

# First report of *Tragopogon dubius* witches' broom disease associated with a subgroup 16SrI-B phytoplasma in Iran

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*Tragopogon dubius* (yellow salsify, Asteraceae) is a perennial herb in Iran. In a survey conducted for phytoplasma diseases in 2014, *T. dubius* witches' broom disease was observed in vineyards and green areas of Bajgah and Zarghan localities (Fars province, Iran). The characteristic symptoms of *T. dubius* witches' broom were flower virescence and phyllody, crown proliferation and witches' broom (Fig. 1). Since the symptoms were suggestive of phytoplasma infection, plants were assayed for presence of phytoplasma by PCR amplification. Total DNA was extracted from 0.3 g of midrib tissue of four witches' broom affected and two symptomless *T. dubius* plants (two plants per location), using the procedure of Zhang *et al.* (1998). Total DNA samples were tested by both direct and nested PCR assays using phytoplasma universal 16S rDNA primer pairs P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and R16F2n/R16R2 (Gundersen & Lee, 1996) that amplify fragments of 1800 bp and 1250 bp, respectively. Expected fragments were amplified following direct and nested PCR from all symptom-bearing but not from symptomless plants.

P1/P7-primed PCR products amplified from four witches' broom-affected *T. dubius* plants were separately cloned and sequenced. The full-length 16S rDNA sequences displayed 100% sequence identity with each other. A consensus sequence corresponding to the Bajgah *T. dubius* witches' broom was deposited in GenBank (Accession No. KR262955). A Blast search showed that this sequence had 100% identity with *Oenothera* phytoplasma ('*Candidatus* Phytoplasma asteris') (M30790), representative of subgroup 16SrI-B. Virtual RFLP analysis using iPhyClassifier online tool (<http://www.ba.ars.usda.gov/data/mppl/iphyclassifier>), carried out with 17 restriction enzymes, indicated the presence of a 16SrI-B phytoplasma in witches' broom-affected *T. dubius* plants (Fig. 2). Phylogenetic analysis of full length 16S rDNA sequences using MEGA version 5 (Tamura *et al.*, 2011) grouped *T. dubius* witches' broom phytoplasma with onion yellows phytoplasma (GenBank Accession No. D12569), a member of 16SrI-B subgroup (Fig. 3). To our knowledge this is the first report of natural occurrence and partial molecular characterisation of a phytoplasma associated with *T. dubius* witches' broom disease. A 16SrI-B subgroup

phytoplasma has previously been reported as causal agent of rapeseed phyllody disease in Zarghan (Salehi *et al.*, 2011). The significance of diseased *T. dubius* as natural reservoir of rapeseed phyllody phytoplasma remains to be determined. Aster yellows phytoplasmas were reported in grapevine in several countries. However, possible transmission of *T. dubius* witches' broom phytoplasma to grapevine is yet to be tested.

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Figure 1

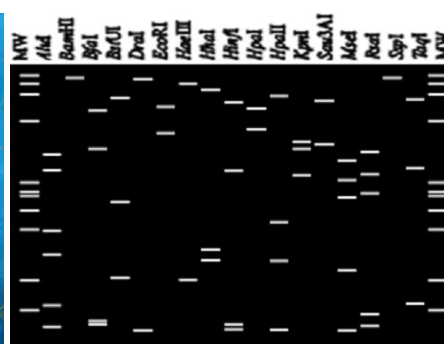


Figure 2

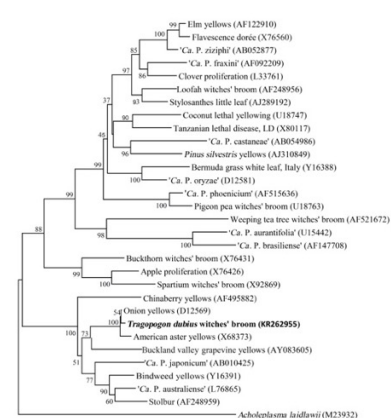


Figure 3

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