



First report of a '*Candidatus Phytoplasma phoenicium*'-related strain (16SrIX-I) associated with yellowing of *Onobrychis viciifolia* in Iran

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Onobrychis viciifolia (common sainfoin) is a forage legume which enhances biodiversity within agro-ecosystems and is an important forage crop in Iran. During 2014-16, *O. viciifolia* plants were observed with symptoms of yellowing (Fig. 1), little leaf and reddening of leaflet margins (Fig. 2), witches' broom and dwarfing compared with healthy plants (Fig. 3). The disease, known locally as sainfoin yellowing (SY), was observed in Shalamzar and Shahre Kian locations in Chaharmahal and Bakhtiari province, Iran. In each area, three 1,000 m² fields were selected randomly and sampled at five points within one square metre on a diagonal transect across each of the fields. The number of diseased plants and the total number of plants in each sampled square metre was determined to calculate the extent of the disease; this being 32% in Shalamzar and 22% in Shahre Kian.

To investigate the possible presence of phytoplasmas, total DNA was extracted from samples collected from 16 diseased and five asymptomatic plants in each location. DNA was tested by direct PCR using P1/P7 primers (Deng & Hiruki, 1991; Schneider *et al.*, 1995), and on these amplicons nested PCR using primers R16mF2/R16mR2 or R16F2n/R16R2 (Gundersen & Lee, 1996). Amplicons of the expected size were obtained from all diseased plants, but not from water used as negative control, or from asymptomatic plants. Restriction fragment length polymorphism (RFLP) analyses on R16F2n/R16R2 amplicons using *KpnI*, *AluI*, *HaeIII*, *HhaI*, *HpaII*, *MseI*, *RsaI*, *TaqI* and *HpaI* restriction enzymes showed that all samples had identical restriction profiles, comparable with reference samples of the 16SrIX phytoplasma group (Lee *et al.*, 1998) (data not shown). Four samples in total from the two sampling areas were directly sequenced using nested PCR products obtained with P1/P7 and R16mF2/R16mR2. After alignment and assembling, the resulting sequences were trimmed to the R16F2n/R16R2 fragment. Obtained sequences showed 100% identity to each other and one sample from Shalamzar (SSY) was submitted to GenBank (Accession No. KX461906). Sequence comparison by BLAST analysis showed highest sequence identity with phytoplasmas in group 16SrIX (pigeon pea witches' broom). Phylogenetic analysis using the neighbour-joining method (with MEGA software version 6.0) confirmed that the SSY phytoplasma clustered with '*Candidatus Phytoplasma phoenicium*' (Fig. 4), with 99.4% sequence identity to the reference strain for this phytoplasma (AF515636), and is therefore a '*Ca. P. phoenicium*'-related strain. Computer-simulated analysis with 17 restriction endonucleases using *iPhyClassifier* (Zhao *et al.*, 2009)

showed that the RFLP pattern derived from SSY was not referable to any reported subgroup in 16SrIX group, although the highest similarity was with the 16SrIX-E subgroup (GQ925918), with a similarity coefficient of 0.95. Therefore SSY phytoplasma represents a new subgroup within the 16Sr group IX that was designed as 16SrIX-I.

'*Candidatus P. phoenicium*' is widespread in Iran, and has been reported in GF-677 (*Prunus amygdalus* × *Prunus persica*) and *Prunus amygdalus* in Fars province (Salehi *et al.*, 2011). To our knowledge, this is the first report of a phytoplasma associated with *O. viciifolia* in Iran or worldwide, and indicates that it may serve as phytoplasma reservoir for other agricultural relevant plant species.

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Figure 1



Figure 2



Figure 3

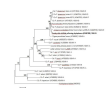


Figure 4

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