New Disease Reports

First report of a '*Candidatus* Phytoplasma asteris' isolate associated with banana elephantiasis disease in Colombia

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Colombia is one of the largest banana exporters in Latin America (FAO, 2017), the main destinations being Europe and the USA. Banana (*Musa acuminata*) is severely affected by banana elephantiasis disease (BED), a disease first reported in 1911 in Suriname. BED has significantly lowered both yield and commercial value of the cultivars 'Gros Michel' (AAA) and 'Dominico Harton' (AAB). Yield reductions of 9 to 71.6% have been reported in eight municipalities of Ulloa and Alcalá in the Department of Valle del Cauca in Colombia from surveys conducted during 2016 and 2017. BED causes an overgrowth of the pseudostem-rhizome junction generating longitudinal and transverse ruptures (Fig. 1) that lead to the collapse of the whole plant. The banana suckers exhibit necrotic tips which limit the plant's development, the petioles remain rigid with a bunchy appearance (Fig. 2), fruit size is reduced (Fig. 3) and finally, the rhizome develops conically.

Total DNA was extracted using the CTAB protocol (Daire *et al*., 1997) from rhizome tissues of the 16 symptom-bearing and four symptomless plants of banana cv. 'Gros Michel'. DNA extracts were used as templates in conventional and quantitative PCR (qPCR) assays for phytoplasma detection. Conventional PCR used primers that amplify the phytoplasma 16S ribosomal RNA gene, P1/Tint (Smart *et al*., 1996), followed by primers fU5/rU3 (Lorenz *et al*., 1995) in a nested PCR reaction. DNA was tested by qPCR with phytoplasma universal primers (Christensen *et al*., 2004). DNA extracts corresponding to an isolate of group 16SrIII, '*Candidatus* Phytoplasma pruni' obtained from root tissue from cassava plants infected with the cassava frog skin disease phytoplasma were used as a positive control. *In vitro* banana plants were used as negative controls.

Nested and qPCR amplicons were obtained for all the 16 symptom-bearing samples but not from the symptomless plants. Nested PCR amplicons were directly sequenced (Iowa State University, USA), and two consensus sequences were produced and deposited in GenBank (Accession Nos. MF629790 and MF662673). BLAST analysis of the BED phytoplasma 16S rDNA sequences showed 99% sequence identity with those of phytoplasmas of group 16SrI '*Ca*. P. asteris'. BLAST comparisons were confirmed by phylogenetic analysis (MEGA 7.0), which supported the grouping of the BED phytoplasma within the 16SrI cluster (Fig. 4). Results were also supported by *in silico* restriction fragment length polymorphism analysis (pDRAW32, http://www.acaclone.com) with *Alu*I, *Mse*I and *Rsa*I restriction endonucleases, which yielded profiles identical to members of phytoplasma group 16SrI.

'*Candidatus* P. asteris' is found in several tree host species in Colombia and represents a potential threat to other plant species (Perilla-Henao *et al*., 2012) in addition to the potential for BED to emerge as an important disease. This is the first report of a '*Ca*. P. asteris' associated with BED in banana in Colombia.

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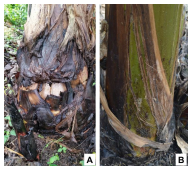

Figure 1

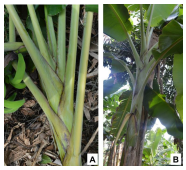

Figure 2

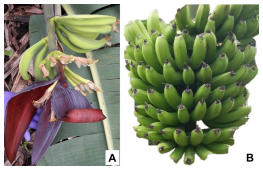

Figure 3

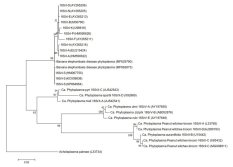

Figure 4

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