



First report of a 16SrII-D phytoplasma associated with *Calendula officinalis* phyllody in Iran

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Calendula officinalis, commonly known as marigold, belongs to the family Asteraceae. In Iran, this species is cultivated for ornamental purposes and used for many medicinal, culinary and cosmetic uses. From 2006, *Calendula officinalis* phyllody (CaoP) has been observed in different areas of Yazd province, Iran and association of a phytoplasma with the disease has been reported (Esmailzadeh Hosseini *et al.*, 2011). Disease incidence of up to 12% and symptoms including leaf size reduction, yellowing, phyllody, virescence, proliferation and sterility in the flower, proliferation of axillary buds along the stem, witches' broom and stunting were observed (Fig. 1).

Total DNA was extracted from 0.2 g of fresh leaves from both diseased and symptomless plants. DNA samples were tested for phytoplasma presence by direct PCR using P1/P7 primers (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and nested PCR using primers P1/P7 and R16F2n/R16R2 (Gundersen & Lee, 1996). PCR amplicons of ~1.8 and ~1.25 kb respectively were obtained from six diseased *C. officinalis* plants from Ashkezar and Yazd (two areas in Yazd province) but not from four symptomless marigold plants. The ~1.25 kb amplicons from the six diseased plants were directly sequenced and shown to have 100% identity. A consensus sequence of 1,248 bp (CaoP1) was deposited in GenBank (Accession No. KU297202). Sequence comparison by BLAST analysis showed the highest sequence identity with members of phytoplasma group 16SrII (peanut witches' broom). Phylogenetic analysis using the neighbour-joining method (MEGA software version 6.0) confirmed that the CaoP1 phytoplasma clustered within the group 16SrII phytoplasma clade, and closer to strains affiliated to the 16SrII-D subgroup than to other strains in the same group (Fig. 2). Computer-simulated analysis with 17 restriction endonucleases using iPhyClassifier (Zhao *et al.*, 2009) showed that the RFLP pattern derived from the CaoP1 16S rRNA gene was identical to the papaya yellow crinkle strain classified into subgroup 16SrII-D (Y10097).

This is the first report of a 16SrII-D phytoplasma associated with *Calendula officinalis* phyllody disease in Iran. Due to the occurrence and spread of alfalfa witches' broom phytoplasma associated with 16SrII-D in Iran since

1990 (Esmailzadeh Hosseini *et al.*, 2015) adjacent to plantings of *C. officinalis*, it is possible that alfalfa play a role in the epidemiology of *Calendula officinalis* phyllody disease or *vice versa*. Due to natural transmission of phytoplasma diseases by leafhoppers and psyllids, studies to identify insect vectors are in progress.

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Figure 1

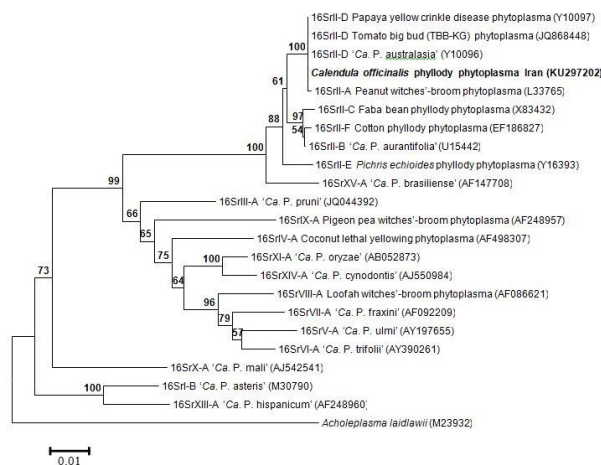


Figure 2

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