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NEW DISEASE REPORT

First report of a '*Candidatus* Phytoplasma aurantifolia' related strain (16SrII-C) associated with *Convolvulus virgatus* witches'-broom disease in Iran

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Convolvulus virgatus (Convolvulaceae) is a perennial shrub which grows mainly in desert and shrubland biomes in Iran, the Arabian Peninsula and Pakistan (Wood et al., 2015). In a 2020 survey for phytoplasma diseases, witches'-broom disease of *C. virgatus* (WBDCV) was observed in the Roodan area (Hormozgan province, Iran). The main symptoms of WBDCV were flower and stem proliferation, shortened internodes, little leaf, severe witches'-broom, yellowing, reddening, plant sterility and decline (Figs. 1, 2).

Total DNA was extracted from leaves and leaf petioles of five symptom-bearing and five symptomless *C. virgatus* plants using a CTAB-based method. DNA samples were tested for the presence of phytoplasmas by direct-PCR using primer pair P1/P7 (Lee et al., 1998) and nested PCR using primer pairs P1/P7 and R16F2n/R16R2 (Gundersen & Lee, 1996). The expected amplicons of c. 1,800 bp and 1,250 bp were obtained in direct and nested-PCR respectively, from all diseased plants but not symptomless ones. Two R16F2n/R16R2 amplicons were randomly selected and cloned into *Escherichia coli* DH5α cells using the PCR TA Cloning Kit (SinaClon, Cat. No.:CL5841, Iran) and three plasmid DNAs from recombinant colonies were sequenced. The obtained sequences were identical, and a consensus sequence was deposited in GenBank (Accession No. ON361565). BLASTn analysis of the obtained sequence showed the highest sequence identity (98.7-99.9 %) with phytoplasmas related to '*Ca.* P. aurantifolia' (16SrII group). Phylogenetic analysis with MEGA11 software using the neighbour-joining method Fig. 3 revealed that the WBDCV phytoplasma clustered within the '*Ca.* P. aurantifolia' (16SrII) group clade, closest to the strains of subgroup 16SrII-C.

In Iran, phytoplasma strains belonging to 16SrII-C subgroup have been reported previously in Bushehr, Fars (adjacent to Hormozgan province) and Yazd provinces in several plant species including *Daucus carota*, *Medicago sativa*, *Prunus dulcis*, *Vicia faba* (Siampour et al., 2019), *Alhagi maurorum* and *Artemisia sieberi* (Esmaeilzadeh-Hosseini & Salehi, 2021); however, this is the first report of a '*Ca.* P. aurantifolia'-related strain in *C. virgatus* associated with WBDCV in Iran and most probably, in the world.

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FIGURE 1 Symptoms of witches'-broom disease in *Convolvulus virgatus* including flower and stem proliferation, shortened internodes, little leaf, witches'-broom, yellowing, reddening, plant sterility and decline (a), compared with a healthy plant (b)



FIGURE 2 Severe witches'-broom, stem proliferation, little leaf and reddening of the leaves on the branches of an infected plant of *Convolvulus virgatus*

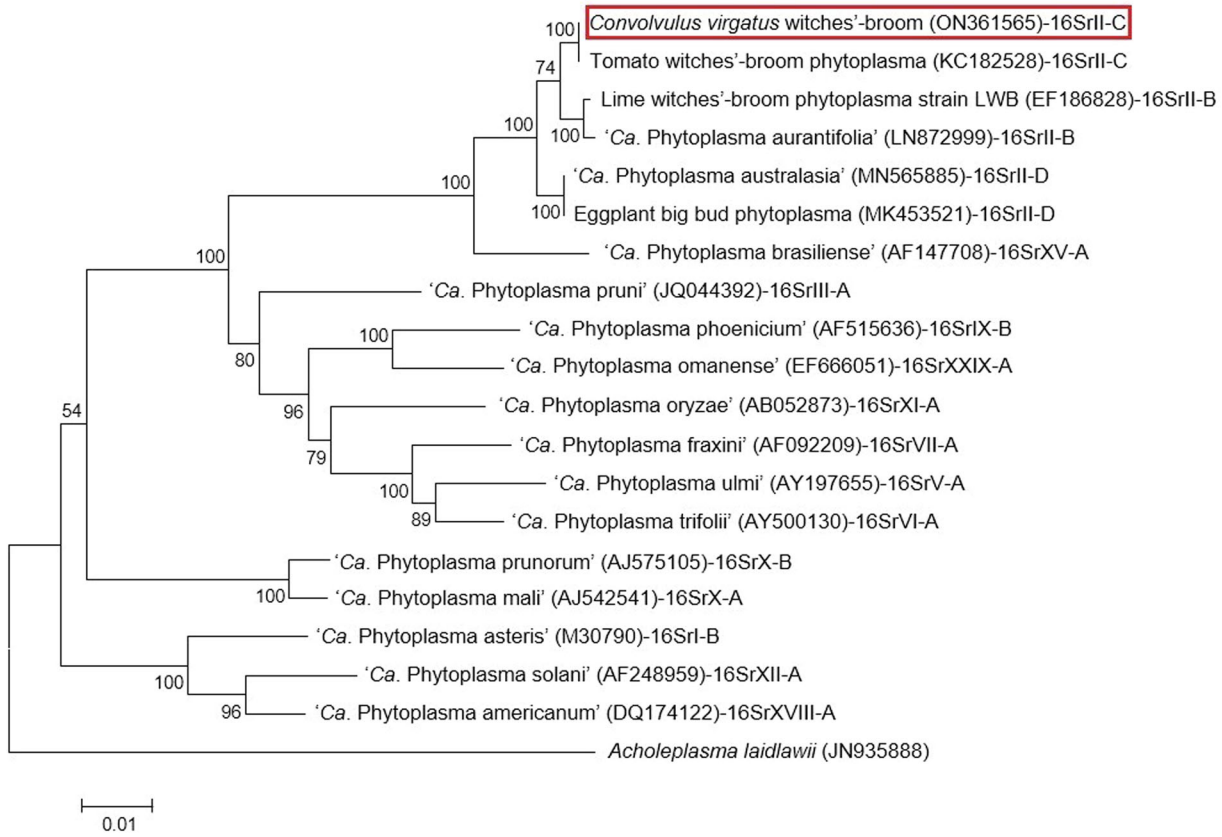


FIGURE 3 Phylogenetic tree based on partial 16S rDNA sequences (1,247 bp) from the *Convolvulus virgatus* witches'-broom phytoplasma identified in this study (red rectangle) and selected phytoplasma sequences from GenBank (accession numbers in parentheses). Tree constructed by the neighbour-joining method using MEGA 11 software. Numbers on branches are bootstrap values of 1,000 replicates. *Acholeplasma laidlawii* was used as an outgroup. Scale bar indicates nucleotide substitutions per site

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