New Disease Reports

Association of a '*Candidatus* Phytoplasma solani'-related strain with pistachio in Iran

M. Ghayeb Zamharir

Plant Disease Department, Iranian Research Institute of Plant Protection, AREEO, Tehran, PO Box 19395-1454, Iran

*E-mail: zamharir2005@yahoo.com

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Pistachio (*Pistachia vera*) is one of the most important horticultural plants in Iran, growing in the majority of the regions throughout the country. The main cultivation centres are the provinces of Kerman, Semnan, Yazd, Qom and Qazvin. Previously, a phytoplasma of an undetermined species has been identified in pistachio showing witches' broom symptoms in Iran (Zamharir & Mirabolfathi, 2011), whilst group 16SrI (Marzachi *et al*., 1999) and 16SrII (Khodaygan *et al*., 2014) phytoplasmas have been identified in pistachio in Italy and Iran, respectively.

In recent years, yellowing and scorch symptoms (Figs. 1-2) were observed in some pistachio-cultivation areas in Yazd. Given the increasing importance of phytoplasma-associated diseases in Iran, a survey was carried out during 2011-2015. Diseased trees developed every year during the five-year survey. Samples from twenty symptom-bearing pistachio trees and five asymptomatic trees were collected from two pistachio-growing regions in Meibod and Marvast (Yazd province). DNA was extracted from 0.5 g of leaf midribs and petioles using a CTAB-based method (Doyle & Doyle, 1987). Universal phytoplasma PCR was performed for phytoplasma detection. First round PCR used primers P1/P7 (Schneider *et al*., 1995), and the amplicons were re-amplified with the internal primer pair R16mF2n/R16mR1 (Gundersen & Lee, 1996), amplifying approximately 1,200 bp of 16S rRNA gene. Direct sequencing was done with primers R16mF2n/R16mR1. The sequences were assembled using MEGA6 and compared with selected nucleotide sequences in the GenBank database using BLAST. Phylogenetic analyses were done with 16S rDNA sequence from pistachio sample PM3 and from sixteen '*Candidatus* Phytoplasma' strains. The majority of the symptom-bearing samples (90%) were positive in nested PCR, and no amplification was obtained from negative controls (without template DNA) and the asymptomatic samples. The sequence of sample PM3 was submitted to GenBank (Accession No. KX417297). The sequence showed 99% homology with many strains affiliated with '*Candidatus* Phytoplasma solani' and with the reference strain for the species STOL11 (AF248959). The phylogenetic analysis confirmed its placement as a '*Ca*. P. solani'-related strain (Fig. 3).

On the basis of disease symptoms and molecular analyses, the pistachio scorch disease in the two regions of Yazd was associated with the presence of a phytoplasma. The epidemiology of pistachio scorch disease in Iran and the presence of possible insect vectors requires further study.

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Figure 1

Figure 2

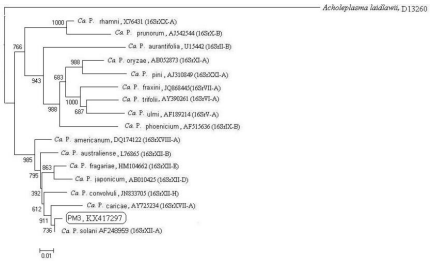

Figure 3

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