



First report of a 16SrII-D phytoplasma associated with *Albizia lebbek* witches' broom disease in Iran

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The lebbek tree, *Albizia lebbek*, family Fabaceae is a deciduous, perennial tree mainly distributed in tropical and subtropical areas of the world. Lebbek is widely used as a shade tree on the margin of agricultural fields and urban parks in the south of Iran.

In the spring of 2018, two *A. lebbek* trees showing shoot proliferation, short internodes, witches' broom and twig dieback symptoms (Figs. 1-2) were observed in Jiroft, Kerman province, Iran. Total genomic DNA was extracted from leaf midribs of the symptomatic and two symptomless lebbek trees according to a CTAB-based method (Zhang *et al.*, 1998). The DNA was used for nested PCR testing with P1/P7 (Schneider *et al.*, 1995) followed by R16F2n/R16R2 universal primer pairs (Gundersen & Lee, 1996) for phytoplasma detection as previously described (Azadvar *et al.*, 2011). The amplified PCR products were directly sequenced and the nucleotide sequences obtained were analysed by BLAST and *iPhyClassifier* (Zhao *et al.*, 2008) for phytoplasma identification and group/subgroup classification, and using the MEGA 6.0 programme (Tamura *et al.*, 2013) for phylogenetic analysis.

Results indicated that 1.25 kb nested PCR amplicons corresponding to the phytoplasma 16S rRNA gene were amplified from each symptomatic but not from the symptomless lebbek trees. BLAST analyses of the consensus sequences showed that the phytoplasma associated with lebbek witches' broom disease in Iran (GenBank Accession No. MN121115) was identical to that of the 16SrII phytoplasma '*Candidatus* Phytoplasma aurantifolia' (MH547068).

Virtual restriction fragment length polymorphism (RFLP) analyses of the 16S rDNA F2nR2 sequences by *AluI*, *BamHI*, *BfaI*, *BstUI* (*ThaI*), *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI* (*MboI*), *MseI*, *RsaI*, *I* and *TaqI* restriction enzymes using *iPhyClassifier* (Zhao *et al.*, 2009) confirmed that the RFLP profile of the phytoplasma associated with lebbek witches' broom disease in Iran was identical (similarity coefficient 1.00) to that of the 16Sr group II, subgroup D (Y10097). Moreover, the phylogenetic tree (Fig 3) constructed based on the partial 16S rDNA sequences confirmed that the phytoplasma associated with *A. lebbek* witches' broom disease is clustered with phytoplasmas belonging to the

16SrII group, including the reference strain of subgroup D.

To our knowledge, this is the first report of *A. lebbek* witches' broom disease and its association with a 16SrII-D phytoplasma in Iran. Phytoplasma-infected *A. lebbek* trees may pose an epidemiological threat to other crops or nearby plant species. It will be important to identify the natural hosts of 16SrII-D phytoplasma and to determine the insect vectors of the phytoplasma.

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Figure 1



Figure 2

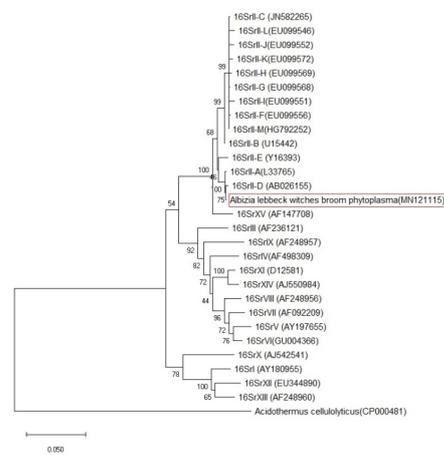


Figure 3

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