

Molecular characterization of a phytoplasma associated with Coconut Yellow Decline (CYD) in Malaysia

ABSTRACT

Problem statement: Molecular methods have been used to detect phytoplasma in Malayan Red Dwarf (MRD) ecotype of coconut for the first time in Malaysia. Detect phytoplasma from coconut showing yellowing symptoms by nested PCR and Classify phytoplasma associated with disease of coconut palms, based on analysis of 16S rRNA gene operon sequences and virtual RFLP. Approach: Twenty MRD symptomatic palms were tested. Leaf spear, inflorescence and wood shavings from trunks of coconut palms showing yellowing symptoms were harvested from Serdang located in Selangor state. PCR assays and sequence analysis were carried out. Results: Nested PCR with primer pairs R16F2n/R16R2 and fU5/rU3 resulted in amplification of products of approximately 1.2 kb and 890 bp respectively, from 8 out of 20 MRD symptomatic palms tested. Sequence analysis of the 16S rDNA PCR products determined that the phytoplasma strain associated with Coconut Yellow Decline (CYD) in MRD ecotype belongs to the 'Candidatus Phytoplasma cynodontis' (16SrXIV) group of phytoplasmas. In addition, nested R16F2n/R16R2 PCR products from 6 spear leaves and 2 inflorescences from MRD palms showed high sequence similarity to the 16S rRNA gene from coconut chloroplasts, with a similar size (approximately 1.3 kb) and a further 5 R16F2n/R16R2 PCR products from MRD inflorescences showed high sequence similarities to *Bacillus* spp. 16S rRNA gene sequences. Conclusion: These results indicate that sequencing is a reliable method for the detection. Furthermore, trunk borings are the most reliable source of DNA for phytoplasma detection in coconuts using 16S rRNA gene primers, since there is less co-amplification of PCR products from other organisms when compared to spear leaves and inflorescences, nor from the spear leaves and inflorescences of MRD palms when primers fU5/rU3 were used in nested PCR.

Keyword: Cloning, Coconut palm, Nested PCR, Phytoplasma, Sequencing