dutta et al. 2020). To the best of our knowledge, this is the first report of the association of the 16SrVI-D subgroup of the phytoplasma strain with *S. violaceum*, which is an important medicinal plant widely used in folk and traditional Indian systems of medicine. Since this study has confirmed *S. violaceum* as a new host of the 16SrVI-D subgroup of phytoplasma, further studies on the disease epidemiology and insects vectoring the phytoplasma would help to formulate effective management strategies to prevent further spread to other hosts.

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