

ISSN: 0191-2917

## SEARCH

Enter Keyword



Phytopathology



Plant Disease



MPMI

[Advanced Search](#)

Inside the Journal

## BACK ISSUES

(Issues before 1997)

[First Look](#)[View Most Downloaded Articles](#)[About Plant Disease](#)[Editorial Board](#)[Submit a Manuscript](#)[Author Instructions](#)[Policies/Procedures](#)[Online e-Xtras](#)

= "Open" Access

Editor-in-Chief: R. Michael Davis

Published by The American Phytopathological Society

&gt;

&gt;

&gt; Abstract

[Previous Article](#)[Next Article](#)May 2010, Volume 94, Number 5  
Page 636

DOI: 10.1094/PDIS-94-5-0636B

## Disease Notes

**Molecular Detection of 16SrXI Group Phytoplasma Associated with Root (Wilt) Disease of Coconut (*Cocos nucifera*) in India****R. Manimekalai, V. P. Soumya, and R. Sathish Kumar**, Central Plantation Crops Research Institute, Kasaragod, Kerala, India; **R. Selvarajan**, National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India; **K. Reddy**, Indian Institute of Horticultural Research, IHR, Bengaluru, India; **G. V. Thomas, M. Sasikala, and G. Rajeev**, Central Plantation Crops Research Institute, Kasaragod, Kerala, India; and **V. K. Baranwal**, Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India

Coconut palm (*Cocos nucifera* L.), a versatile tree crop with multifarious uses, is important for the livelihood security of millions of people in India. Root (wilt) disease (RWD) is a major production constraint causing an estimated yield loss of 968 million nuts in southern India. Affected palms show bending of leaflets (flaccidity), foliar yellowing, and marginal necrosis. Phytoplasmas have been observed to be associated with this disease by electron microscopy (EM) and transmission (3) but not characterized. Attempts made in the past decade to detect a phytoplasma associated with RWD through PCR using universal primers had inconsistent results so we designed two primer sets (1F7 [AGTGCTTAACACTGTCCTGCTA]/7R3 [TTGTAGCCCAGATCATAAGGGGCA], 3Fwd [ACCTGCCTTAAGACGAGGA]/3Rev [AAAGGAGGTGATCCATCCCCACCT]) and seminested primer pair 1F7/7R2 (GACAAGGGTTGCGCTCGTTTT), 3Fwd/5Rev (ACCCGAGAACGTATTCACCGCGA) from sequencing of a 1.8-kb fragment (GenBank No. FJ794816) amplified by primers P1/P7 from a diseased sample. These new primer pairs were used for the detection of phytoplasma from five symptomatic and five asymptomatic palms from Kasaragod (where disease is not endemic), 14 symptomatic palms from Kayamkulam (endemic area), and 10 palms from disease-free areas (Kidu, Karnataka) using PCR. DNA was extracted from 3 g of spindle leaf (two to three leaflets) midrib tissues using a modified phytoplasma enrichment protocol in which an addition of 5% polyvinylpyrrolidone (MW of 40,000) during tissue grinding was essential. PCR was performed for 35 cycles with an annealing temperature of 63°C to avoid nonspecific amplification. A 1.3-kb amplicon was seen in two of the five samples and the positive control sample (sugarcane grassy shoot DNA) using the seminested primer pair 3Fwd/3Rev–3Fwd/5Rev. The amplicons were cloned and sequenced and a representative sequence was deposited in GenBank (GQ850122). With the 1F7/7R3–1F7/7R2 seminested primers, a 493-bp product was obtained from 13 of 14 palms from Kayamkulam and all five diseased palms from Kasaragod. No amplification was seen from healthy palms. A BLAST search showed that the RWD phytoplasma 16S rRNA gene sequence has >96% nt identity with 16SrXI

and 16SrXIV group phytoplasmas and 99% identity with sugarcane white leaf phytoplasma (AB052874), On the basis of the identity of the 16Sr RNA gene 3Fwd/5Rev region, RWD phytoplasma belongs to the 16SrXI group. A phylogenetic tree (neighbor-joining method) also revealed clustering of the coconut phytoplasma with the 16SrXI group phytoplasmas and virtual restriction fragment length polymorphism analysis (4) also placed it into group 16SrXI. Other phytoplasmas infecting coconut are found in groups 16SrIV (1) and 16SrXIV (2). Our RWD phytoplasma sequence does not match an earlier reported Kerala (wilt) coconut phytoplasma sequence (AY158660) and the latter sequence does not have similarity with any known phytoplasma sequences in the database. To our knowledge, this is first report of the association of 16SrXI group phytoplasma with the root wilt disease of coconut in India. These findings could be used for the early detection of root wilt disease phytoplasma in breeding materials and to develop a DNA-based diagnostic kit.

*References:* (1) N. A. Harrison et al. *Ann. Appl. Biol.* 153:85, 2008. (2) N. Nejat et al. *Am. J. Appl. Sci.* 6:1331, 2009. (3) M. Sasikala et al. *Eur. J. Plant Pathol.* 94:191, 2005. (4) Y. Zhao et al. *Int. J. Syst. Evol. Microbiol.* 59:2582, 2007.

- [Journals Home](#)
  - [APSnet](#)
  - [IS-MPMInet](#)
  - [Contact Us](#)
  - [Privacy](#)
- [Copyright The American Phytopathological Society](#)