



First report of a 16SrI-B subgroup-related phytoplasma associated with *Eruca sativa* phyllody in Iran

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During a 2012-14 survey for phytoplasma diseases, *Eruca sativa* phyllody (ESP) disease was observed in Abarkooh (Yazd province, Iran). Infected plants showed crown proliferation, witches' broom, little leaf, flower virescence, phyllody, sterility and stunting (Fig. 1). Total DNA was extracted from 0.2 g of fresh leaves and flowers from both infected and symptomless plants (Zhang *et al.*, 1998). DNA samples were tested for phytoplasma by direct PCR using primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and nested PCR using primer pairs P1/P7 and R16F2n/R16R2 (Gundersen & Lee, 1996). Primer pair P1/P7 amplifies an 1.8 kb fragment of the phytoplasma ribosomal RNA operon which includes the 16S rRNA gene, the 16S-23S intergenic spacer region and a portion of the 5' region of the 23S rRNA gene. The R16F2n/R16R2 primer pair amplifies approximately 1.25 kb of the phytoplasma 16S rRNA gene. PCR amplicons of ~1.8 and ~1.25 kb respectively were obtained from all 10 of the phyllody-affected *E. sativa* (two per location) but not from symptomless plants. Five R16F2n/R16R2 amplicons from Esfandabad, Harooni, Bedaf, Taqhiabad and Mehrdasht locations were directly sequenced and showed 100% sequence identity to each other. A consensus sequence corresponding to the Aliabad-e Shams (Esfandabad, Abarkooh, Yazd province) *E. sativa* phyllody (AESP) phytoplasma was deposited in GenBank (Accession No. KT626568). A BLAST search showed that the AESP phytoplasma had 100% sequence identity with those of the 16SrI 'Candidatus Phytoplasma asteris' group (Aster Yellows group), including the onion yellows phytoplasma mild strain (OY-M) (NC_005303) representative of the 16SrI-B subgroup. Phylogenetic analysis (MEGA software version 6.0) confirmed that the AESP phytoplasma clustered within the 16SrI phytoplasma clade (Fig. 2) closer to the 16SrI-B strains, OY-M and 'Ca. P. asteris' (M30790). Computer-simulated restriction analysis (Fig. 3) with 17 restriction endonucleases using iPhyClassifier (Zhao *et al.*, 2009) revealed that virtual RFLP patterns of the AESP phytoplasma were identical (similarity coefficient 1.00) to those of OY-M. This is the first report of a 16SrI-B phytoplasma associated with ESP disease in Iran. A 16SrI-B related

phytoplasma has been reported associated with rapeseed (*Brassica rapa*) phyllody in Iran (Salehi *et al.*, 2011). *Eruca sativa* may be a secondary host for the 16SrI-B phytoplasma associated with rapeseed phyllody.

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Figure 1

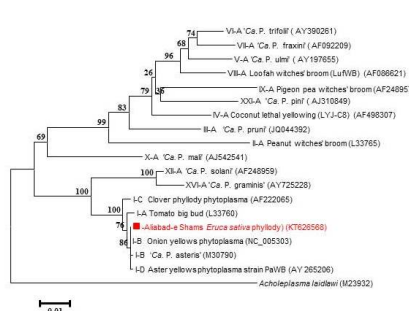


Figure 2

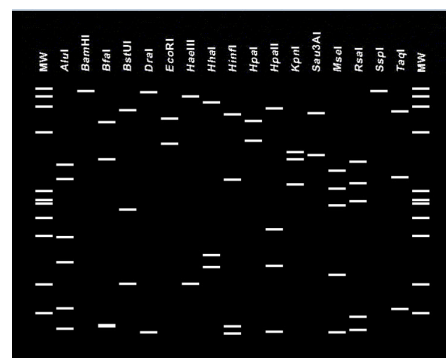


Figure 3

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