



## First report of a '*Candidatus* Phytoplasma phoenicium'-related strain associated with *Bidens alba* phyllody in Iran

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*Bidens alba* (family Asteraceae), commonly known as shepherd's needle, is grown in tropical and subtropical regions of North and South America, Asia and Africa (Hall *et al.*, 2009). Some studies indicate that it can be used as a remedy for cuts, swelling, hypertension, jaundice and diabetes (Ong *et al.*, 2008).

In July 2016, typical symptoms of phytoplasma diseases including phyllody, virescence, witches' broom and little leaf (Figs. 1-2) were observed in *Bidens alba* growing as weeds in citrus orchards of Seyahoo district in Hormozgan province, Iran. Total DNA was extracted separately from five symptom-bearing and two symptomless plants (Fig. 3) using the CTAB method (Doyle & Doyle, 1990). Samples were tested for the presence of phytoplasma DNA by direct PCR using the universal primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995), followed by nested PCR using primer pair R16F2n/R16R2 (Gundersen & Lee, 1996). Amplicons of c. 1.8 (direct PCR) and 1.25 kb (nested PCR) were obtained from all symptom-bearing but not from symptomless plants. PCR products from both PCR rounds obtained from one infected plant were directly sequenced on both strands. The sequences were edited, assembled and deposited in GenBank (Accession No. KY358007). BLAST analysis of this sequence revealed that the phytoplasma associated with *B. alba* phyllody (BaP) shared 99% identity with phytoplasmas related to the 16SrIX phylogenetic group, '*Candidatus* Phytoplasma phoenicium', such as Iranian *Lactuca serricola* phyllody phytoplasma (DQ889749; Iran), Eggplant big bud (JX483702; Iran) and Periwinkle virescence (HQ589191; Germany). Phylogenetic analysis using the neighbour-joining method (MEGA 6 software) based on the sequenced fragment of the BaP phytoplasma and other phytoplasmas confirmed that the BaP phytoplasma is a '*Ca. P. phoenicium*'-related strain, with 99.2% sequence similarity to the reference strain for the species (AF515636) (Fig. 4).

In Iran, 16SrIX group phytoplasmas have been reported on several hosts such as aubergine, chrysanthemum, grapevine and lucerne. Results obtained

from our findings suggest that *B. alba* may act as a natural intermediate host for the 16SrIX phytoplasma in the Hormozgan province of Iran. To our knowledge, this is the first report of a phytoplasma associated with *B. alba* phyllody in Iran and most probably in the world; the potential for *B. alba* to act as a host for '*Ca. P. phoenicium*' is a concern.

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Figure 1



Figure 2

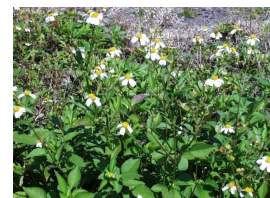


Figure 3

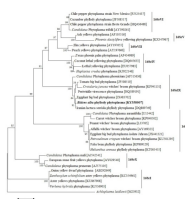


Figure 4

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