

plant disease

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DISEASE NOTES

Molecular and Scanning Electron Microscopic Proof of Phytoplasma Associated With Areca Palm Yellow Leaf Disease in Sri Lanka

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The areca palm, *Areca catechu* L., of the Arecaceae family, is an important commercial crop in the world, where the drupe, commonly called the areca nut, is mainly used in betel quid chewing in many Asian and Oceanic countries. Areca palms have a low commercial value in Sri Lanka; they are largely grown in wet and intermediate zones on a total area of 11,968 ha in home gardens, as mixed cultivations, and seldom as small scale commercial

cultivations. Although areca yellow leaf disease (AYLD) was not previously reported in Sri Lanka, it is a serious disease in neighboring India, reducing yield by much as 50% (±). After an occurrence of Weligama coconut leaf wilt disease (WCLWD) in the southern province of Sri Lanka (±), areca palms with similar symptoms were noted in the vicinity of the WCLWD-affected plantations. Foliar yellowing of areca palms, the most conspicuous symptom, began from the inner whorl and spread to the outer parts of the crown. Chlorosis was observed on almost all leaves in the whorl. The association of a phytoplasma strain with this yellowing disease was investigated by molecular characterization based on the *secA* gene in conjunction with scanning electron microscopy (SEM). Symptomatic palms were selected from the vicinity of WCLWD-affected plantations in the Matara District. Symptomless, apparently healthy areca palms were selected from the Kandy District of the central province of the island. Total DNA was extracted from midrib of areca pinnae using the small scale DNA extraction method (±). Total DNA derived from symptomatic and apparently healthy leaf samples were analyzed by primary PCR using a phytoplasma-specific universal primer pair, *SecAfor1/SecArev3* (±) in the primary PCR, and a 1/10 dilution of this amplicon used as the template to perform the nested PCR using *RicesecAfor2/RiceseArev3* primers specific for phytoplasma of the 16SrXI and 16SrXIV groups (±). Twelve of 15 symptomatic samples yielded an amplicon of 420 bp; no amplicon was generated by DNA templates from symptomless plants ($n = 15$). Analysis of these partial *secA* sequences (NCBI Accession No. KM978910) of this characteristic band of AYLD confirmed the association of a phytoplasma strain that shared 99% nucleotide homology with that of '*Candidatus* Phytoplasma cynodontis' reference strain and 100% identity with WCLWD phytoplasma (NCBI Accession No. KM978909). Observations of transverse sections of midribs of pinnae of infected spear leaves by SEM revealed the presence of spherical, pleomorphic phytoplasma bodies ranging in diameter from 100 to 1000 nm. These appeared as separate or clustered particles, adhering to the inner surface of sieve tubes of diseased plants. The uneven distribution of phytoplasma bodies in sieve tubes as well as a developmental stage of the phytoplasma (i.e., those undergoing budding) was observed. Phytoplasma bodies were categorically absent in transverse sections of midrib of pinnae of healthy areca leaf samples. To our knowledge, this is the first report of AYLD in Sri Lanka as well as the detection of a 16SrXIV group phytoplasma strain associated with this disease. It is plausible that the same phytoplasma strain is associated with both AYLD and WCLWD. Thus, identification of areca palm as an alternative host of WCLWD phytoplasma will not only increase the complexity of the epidemiology of this disease, but will be more important in implementing control strategies for WCLWD.

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Refinement of the Taxonomic Structure of 16SrXI and 16SrXIV Phytoplasmas of Gramineous Plants using Multilocus Sequence Typing

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