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NEW DISEASE REPORT

First report of a '*Candidatus* Phytoplasma aurantifolia' (16SrII-D) isolate associated with little leaf and stunting of *Dracaena reflexa* in India

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Dracaena reflexa commonly known as "song of India" is an important ornamental plant grown both outdoors and as a house plant. During October 2021, typical phytoplasma symptoms including little leaf and stunting were observed in *D. reflexa* plants growing in the landscape around the Dr.Y.S.R. Horticultural University, Anantharajupeta, Andhra Pradesh. Eight diseased plants were observed amongst the 100 surveyed (Figure 1).

Total DNA was extracted from leaf samples collected from two diseased and one symptomless plant using the CTAB method (Murray & Thompson, 1980). DNA extracts were subjected to nested PCR using universal phytoplasma-specific primer pairs P1/P7 (Deng & Hiruki, 1991) for the direct round and R16F2n/R16R2 for the nested amplification of the 16SrRNA gene (Gundersen & Lee, 1996). Primers rp(II)F/rp(I)R1A followed by semi-rp(II)F2/ rp(I)R1A) were used for the amplification of the ribosomal proteins (*rp* gene) (Martini *et al*., 2004). Amplicons of the expected size (c. 1.25 Kb from the 16S rRNA gene and c. 1.29 Kb from the *rp* gene) were obtained from diseased plants but not from the symptomless samples. PCR products were cloned (pGEM-T Easy Vector; Promega, USA) and directly sequenced (ABA Biotech, India). Sequences were deposited in GenBank with the Accession Nos. ON787808 and ON787809 (16SrRNA gene) and ON791584 and ON791585 (*rp* gene). BLASTn analysis showed that the partial 16Sr RNA gene sequences of the *D. reflexa* phytoplasma isolates shared 100% identity with that of '*Candidatus* Phytoplasma aurantifolia' (MT420677, MT420671). The *rp* gene sequence identities



FIGURE 1 Symptoms on *Dracaena reflexa*: (a) symptomless plant, and (b, c) little leaf and stunting symptoms, both observed in Anantharajupeta, Andhra Pradesh, India.

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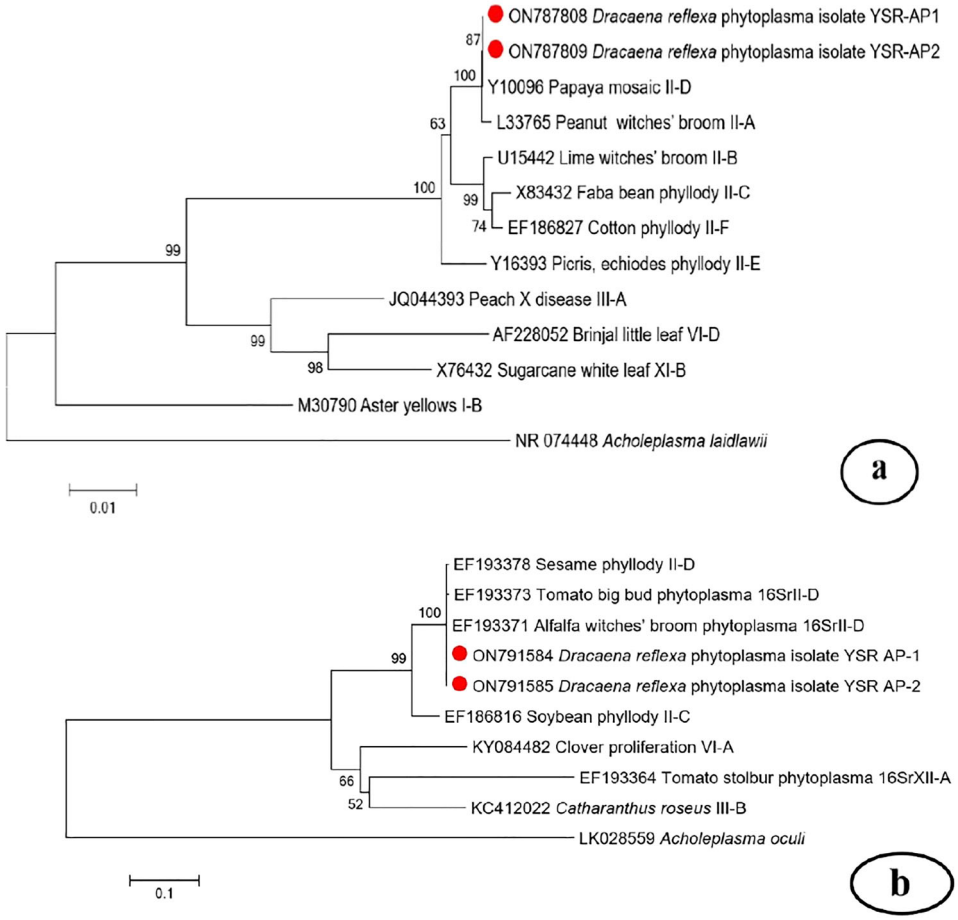


FIGURE 2 Phylogenetic trees constructed by the neighbour-joining method and the bootstrap model (1000 replicates) using the MEGA 7 software showing the phylogenetic relationships among the identified *Dracaena reflexa* phytoplasma isolates (red) and reported phytoplasma strains of different groups, based on (a) 16S rRNA gene sequences rooted with *Acholeplasma laidlawii* and (b) *rp* gene rooted with *A. oculi*.

ranged from 99.92-100% compared with that of the '*Ca.* P. aurantifolia', 16SrII group (EF193377, EF193371). A 16S rRNA and *rp* gene sequence-based phylogenetic analyses (Figure 2) showed that the *D. reflexa* phytoplasma associated with little leaf and stunting clustered with the 16SrII group '*Ca.* P. aurantifolia' strains. A virtual RFLP analysis based on the 16S rRNA gene sequences was performed using *i*Phyclassifier (https://plantpathology.ba.ars.usda.gov) which revealed that the RFLP pattern of the *D. reflexa* phytoplasma was identical to that of phytoplasmas of the 16Sr group II, subgroup D (Y10097) with a similarity coefficient of 1.00.

To the best of our knowledge, this is the first report of a 16SrII-D phytoplasma infection in *D. reflexa*. '*Ca.* Phytoplasma aurantifolia' (16SrII-D subgroup) strains have been reported from commercial crops, weed hosts and several leafhopper vectors in India (Reddy *et al.*, 2021). This report indicates that the '*Ca.* P. aurantifolia' related strains (16SrII-D) are widespread and have a wide host range in India. Further research into the epidemiology of the phytoplasma in *D. reflexa* may provide useful information to implement effective management recommendations.

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