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Molecular detection and characterization of phytoplasmas associated with lethal yellowing disease of coconut palms in Jamaica

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Abstract

Tissue samples were collected from coconut palms with symptoms of lethal yellowing (LY) disease in Jamaica. Palms were sampled at 50 sites exhibiting varying levels of mortality due to LY. Samples were assayed for phytoplasma infection by polymerase chain reaction (PCR) assays. Detected phytoplasmas were characterized by dot blot nucleic acid hybridisation, restriction fragment length polymorphism (RFLP) or sequencing of PCR products. A 1.8 kb rDNA was amplified from all samples by PCR employing primer pair P1/P7. Similarly reamplification of P1/P7 products using nested 16SrIV group-specific primer pair LY16Sf/LY16S-23Sr generated a product of expected size (1.7 kb) from all samples. Evaluation of samples by PCR with pathogen-specific non-ribosomal primer pair LYR1/LYF1 amplified a 1.0 kb product thereby confirming that palms contained the LY agent. When P1/P7 products were separately digested with 11 endonucleases and compared, no differences were evident among RFLP patterns of phytoplasmas infecting Maypan or Atlantic tall in Jamaica or between these patterns and those of the LY agent in Atlantic Tall palms in Florida, Honduras or Mexico, respectively. Polymorphisms revealed by *Hinf*I endonuclease digestion of rDNA products, differentiated coconut-associated phytoplasmas in Jamaica from those in Florida, Honduras and Mexico. Sequence analysis of P1/P7-primed rDNA cloned from Jamaican LY samples indicated they were all very similar strains (98.8% - 99.98%). Phylogenetic analysis of rDNA sequences established Jamaican coconut LY phytoplasmas all clustered together with previously characterized strains composing the coconut lethal yellows (16SrIV) phytoplasma group.

Key words: Lethal yellowing, phytoplasmas, detection, molecular identification, sequencing.

Introduction

Phytoplasmas are associated to coconut lethal yellowing disease (LY). They are prokaryote and belong to the class *Mollicutes* (Lee *et al*., 2000). Infection by phytoplasma of coconut palms is usually fatal. Many coconut palms, over the years, have been destroyed by this disease. Phytoplasmas are difficult to isolate and visualize and therefore require DNA-based molecular techniques for diagnosis and research (Lee *et al*., 1998).

Through PCR assays employing primer pairs derived from rRNA sequences LY phytoplasma detection can be achieved (Gundersen and Lee, 1996; Harrison *et al*., 2002). Phytoplasma identity and classification can be obtained using restriction fragment length polymorphism (RFLP) of rDNA amplified products or sequence analyses. Both techniques were employed to study phytoplasma involved in LY epidemic in Jamaica.

Materials and methods

Tissue samples were collected from 354 coconut palms with symptoms indicative of lethal yellowing disease in Jamaica. Palms were sampled at 50 sites exhibiting varying levels of mortality due to LY. Samples were assayed for phytoplasma infection by polymerase chain reaction (PCR) assays using phytoplasma specific oligonucleotide primers P1 (Deng and Hiruki, 1991) and P7 (Smart *et al*., 1996) and LY16Sr/LY16S-23Rs (Harrison *et al*., 2002a, 2002b). Detected phytoplasmas were characterized by dot blot nucleic acid hybridisation, restriction fragment length polymorphism (RFLP) with endonucleases *Alu*I, *BstU*I, *Dde*I, *Dra*I, *Hae*III, *Hha*I, *Msp*I, *Rsa*I, *Sau3*AI, *Taq*I or *Tru9*I or sequencing of PCR products.

Results and discussion

A 1.8 kb rDNA was amplified from all samples by PCR employing primer pair P1/P7. Similarly reamplification of P1/P7 products using nested 16SrIV group-specific primer pair LY16Sf/LY16S-23Sr generated a product of expected size (1.7 kb) from all samples. Evaluation of samples by PCR with pathogen-specific non-ribosomal primer pair LYR1/LYF1 amplified a 1.0 kb product thereby confirming that palms contained the LY agent.

When P1/P7 products were separately digested with endonucleases *Alu*I, *BstU*I, *Dde*I, *Dra*I, *Hae*III, *Hha*I, *Msp*I, *Rsa*I, *Sau3*AI, *Taq*I or *Tru9*I and compared, no differences were evident among RFLP patterns of phytoplasmas infecting Maypan (MPJ) or Atlantic tall (ATF) in Jamaica or between these patterns and those of the LY agent in Atlantic Tall palms in Florida (ATF), Honduras (ATH) or Mexico (ATM), respectively. Polymorphisms revealed by *Hinf*I endonuclease digestion of rDNA products, differentiated coconut-associated phytoplasmas in Jamaica from those in Florida, Honduras and Mexico.

Sequence analysis of P1/P7-primed rDNA cloned from Jamaican LY samples indicated they were all very similar strains (98.8% - 99.98%). Phylogenetic analysis of rDNA sequences established Jamaican coconut LY phytoplasmas all clustered together with previously characterized strains composing the coconut lethal yellows (16SrIV) phytoplasma group (Lee *et al*., 1998).

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