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Disease Notes

Molecular Detection of Phytoplasma Associated with Yellow Leaf Disease in Areca Palms (*Areca catechu*) in India

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The arecanut palm (*Areca catechu* L.), Arecaceae family, is one of the most important commercial crops in the world, which yields fruits called arecanut that are used as a medicine and chewing substance (1). Yellow leaf disease (YLD) is one of the most serious diseases in areca palms in India. It reduces the yield as much as 50% over a period of 3 years immediately following disease incidence. Foliar yellowing, the most conspicuous symptom, begins from the inner whorl and spreads to the outer parts of the crown. Chlorosis is observed on almost all leaves in the whorl from edges of the leaflet to the midrib region. Stems become spongy and friable and the conducting strands are destroyed. Microscopic detection is evidence of the association of phytoplasma in YLD-affected areca palms (3). There is no evidence for molecular level detection of phytoplasma in YLD-affected palms. To prove the phytoplasma association in YLD-affected palms in India, samples (inflorescence, spindle leaf, mature leaf, and root) were collected from 15 (5 severe, 5 middle, and 5 early stage of the disease) YLD-affected areca palms and two symptomless palms at Sullia District, Karnataka. DNA was extracted from rachis of inflorescence, midrib of spindle leaf, and meristem of root samples as previously described (2). With universal primers there was no consistency in amplification. Then we used two sets of seminested primers, 1F7/7R3–1F7/7R2 and 4Fwd/3Rev–4Fwd/5Rev, which were designed to amplify the coconut root (wilt) disease (RWD) phytoplasma (2). With the seminested primers, 1F7/7R3–1F7/7R2, a 493-bp amplicon was obtained from 15 of 15 palms. With the seminested primers, 4Fwd/3Rev-4Fwd/5Rev, a 1.3-kb amplicon was seen in 11 samples and the positive control sample (sugarcane grassy shoot DNA). The amplicons were cloned and sequenced and two representative sequences were deposited in GenBank (GU552782 and HM215624). A BLAST search showed that the sequence has 99% nt identity with sugarcane white leaf phytoplasma (FM208260, 16sr XI), coconut RWD phytoplasma (GQ850122, 16sr XI), 98% nt identity with bermuda grass white leaf phytoplasma (AJ550986, 16sr XIV), and only 91% nt identity with YLD-affected areca phytoplasma reported from China (FJ998269 and FJ694685). The phylogenetic analysis revealed the clustering of YLD phytoplasma with 16s

rRNA XI and 16s rRNA XIV groups. However, the YLD phytoplasma is closely related to the 16s rRNA XI group. PhytoDB-group identifier tool (http://220.227.88.253/phytodb) showed YLD phytoplasma from India belongs to the 16sr XI group. Earlier we reported the association of 16sr XI group phytoplasma with coconut RWD in India (2) and the YLD phytoplasma reported here has 99% nt identity with RWD phytoplasma. In southern India, coconut and arecanut are grown together in adjacent fields and there is a possible occurrence of the same phytoplasma in two different hosts. The current study proved the association of phytoplasma through nested PCR in YLD-affected areca palms in India and it is clustered with 16sr RNA XI group. Purushothama et al. (4) couldn't detect the phytoplasma with YLD-affected areca palms. To our knowledge, this is first report of the association of 16SrXI group phytoplasma with the arecanut YLD in India.

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ERRATUM: On 27 October 2010, at the request of the authors, the title of this note was changed.

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