



Characterisation of a phytoplasma associated with *Petunia witches' broom* disease in Iran

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Petunia hybrida (Solanaceae) is an economically important ornamental plant species that is used in residential and commercial landscapes. Varieties are diverse and available in a range of colours (Abu-Qaoud *et al.*, 2010; Hiuts *et al.*, 1994). In July 2012, symptoms of witches' broom, yellowing, little leaf, phyllody and virescence were observed in *Petunia* in Sistan and Baluchestan province, Iran (Fig. 1a, b). Total DNA was extracted separately from 0.5 g of shoot material of two symptom-bearing and one symptomless plant (Fig. 1c) using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990). The samples were analysed for detection of phytoplasma DNA by both direct and nested PCR assays using phytoplasma universal 16S rDNA primer pairs P1/P7 and R16F2n/R16R2 that amplify fragments of 1800 bp and 1250 bp, respectively (Lee *et al.*, 1998). The 1800 and 1250 bp PCR products were obtained following direct and nested PCR from all symptom-bearing but not from symptomless plants. The 1250 bp PCR product was directly sequenced. BLAST analysis of the partial 16S rDNA sequence (GenBank Accession No. JX570935) revealed that the phytoplasma associated with *Petunia witches' broom* (PtWB) shared 100% identity with some phytoplasmas related to 16Sr-II phylogenetic group, '*Candidatus* Phytoplasma aurantifolia', such as chickpea phyllody phytoplasma (FJ870549.1), tomato big bud phytoplasma (JQ868448), '*Ca. P. aurantifolia*' (JX104335) and Echinacea witches' broom phytoplasma (JF340075.1). This result was further confirmed by the analysis using iPhyClassifier online tool (<http://www.plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>) where it was determined that PtWB phytoplasma is related to 16Sr group-II, subgroup D. The

phylogenetic neighbour-joining tree (MEGA 5 software) based on the partial 16S rDNA sequences (1250 bp) of the PtWB phytoplasma and several reference phytoplasmas corroborated these results and showed that PtWB phytoplasma is closely related to '*Candidatus* Phytoplasma australasiae' in the 16Sr-II phytoplasma clade (Fig. 2). Salehi *et al.* (unpublished) have recently detected a phytoplasma associated with phyllody symptoms in *Petunia* by PCR in Fars province, Iran. However, this is the first report of molecular characterisation of a phytoplasma associated with PtWB disease in Iran.

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Figure 1

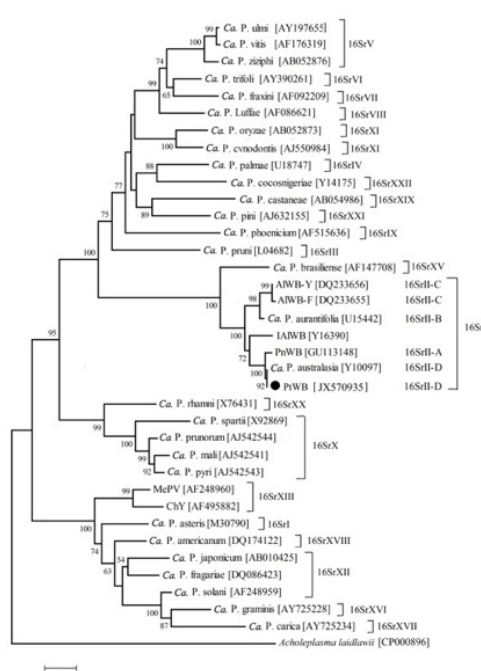


Figure 2

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