



First report of a '*Candidatus Phytoplasma phoenicium*'-related strain (16Sr IX) associated with *Salix witches'* broom in Iran

M. Ghayeb Zamhari

Plant Disease Department, Iranian Research Institute of Plant Protection, AREEO, Tehran, PO Box 19395-1454, Iran

*E-mail: zamharir2005@yahoo.com

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In Iran *Salix acmophylla*, *S. aegyptiaca*, *S. alba* and *S. babylonica* are trees traditionally grown in urban areas. Over the last few years witches' broom symptoms (Fig. 1) were observed in 12 out of 20 trees of *S. alba* growing along the Chalus Road in Alborz province, Iran. The symptomatology observed suggested the presence of a phytoplasma; there have been previous reports of an aster yellows group phytoplasma (16SrI-B) in *Salix tetradenia* (black mountain willow) in China (Muo *et al.*, 2014), a clover proliferation phytoplasma (16SrVI) in *Salix bebbiana*, *S. discolor*, *S. exigua* and *S. petiolaris* in Canada (Khadhair & Hiruki, 1995), and a stolbur group phytoplasma (16SrXII) in *Salix babylonica* in Spain (Alfaro-Fernandez *et al.*, 2011).

Leaf samples from the twelve trees (*S. alba*) showing witches' broom symptoms and five asymptomatic trees were collected in different areas in Alborz province. Leaf tissue was subjected to DNA extraction immediately after collection according to the procedure described by Doyle & Doyle (1987). The partial 16Sr DNA was amplified with phytoplasma universal primers using primers P1/tint in the first round. The resultant PCR products were diluted with sterile distilled water (1:29) prior to nested PCR using primers R16F2/R2 (Gundersen & Lee, 1996). Nested PCR gave positive results from all twelve *Salix* trees with witches' broom symptoms. No PCR products were obtained from the five asymptomatic *Salix* trees or negative controls.

The PCR product from one randomly selected positive *Salix* sample (reference BT18) was cloned, sequenced and submitted to GenBank (Accession No. KX500119). It showed a 99% sequence identity to the reference isolate of '*Candidatus P. phoenicium*' (AF515636), and is therefore a '*Ca. P. phoenicium*'-related strain. Phylogenetic analysis with selected reference strains indicated that the phytoplasma clustered together with member strains of '*Ca. P. phoenicium*' (16SrIX) (Fig. 2). Other hosts

of '*Ca. P. phoenicium*' include almond in Lebanon, and numerous hosts including almond, grapevine, peach, pistachio, *Bidens alba* and *Chrysanthemum morifolium* in Iran (Ghayeb Zamharir *et al.*, 2017). To our knowledge this is the first report of a '*Ca. P. phoenicium*'-related strain associated with *Salix* worldwide.

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Figure 1

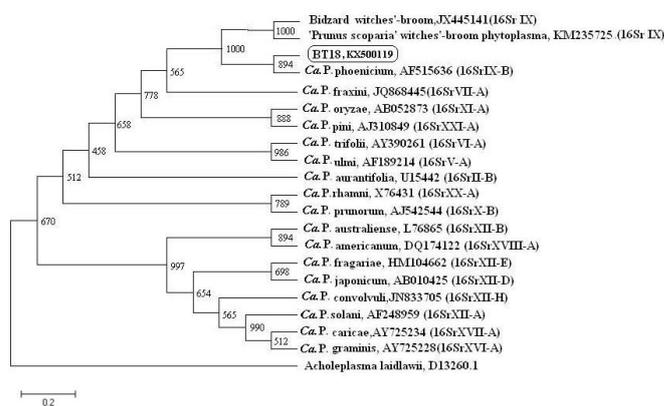


Figure 2

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