New Disease Reports

First report of a '*Candidatus* Phytoplasma ulmi' isolate affecting sapodilla trees in western Cuba

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The cultivation of sapodilla (*Manilkara zapota*) has increased in the last five years in western Cuba as part of a fruit diversification programme to support the tourism industry, and as a potential export crop. Symptoms of witches' broom have recently been observed in sapodilla trees in a fruit farm located at Ceiba, in Artemisa western province (Fig. 1A) strongly contrasting with those healthy-looking sapodilla trees (Fig. 1B). Similar symptoms were previously associated with a phytoplasma in eastern Cuba (Acosta *et al*., 2008). Therefore sapodilla plants from Ceiba were surveyed and tested for phytoplasmas.

Total DNA was extracted from 0.5 g of leaf midribs of five symptom-bearing and five symptomless sapodilla plants collected (Murray & Thompson, 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 five symptom-bearing plants. PCR products were purified (Wizard SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA) and cloned (pGEMT-Easy Vector, Promega). One individual clone per infected plant was sequenced using Macrogene Inc. Sequencing Service (Korea). The R16F2n/R16R2 sequences were trimmed, assembled into a consensuses using the Sdaten package (Bonfield & Whitwham, 2010) and compared with reference sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov). Sequences were subjected to *in silico* restriction fragment length polymorphism (RFLP) analysis with endonucleases *Alu*I, *Bfa*I, *BstU*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Mse*I and *Tsp*509 (pDRAW32 AcaClone Software), and then subjected to phylogenetic analysis with the Mega version 5.0 software (USA).

The R16F2n/R16R2 sequences of the phytoplasma detected in the symptom-bearing sapodilla plants were 100% identical to each other. The consensus sequence (1171 nt) of the sapodilla phytoplasma (GenBank Accession No. KF500549) showed the highest sequence identity (98.04%) with the elm yellows (EY) phytoplasma strains from the 16SrV group '*Candidatus* Phytoplasma ulmi', including the strain EY1 (AY197655). Phylogenetic analysis confirmed both sequence and RFLP results (Fig. 2) since the sapodilla phytoplasma clustered within the 16SrV phylogenetic branch closely related to the EY1 strain (AY197655). All RFLP profiles of the sapodilla phytoplasma were identical to those of the 16SrV group used as reference, except for the *Alu*I, *Mse*I and *Bst*UI RFLP profiles. This suggests that the phytoplasma detected in sapodilla trees of western Cuba may be a member of a new subgroup. A phytoplasma of group 16SrII '*Ca*. Phytoplasma aurantifolia' was previously identified in sapodilla plants of eastern Cuba showing witches' broom and leaf yellowing symptoms (Acosta *et al*., 2008). However, this is the first record of a '*Ca*. Phytoplasma ulmi' isolate identified in western Cuba affecting sapodilla plants.

Figure 1

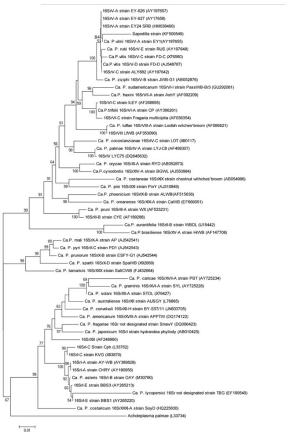

Figure 2

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