New Disease Reports

First finding of a '*Candidatus* Phytoplasma fraxini'-related strain associated with disease of olive in Iran

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Iran is becoming a globally significant olive producer. During a survey in 2015, olive trees with symptoms of shoot proliferation, stunting, yellowed and little leaves (Fig. 1) typical of phytoplasma infection were observed in Roudbar region, Gilan province, Iran. Approximately 35% of the samples collected showed symptoms.

To determine if a phytoplasma was associated with the disease, total DNA was extracted using a CTAB-based method (Doyle & Doyle, 1987) from leaves of five healthy and 20 diseased trees. DNA was analysed by PCR using phytoplasma universal primers P1/P7 (Ahrens & Seemuller, 1992) in first round PCR, and R16F2n/R16R2 (Gundersen & Lee, 1996) in nested PCR. This resulted in fragments of the expected size in all symptomatic plants. All of the healthy plants tested negative for phytoplasmas. The nested PCR product of one sample was sequenced directly (using primers R16F2n/R16R2) on both strands by Macrogen Co. (Korea). Sequences were assembled using SeqMan (Lasergene), manually adjusted when necessary, and deposited in GenBank (Accession No. KX298840). BLAST analysis of the 16Sr DNA showed the highest identity (99%) with members of the 16SrVII phytoplasma group. Phylogenetic analysis with other related phytoplasmas using ClustalX and MEGA6 identified the phytoplasma, designated olive little leaf phytoplasma, as a '*Candidatus* Phytoplasma fraxini'-related strain (Fig. 2), with 99% sequence identity to the reference strain of the species (Ashy4, JQ868445).

These results extend the geographical distribution and host range of '*Ca*. P. fraxini'. To our knowledge this is the first report of a phytoplasma related to '*Ca*. P. fraxini' infecting olive, and the study of natural reservoirs and vectors will be important to understand this disease.

References

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Doyle JJ, Doyle JL, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**, 11-15.

Gundersen DE, Lee IM, 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea* **35**, 144-151.

Figure 1

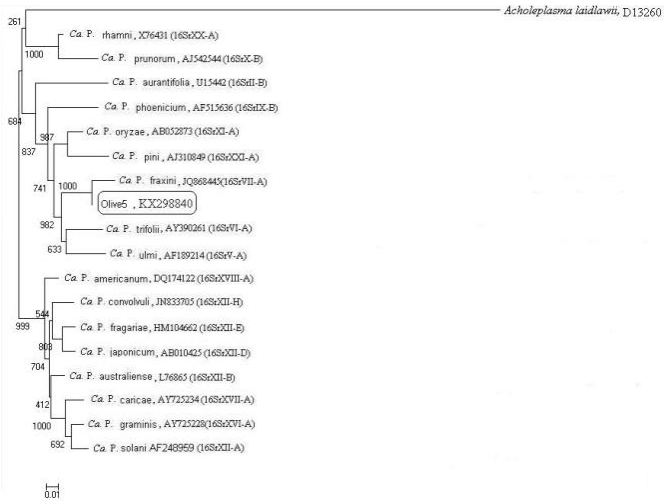

Figure 2

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