



First report of a '*Candidatus* Phytoplasma australasia'-related strain (16SrII-D subgroup) associated with alfalfa leaf chlorosis disease in Turkey

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Alfalfa (*Medicago sativa*) is an important forage crop in Turkey. Its high nutritional value and adaptation to harsh conditions make it an important crop for animal fodder. Şanlıurfa province is the second most important area for alfalfa seed production in Turkey. However, in recent years, alfalfa plants have exhibited disease symptoms including the production of small leaves, leaf chlorosis and witches' broom (Figs. 1-3).

Samples were collected from diseased plants to determine if a phytoplasma was associated with these symptoms. Total nucleic acid was isolated using the CTAB buffer method. PCR was done on the extracted nucleic acid using the R16F0-R16R1 primer pair followed by R16F2n-R16R2 primers in nested PCR to amplify the 16S rRNA gene (Davis & Lee, 1993; Duduk *et al.*, 2013). Amplicons of c. 1250 bp were obtained from diseased samples, but not from symptomless plants. The resulting amplicons were purified, cloned and sequenced in both directions. The sequences were assembled and compared to those of reference strains using MEGA 7 software (www.megasoftware.net). The three isolates sequenced in this study (HM1YON, HM2YON and HM3YON) had 99.9% identity with phytoplasmas of the 16SrII-D subgroup according to a BLAST analysis. The 16S rDNA sequences were deposited in GenBank under Accession Nos. MW048988, MW048989 and MW048990. A simulated RLFP analysis using 17 restriction enzymes was made with iPhyClassifier software (<https://plantpathology.ba.ars.usda.gov>) and the results confirmed that the three isolates are related to 16SrII-D subgroup phytoplasmas. A phylogenetic tree was constructed using the MEGA7 programme (neighbour-joining method) and the analysis confirmed that the phytoplasma isolates grouped with phytoplasma strains in group 16SrII and are closely related to '*Candidatus* Phytoplasma australasia'.

Damaging witches' broom diseases were reported several times in alfalfa in Iran (Hosseini *et al.*, 2016) and in Wisconsin (USA), associated with '*Candidatus* Phytoplasma asteris' (Peters *et al.*, 1999). Symptoms such as leaf stunting, yellowing and witches' broom were observed in alfalfa in Iran associated with phytoplasmas in the 16SrII-D group (Hosseini *et al.*, 2013). Leaf yellowing and plant stunting were also associated with 16SrII-D group phytoplasmas infecting alfalfa in Sudan (Tahir *et al.*, 2017).

To the best of our knowledge this is the first report of a phytoplasma

disease in alfalfa-growing areas in Turkey. In our study, symptoms in diseased plants were associated with a '*Candidatus* Phytoplasma australasia'-related strain. We predict that yield losses in alfalfa could reach significant levels. Disease epidemiology and the economic impact on crop yield, as well as the dissemination of the pathogen are the subject of ongoing research.

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Figure 1



Figure 2



Figure 3

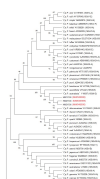


Figure 4

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