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Epidemic of lethal yellowing disease affecting *Phoenix dactilyfera* and *Sabal mexicana* in Central Mexico

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Abstract

Phytoplasmas are nonculturable pleomorphic prokaryotes associated to plant diseases worldwide. Lethal yellowing of coconut palms disease has high economic impact of the palm population affected in several countries. *Phoenix dactilyfera* and *Sabal mexicana* plants showed symptoms of lethal decline in central Mexico at locations that were approximately 500 km from the coast and altitudes ~ 1700 masl which are unusual for putative lethal yellowing. We identified two strains that fit within the group 16SrI, subgroup D. This is the first report of the presence of the LY phytoplasmas far from the coasts and at extreme high altitudes. The vector *Myndus crudus* is only present in warm areas due to its high susceptibility to low temperatures. Our findings could indicate the presence of a new unknown vector or that a new biotype of *M. crudus* is spreading in central Mexico.

Key words: mollicutes, PCR, detection, vector.

Introduction

Phytoplasmas are pleomorphic prokaryotes associated to plant diseases worldwide. Infected plants show abnormal growth with excessive bud proliferation, yellow stems, yellow mosaics and unusual colorations. These pathogens induce hormonal disorders on their hosts but they normally do not cause premature death of affected plants. An exception to this however, is the lethal yellowing (LY) of coconut palms. LY appeared in Mexico in 1971 devastating palm plantations from the States of Yucatán, Chiapas and Campeche (Martínez-Soriano *et al*, 1994) and recently in the Pacific coasts of Oaxaca and Guerrero. This disease has high economic impact with up to 68% of the palm population being affected. In 2009 *Phoenix dactilyfera* and *Sabal mexicana* plants showed symptoms of lethal decline in central Mexico at locations that were approximately 500 km from the coast and altitudes ~ 1700 m asl which are unusual for putative LY. Symptoms observed were fruit drop as well as flower necrosis. These were followed by appearance and chronological progression of foliar yellowing. First younger leaves turn reddish-brown to dark brown, finally inducing death of apical meristem.

Materials and methods

DNA extraction

Plant samples of *Phoenix dactilyfera* and *Sabal mexicana* were taken at the municipalities of Abasolo, Salamanca and Irapuato, State of Guanajuato in Mexico. Total DNA was extracted as described by Lopez and Larkins (1993).

PCR assays

Universal primer pairs targeting the 16S rRNA gene in the initial reaction primers R16mF2 and R16mR1 (Gundersen and Lee, 1996) were used. Then sequential nested PCRs with the primers R16F2/R2 (Lee *et al.*, 1993) were performed. PCR was performed in a 25 µl total volume of reaction, containing approximately 50 ng of genomic DNA, 2 mM $MgCl_2$, 10 pmol of each primer, 1X of PCR buffer solution, 200 mM of dNTPs and 2.5 units of Platinum®*Taq*DNA polymerase (Invitrogen). PCR rounds were as conventional programs. This amplification products were used to made 1:20 dilutions as DNA template for second amplification reaction. All products were visualized in 1% agarose gels by staining with ethidium bromide.

Cloning and nucleotide sequencing

RFLP and DNA sequence analysis were conducted for molecular characterization. PCR products were purified with Pure Link PCR purification kit and inserted into pCR TOPO TA cloning 2.1 vector (Invitrogen) used to transform *E. coli* DHα cells. Plasmids were sequenced using an ABI PRISM 377 PERKIN-ELMER DNA sequencer.

Sequence alignment and phylogenetic analysis

Restriction DNA patterns of 16S rDNA amplicons were generated using the iPhyclassifier software. DNA sequences were analysed by NCBI Blastn algorithm.

Results

Nucleotide sequences were determined for 16S rDNA fragments amplified after nested PCRs were deposited at the GenBank database (accession numbers JF431249 and JF431250). Sequence similarity among the 16S rDNAs of *Sabal mexicana* strain and others reported for the States of Guerrero (Mexico) and Florida (USA) were 99% identity of 98% coverage. The *Phoenix dactilyfera* strain showed 99% identity of 98% coverage

with Texas Phoenix (USA) and Yucatán (Mexico) strains. On the basis of RFLP patterns, these two strains of the lethal yellowing phytoplasmas fit within the group 16SrI, subgroup D (Lee *et al*., 1998).

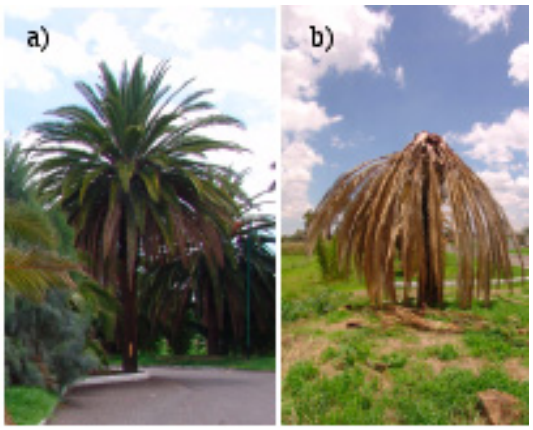

Figure 1. Palms showing coconut lethal yellowing phytoplasma associated diseases. a) Symptomless, b) lethal yellowing in terminal stage.
(In colour at www.bulletinofinsectology.org)

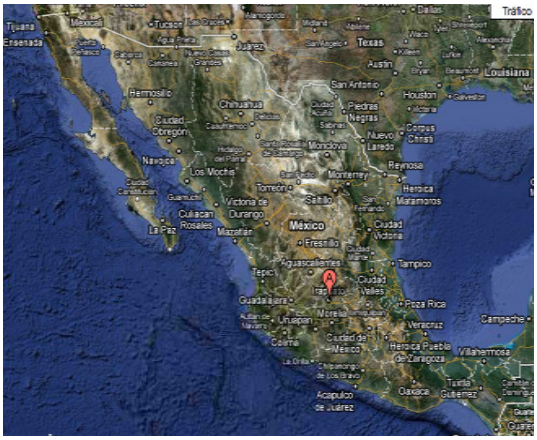

Figure 2. Presence of coconut yellowing phytoplasma far from Mexican coasts.
(In colour at www.bulletinofinsectology.org)

Discussion

This is the first report worldwide of the presence of the LY phytoplasmas far from the coasts and at extreme high altitudes. The vector *Myndus crudus* is only present in warm areas due to its high susceptibility to low temperatures. Our findings could indicate the presence of a new unknown vector or that a new biotype of *M. crudus* is spreading in central Mexico.

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