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Disease Notes

First Report of *Candidatus* Phytoplasma solani Associated with Potato Plants in Greece

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e-Xtra

In August 2013, potato plants (*Solanum tuberosum*) cv. Banba displaying symptoms resembling those caused by *Candidatus* Phytoplasma solani (potato stolbur phytoplasma) were observed in a 2-ha field in the area of the Peripheral Unit of Drama (northern Greece). The plants were 10 weeks old and their symptoms included reddening and upward rolling of leaflets, reduced size of leaves, shortened internodes, and aerial tuber formation. Incidence of affected plants was estimated to be 40% in the field. Four symptomatic potato plants were collected for laboratory testing of possible phytoplasma infection. From each of these four plants, total DNA was extracted from mid veins of reddish leaflets from apical shoot parts and of leaflets emerging from aerial tubers, using a phytoplasma enrichment procedure (1). A nested PCR using the phytoplasma universal 16S rRNA primer pairs: P1/P7 followed by R16F2n/R16R2 (3) amplified the expected ~1.2-kb 16S rDNA fragment in all four symptomatic potato plants. No amplification was observed with DNA similarly extracted from leaflets of asymptomatic potato plants of the same variety collected from an apparently healthy crop. One of the four 1.2-kb nested 16S rDNA PCR products was gel purified, cloned into the pGEM-T-easy plasmid vector (Promega, Madison, WI), and sequenced by Beckman Coulter Genomics (United Kingdom). At least twofold coverage per base position of the cloned PCR product was achieved. BLAST analysis showed that the obtained sequence of the PCR 16S rDNA product was: i) 100% identical to several GenBank sequences of *Ca.* P. solani strains, including strains detected previously in Greece infecting tomato (GenBank Accession No. JX311953) and *Datura stramonium* (HE598778 and HE598779), and ii) 99.7% similar to that of the *Ca.* P. solani reference strain STOL11 (AF248959). Furthermore, analysis by *i*PhyClassifier software showed that the virtual restriction fragment length polymorphism (RFLP) pattern of the sequenced PCR 16S rDNA product is identical (similarity coefficient 1.00) to the reference pattern of the 16SrXII-A

subgroup (AF248959). The sequence of this PCR product was deposited in NCBI GenBank database under the accession no. KJ810575. The presence of the stolbur phytoplasma in all four symptomatic potato plants examined was further confirmed by nested PCR using the stolbur-specific STOL11 primers (3) targeting non-ribosomal DNA. Based on the observed symptoms in the field and laboratory molecular examinations, we concluded that the potato plants were infected by a *Ca*. P. solani related strain. The stolbur disease has been previously reported in Greece affecting tomato (2,5) and varieties of *D. stramonium* (4). To our knowledge, this is the first report of a *Ca.* P. solani related strain infecting a potato crop in Greece. As northern Greece is a center of potato production, the source of this pathogen is to be investigated.

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