Diseases Caused by Bacteria and Phytoplasmas

First Report on Clover Proliferation Phytoplasma Related Strain Associated with *Matthiola incana* Floral Virescence in Uttar Pradesh, India

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Matthiola incana (L.) R. Br (family Brassicaceae) is an ornamental commonly known as hoary stock and has extremely fragrant flowers, which bloom in dense clusters in a large variety of colors. During a survey of flower nurseries in March 2019 at the Indian Institute of Sugarcane Research campus, Lucknow, flower virescence (MiV) symptoms were observed in *M. incana* pots with an incidence of over 40%. Leaf yellows symptoms were also observed on a weed, *Acalypha indica* (AiLY), in the *Matthiola* nursery. Nested PCR assays were carried out to detect and identify the possible association of phytoplasmas with MiV and AiLY symptoms. Symptomatic MiV and AiLY samples and nonsymptomatic samples were collected and processed for DNA extraction from the leaf midrib by the CTAB method. *Hishimonus phycitis* (HP) (Hemiptera: Cicadellidae) leafhoppers feeding on MiV symptomatic plants were also collected and DNA extracted. The DNA of eight symptomatic and four nonsymptomatic plants and from the 10 leafhoppers was used as a template for PCR assays. Phytoplasma specific primers for the 16S rRNA gene (P1/P7 and 3Fwd/3Rev; Schneider et al. 1995; Manimekhalai et al. 2010) and multilocus gene specific primer pairs for *secA* (SecAfor1/SecArev3; SecAfo5r/SecArev2; Bekele et al. 2011), *secY* (SecYF1(VI)/SecYR1(VI); SecYF2(VI)/SecYR1(VI); Lee et al. 2010), and *rp*

genes (rpFIC/rp(I)R1A; rp(VI)F2/rp(VI)R2; Martini et al. 2007) were employed as previously described. Amplified products of ~1.3 kb, ~600 bp, ~1.7 kb, and ~1.0 kb of 16S rRNA, *secA*, *secY*, and *rp* genes of phytoplasma were consistently amplified in all the MiV and AiLY samples and in the HP leafhopper. No amplifications were achieved in any of the asymptomatic plant samples. Amplified products of all the four genes of MiV, AiLY, and HP isolates were purified, sequenced, and submitted in GenBank. Sequence comparison and phylogeny analysis of the sequences of the four genes of MiV, AiLY, and HP isolates revealed 99 to 100% sequence identity and clustering with clover proliferation phytoplasma related strains (16SrVI group). The virtual restriction fragment length polymorphism analysis with 17 restriction endonucleases corresponding to the 16S rRNA gene sequences of MiV, AiLY, and HP phytoplasma strains by pDraw program assigned them into a novel phytoplasma subgroup strain under 16SrVI group. Because its *HpaII* restriction profile was different from earlier classified 16SrVI subgroups but was very close to 16SrVI-E (GenBank accession no. AY270156). Earlier, peanut witches' broom (16SrII-A) phytoplasma was identified associated with *M. incana* from Italy (Davino et al. 2007). However, the association of clover proliferation phytoplasma (16SrVI) related strain associated with virescence symptom of *M. incana* is the first report in world. The weed (*A. indica*) and HP leafhopper were also reported as additional host and putative vector of the 16SrVI subgroup related new strain in India, which needs further investigation. The report of a new host and new subgroup of clover proliferation phytoplasma related strain in India has an epidemiological significance and warrants attention.

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