New Disease Reports

First report of a '*Candidatus* Phytoplasma asteris' isolate affecting macadamia nut trees in Cuba

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Symptoms of growth abnormalities, including leaf hardness, phyllody and shoot proliferation in eight years old macadamia trees (*Macadamia integrifolia*) were observed in an *ex situ* tropical and subtropical fruit trees collection in Artemisa, Cuba (Fig. 1A). The appearance of the affected trees contrasted with healthy-looking ones (Fig. 1B). There are no previous reports of such disease in macadamia trees. However, similar symptoms have been associated with phytoplasmas affecting other fruit trees (Verdin *et al*., 2003).

DNA was extracted from 0.5 g of leaf midribs of twelve macadamia plants collected (Murray & Thomsom, 1980), and used as a template for a nested PCR assay. Universal primer pairs that target the phytoplasma 16S rRNA gene, P1 (Deng & Hiruki, 1991) and P7 (Schneider *et al*., 1995) were used for the first reaction, and R16F2n/R16R2 (Gundersen & Lee, 1996) for the nested reaction. Nested PCR products of expected size (approximately 1250 bp) were obtained from ten symptom-bearing plants. PCR products were purified (Wizard SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA), cloned (pGEMT-Easy Vector, Promega), and two individual clones per infected plant were sequenced using Macrogene Inc. Sequencing Service. The R16F2n/R16R2 sequences were subjected to *in silico* restriction fragment length polymorphism (RFLP) analysis with endonucleases *AluI, BfaI, BstUI, HaeIII, HhaI, HinfI, HpaII, MseI* and *Tsp509* (pDRAW32 AcaClone Software, http://www.acaclone.com). Phylogenetic relationships were established between the macadamia phytoplasma and those of 16SrI and other phytoplasma groups (Mega 5.0, USA).

The R16F2n/R16R2 sequences of phytoplasmas detected in the symptom-bearing samples were 100% identical to each other. The consensus sequence (1262 nt) of the macadamia phytoplasma was deposited in GenBank (Accession No. KC513772) and showed 99% of sequence identity with those of the 16SrI group '*Candidatus* Phytoplasma asteris', including phytoplasma strains AY-CVB (AY265212), ACLR-AY (AY265211) and Baigah periwinkle little leaf phytoplasma (DQ266089), all members of subgroup 16SrI-F. The virtual RFLP profile of the macadamia phytoplasma was identical to those of the 16SrI-F subgroup, which suggests that the phytoplasma strain detected in Cuban macadamia trees may be a member of this subgroup. The results of sequence and RFLP analyses were confirmed by phylogeny (Fig. 2) since the macadamia phytoplasma grouped within the cluster corresponding to the 16SrI group, closely related to those of subgroup 16SrI-F. The group 16SrI was first recorded in Cuba in 1999 (Arocha *et al*., 1999) and since then in several other plant species. However, this is the first report of a phytoplasma of group 16SrI associated with symptoms in macadamia nut trees, a possible new host for 16SrI phytoplasmas. These results have a significant impact for Cuban agriculture since phytoplasma group 16SrI is well known by its widest plant host range and the most complex epidemiology worldwide, and macadamia may become an important future export crop for the Cuban fruit industry.

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References

Arocha Y, Gonzalez L, Peralta EL, Jones P, 1999. First report of virus and phytoplasma pathogens associated with yellow leaf syndrome of sugarcane in Cuba. *Plant Disease* **83**, 1177. [http://dx.doi.org/10.1094/PDIS.1999.83.12.1177B]

Deng S, Hiruki C, 1991. Amplification of 16 S rRNA genes from culturable and non-culturable mollicutes. *Journal of Microbiological Methods* **14**, 53-61. [http://dx.doi.org/10.1016/0167-7012(91)90007-D]

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

Murray MG, Thompson WF, 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*. **239**, 487-491.

Schneider B, Cousin M, Klinkong S, Seemüller E, 1995. Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunhemp, sesame, soybean and eggplant. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz***102**, 225-232.

Verdin B, Salar P, Danet JL, Choueiri E, Jreijiri F, El Zammar R, Gélie B, Bové JM, Garnier M, 2003. '*Candidatus* Phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran . *International Journal of Systematic and Evolutionary Microbiology* **53**, 833-838. [http://dx.doi.org/10.1099/ijs.0.02453-0]

Figure 1

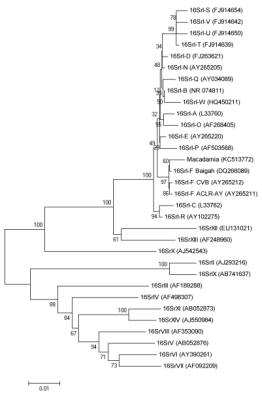

Figure 2

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