

NEW DISEASE REPORT

First report of a '*Candidatus Phytoplasma asteris*'-related strain (16SrI-B) associated with *Zinnia elegans* witches'-broom disease in Iran

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KEYWORDS

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Zinnia elegans (Asteraceae) is a widely used ornamental plant in Iran. Surveys of parks in Shiraz city (Fars province, Iran) for phytoplasma diseases were done in 2019 and a witches'-broom disease of *Z. elegans* was observed. The symptoms of the disease were witches'-broom, stem proliferation, floral virescence, phyllody, little leaf, yellowing and stunting (Fig. 1). Disease incidence reached 8% in one park, Azadi Park, in Shiraz.

Total DNA was extracted from 0.2 g of leaf midribs of six symptom-bearing and four symptomless *Z. elegans* plants (grown from seed in a greenhouse) using the CTAB method described by Zhang *et al.* (1998). DNA samples were tested for the presence of phytoplasmas by a nested-PCR assay primed by primer pairs P1/P7 in the first round followed by R16F2n/R16R2 (Lee *et al.*, 1998). Amplicons of c. 1.25 Kb were obtained in the nested round from all the diseased plants but not the control plants. Six R16F2n/R16R2 amplicons were cloned into *Escherichia coli* DH5 α cells using an Inst/A Clone PCR product Cloning Kit (Metabion, Germany) and plasmids from three of the six cloned samples were sequenced. The sequences had 100% identity with each other and a consensus sequence was deposited in GenBank (Accession No. MZ616665). A BLASTn analysis using the obtained sequence showed the highest sequence identity with phytoplasmas related to 16SrI group. Phylogenetic analysis with MEGA7 software using the neighbour-joining method (Fig. 2) showed that the *Z. elegans* witches'-broom phytoplasma clustered within the 16SrI group close to the *Oenothera* phytoplasma (M30790), representative of subgroup 16SrI-B ('*Candidatus Phytoplasma asteris*'-related). A virtual

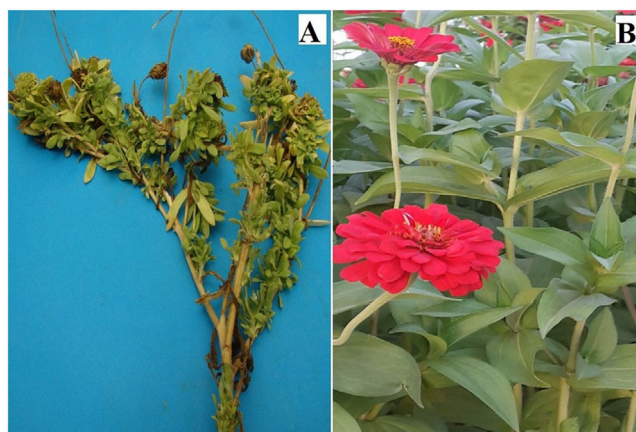


FIGURE 1 Symptoms of witches'-broom disease in *Zinnia elegans* including witches'-broom, stem proliferation, little leaf, shortened internodes, floral virescence and phyllody (A), compared with healthy plants (B).

RFLP analysis with 17 restriction endonucleases using *iPhyClassifier* (<https://plantpathology.ba.ars.usda.gov>) indicated that the RFLP pattern derived from the 16S rDNA F2nR2 fragment of the *Z. elegans* witches'-broom phytoplasma (MZ616665) was identical to the reference pattern of 16Sr group I, subgroup B (AP006628).

A phytoplasma strain related to 16SrII group has been reported previously in *Z. elegans* in Iran (Hemmati *et al.*, 2017); however, to our

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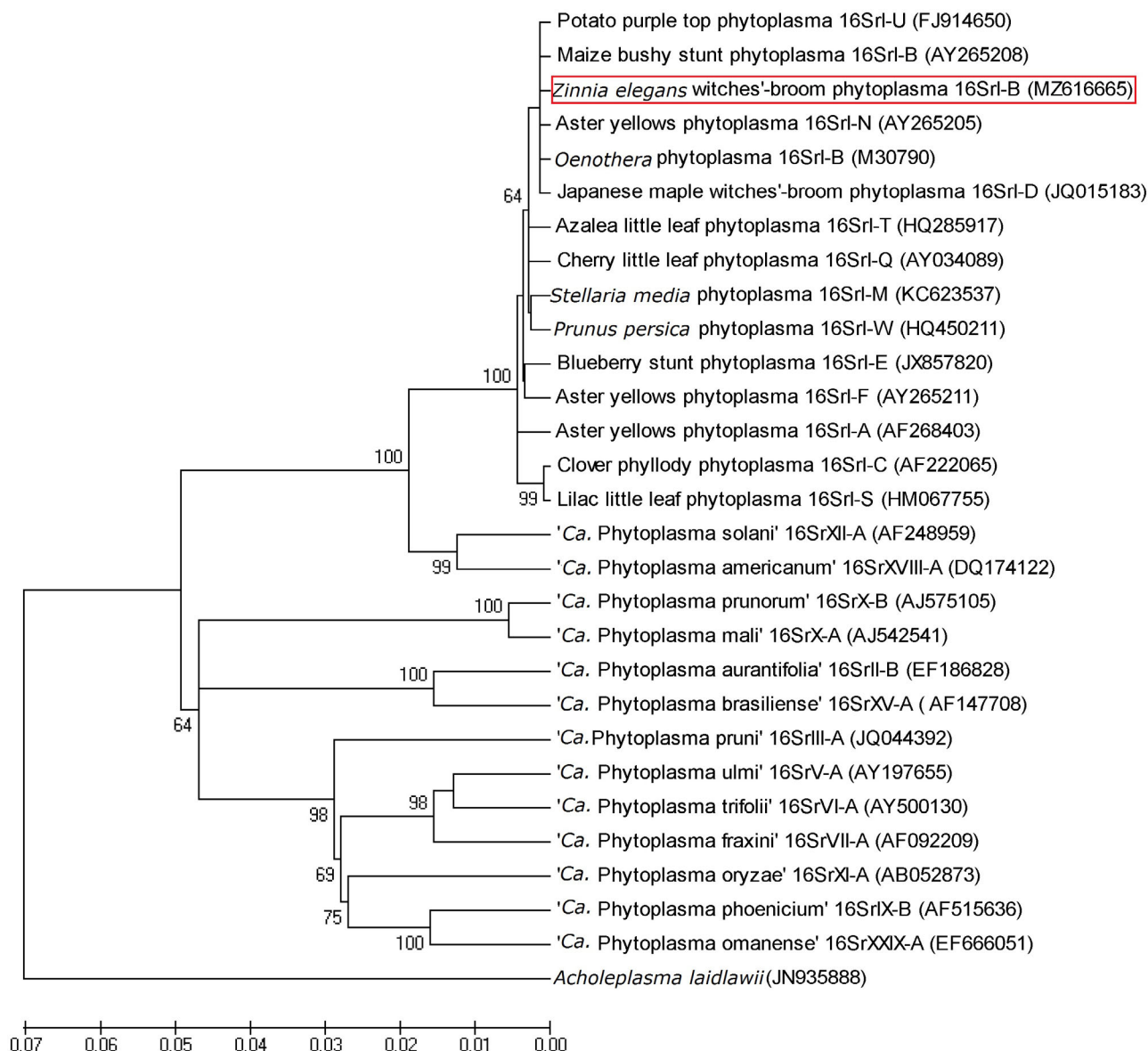


FIGURE 2 Phylogenetic tree based on partial 16S rDNA sequences (1239 bp) from the *Zinnia elegans* witches'-broom phytoplasma in this study (red rectangle) and selected phytoplasma sequences from GenBank (accession numbers in parentheses). The tree was constructed by the neighbour-joining method using MEGA 7 software. Numbers on branches are bootstrap values of 1,000 replicates. *Achleplasma laidlawii* was used as an outgroup. Bar indicates the number of substitutions per nucleotide position.

knowledge this is the first report of a 16SrI-B phytoplasma associated with *Z. elegans* witches'-broom disease in Iran. The occurrence of 16SrI-B phytoplasma strains was reported previously in Iran on *Brassica napus* (Salehi et al., 2011), *Eruca sativa* (Esmailzadeh Hosseini et al., 2017) and *Eucalyptus camaldulensis* (Salehi et al., 2016); *Zinnia elegans* may act as a reservoir host.

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