



First report of a 16SrII phytoplasma associated with a witches' broom disease of *Tamarix aphylla* in Iran

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Tamarisk (*Tamarix aphylla*) has been used for windbreaks near agricultural crops and as shade trees in rural desert margins in Iran. Symptoms associated with tamarisk witches' broom (TWB) disease, including proliferation of shoots with short internodes and little leaf symptoms (Figs. 1-2) were observed in 34% of trees surveyed in an area of 3500 ha in Chah Afzal (Yazd province, Iran) during 2014-15.

Nested polymerase chain reaction using the P1/P7 primers (Deng & Hiruki, 1991; Schneider *et al.*, 1995) followed by the R16mF2/R16mR2 and R16F2n/R16R2 primer pairs (Gundersen & Lee, 1996) was carried out on DNA extracted from six diseased and three symptomless tamarisk plants. After 35 cycles, DNA fragments of c. 1.8, 1.4 and 1.25 kb respectively were obtained from each of the diseased tamarisk samples but not from symptomless samples. Restriction fragment length polymorphism (RFLP) analysis of R16F2n/R16R2 amplicons using *AluI*, *HaeIII*, *HhaI*, *RsaI*, *MseI* and *TaqI* restriction enzymes showed profiles identical to those of phytoplasmas belonging to the 16SrII group. R16F2n/R16R2 sequences from six samples were identical to each other and a representative sequence was deposited in GenBank (Accession No. MF427724). BLAST analysis showed 99% nucleotide identity with other 16SrII-D phytoplasmas including parsley witches' broom phytoplasma (KU501295), which is also found in Iran. Phylogenetic analysis using the neighbour-joining method (MEGA7) showed that the TWB phytoplasma clustered within the 16SrII group closest to papaya yellow crinkle (PYC) phytoplasma (Y10097) which is the type strain of the 16SrII-D group. Computer-simulated analysis with seventeen restriction endonucleases using *iPhyClassifier* (Zhao *et al.*, 2009) showed that the RFLP pattern derived from the TWB phytoplasma 16S rRNA gene was different from the reference patterns of all previously established 16SrII subgroups. The most similar was the RFLP pattern of PYC phytoplasma (Y10097) with a similarity coefficient of 0.97.

This is the first report of a 16SrII phytoplasma associated with TWB disease in Iran and worldwide. TWB disease occurs near field crops heavily

infected with 16SrII phytoplasmas such as alfalfa witches' broom (Esmailzadeh Hosseini *et al.*, 2015) and sesame phyllody (Salehi *et al.*, 2017) in Yazd and therefore tamarisk may have an important role in the epidemiology of the disease.

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Figure 1



Figure 2

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