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NEW DISEASE REPORT

First report of a '*Candidatus* Phytoplasma australasia' (16SrII-D) strain associated with witches' broom and little leaf symptoms of *Martynia annua* in India

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16SrII-D group

Martynia annua (Martyniaceae) is a small, herbaceous annual plant distributed across India. It is used for the treatment of epilepsy, tuberculosis, sore throats and wounds (Kaushik et al., 2021). In August 2021, symptoms of witches' broom and little leaf were observed in *M. annua* in the Anantapur forest area, Andhra Pradesh. India.

Leaves were collected from two diseased and two symptomless plants (Figures 1 and 2). Total DNA was extracted from 100 mg of leaf midribs using a modified CTAB DNA extraction method (Murray & Thompson, 1980). DNA extracts were analysed by direct and nested PCR with universal phytoplasma 16S rRNA primers, P1/P7 (Deng & Hiruki, 1991) and R16F2n/R16R2 (Gundersen & Lee, 1996), respectively. Amplicons of the expected size (c. 1.8 kb after direct PCR and c. 1.3 kb after nested PCR) were obtained from diseased samples but not symptomless plants. Nested PCR R16F2n/R16R2 products



FIGURE 1 *Martynia annua* plant showing witches' broom and little leaf symptoms

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FIGURE 2 Symptomless *Martynia annua* plant

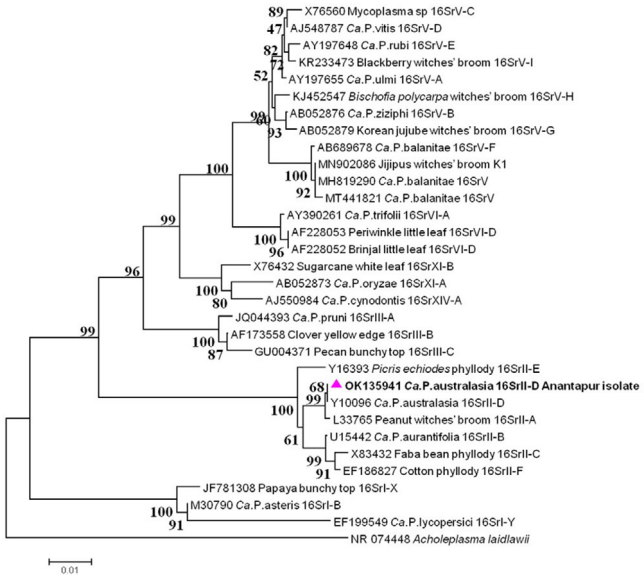


FIGURE 3 Phylogenetic analysis based on the partial 16S rRNA gene sequence OK135941 with the '*Candidatus* Phytoplasma australasia' and other phytoplasma strains. Phylogenetic tree was generated using 1000 bootstrap replicates

were directly sequenced (Eurofins Genomics India Pvt., Ltd., India) and the consensus sequence was deposited in GenBank (Accession No. OK135941).

A BLASTn analysis revealed that the *M. annua* phytoplasma 16Sr DNA sequence shared 100% sequence identity with those of 16SrII group phytoplasmas, including '*Candidatus* Phytoplasma australasia' (MN565885, MN565886) and '*Ca.* Phytoplasma aurantifolia' (MK217101, MK421430). Phylogenetic analysis (Tamura et al., 2007) based on the R16F2n/R2 sequence and performed with the neighbour-joining method (MEGA software version 7.1) revealed that the Anantapur 16SrII phytoplasma had the closest relationship with 16SrII, subgroup D isolates, particularly with the strain '*Ca.* P. australasia' (Y10096) (Figure 3). Virtual RFLP profiles using restriction enzyme *AluI* and the R16F2nR2 sequence were generated with *i*PhyClassifier (https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi). The RFLP patterns of the Anantapur phytoplasma 16S rDNA F2nR2 sequence with *AluI* enzyme agreed with the phylogenetic grouping. The Anantapur phytoplasma was confirmed as a member of the 16Sr group II, subgroup D with a similarity coefficient of 1.00, compared with virtual RFLP profiles of the '*Ca.* P. australasia' strain (Y10097).

This is the first report of a '*Ca.* Phytoplasma australasia' strain (16SrII-D) associated with witches' broom and little leaf symptoms of *M. annua* in India. Since the 16SrII group has a wide host range and occurrence in India, and particularly the subgroup 16SrII-D in the south region, the present finding has a significant impact for future epidemiological studies.

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