



First report of a 16SrII phytoplasma (subgroup D) associated with *Robinia pseudoacacia* witches' broom and dieback in Iran

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Black locust (*Robinia pseudoacacia*) is an important shade tree in urban green spaces in Iran. During 2017-18, *R. pseudoacacia* trees showing witches' broom and dieback symptoms (Fig. 1) were observed in Safayieh, Yazd province, Iran. Twenty-four symptomatic and four asymptomatic plants were surveyed for a phytoplasma and leaf samples were taken from each.

Total DNA was extracted from 0.2 g of fresh leaves from both diseased and symptomless plants. DNA samples were tested for the presence of phytoplasma by direct PCR primed with P1/P7 (Deng & Hiruki, 1991; Schneider *et al.*, 1995) and nested PCR using primers R16mF2/R16mR1 and R16F2n/R16R2 (Gundersen & Lee, 1996). From all symptomatic plants products of the expected size (1.8. 1.4 and 1.2kbp) were detected but not from symptomless plants. Restriction fragment length polymorphism (RFLP) analysis of the amplicons obtained in nested PCR with R16F2n/R16R2 primers was performed using *Rsa*I, *Alu*I, *Mse*I, *Hinf*I and *Hae*III restriction enzymes (Zhao *et al.*, 2009). In all 24 positive samples, RFLP profiles were identical and related to 16SrII phytoplasmas ('*Candidatus* Phytoplasma aurantifolia').

R16mF2/R16mR1 amplicons from four *R. pseudoacacia* phytoplasma-infected trees were sequenced. Consensus sequences showed 100% sequence identity to each other. The sequence of an R16F2n/R16R2 amplicon (1236 bp) from a representative sample was deposited in GenBank (Accession No. MN431432). A BLAST search showed that the *R. pseudoacacia* phytoplasma shared more than 99% sequence identity with those of phytoplasma group 16SrII. Phylogenetic analysis using the neighbour-joining method (MEGA software version 6.0) confirmed that the *R. pseudoacacia* phytoplasma clustered within the 16SrII phytoplasma clade and was closely related to strains in the 16SrII-D subgroup (Fig. 2). Analysis using *iPhyClassifier* suggested that the *R. pseudoacacia* phytoplasma 16S rRNA gene RFLP pattern was identical (similarity coefficient 1.00) to that of a phytoplasma strain in 16Sr group II, subgroup D (Y10097).

A '*Ca. Phytoplasma phoenicium*' strain was reported from *R. pseudoacacia* trees exhibiting yellowing symptoms in Iran (Karimzade *et*

al., 2018). However, this is the first report of a phytoplasma of group 16SrII, subgroup D in *R. pseudoacacia*. The finding has a significant phytosanitary impact since this is the major phytoplasma group associated with diseases in crops and weeds in Iran and therefore poses a serious threat to other plant species growing nearby.

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Figure 1

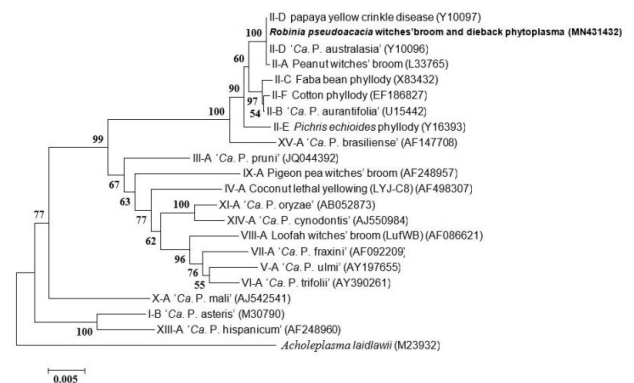


Figure 2

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