



NEW DISEASE REPORT

First report of '*Candidatus* Phytoplasma australasia' related strain associated with little leaf and witches' broom disease of *Hibiscus sabdariffa* in India

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Mesta (roselle; *Hibiscus sabdariffa*) is an important annual vegetable crop cultivated in the Indian subcontinent and the young shoots and leaves of mesta are edible. In July 2021, little leaf and witches'-broom symptoms were observed in a mesta crop in Tirupati, Andhra Pradesh, India (Figure 1).

Leaf samples of two diseased and two asymptomatic plants were collected, and DNA was extracted using a modified CTAB DNA extraction method (Murray & Thomson, 1980). The DNA was used in nested or single PCR assays to detect the 16S rRNA and SAP genes using the phytoplasma 16S rRNA universal primer pairs P1/P7 and R16F2n/R16R2 (Deng & Hiruki, 1991; Gundersen & Lee 1996; Smart et al., 1996) and the SAP11-II-D F1/R1 primer pair (Subhi et al., 2018). Amplicons of the expected size for the 16S rRNA (1.8 and 1.26 kb) and SAP genes (c. 800bp) were produced from DNA of the diseased plants but not from asymptomatic plants. After direct Sanger sequencing (Eurofin Genomics India, Bangalore), a 1260 nt 16S rRNA gene (GenBank Accession No. OP019262) sequence and a 339 nt SAP gene (OP009357) sequence were submitted to GenBank. BLAST analysis revealed that the 16S rRNA nucleotide sequence was identical

to 16SrII group isolates from India that were associated with *Croton bonplandianus* (MT555412), *Solanum lycopersicum* (MN462969) and *Parthenium hysterophorus* (MG748744). The SAP gene sequence was identical to that of two '*Parthenium hysterophorus*' witches'-broom phytoplasma (16SrII) isolates (MT225448, MT225449) from India and to the sequence of *Cicer arietinum* phyllody phytoplasma (KX358621) from Oman. The phytoplasma subgroup was identified as 16SrII-D using the iPhyClassifier online tool (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>) which showed a similarity coefficient of 1.00 with '*Candidatus* Phytoplasma australasia' (Y10097).

To date, the only phytoplasmas associated with diseases in mesta are related to the 16SrI-B group (KF923397) which was found in Taiwan (Tseng et al. 2014) and 16SrV group (JX975061) which was reported in India (Biswas et al., 2013). The 16SrII-D subgroup has been reported previously as widespread and infecting many crops and weeds in India, including Andhra Pradesh (Vijay Kumar Naik et al., 2018), so the detection is not surprising. However, this is the first report of 16SrII-D subgroup phytoplasma in mesta globally and in India.

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FIGURE 1 Mesta infected with a 'Candidatus Phytoplasma australasia'-like phytoplasma showing little leaf (left), and witches' broom (right) symptoms

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