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Genetic studies of Phytophthora on Theobroma cacao from East New Britain and Bougainville (Papua New Guinea)

A thesis presented in partial fulfilment of the degree of

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ABSTRACT

DNA was extracted from 14 Phytophthora isolates from Theobroma cacao plants (SG2 hybrids, hybrid derived clones and Trinitario varieties) collected from New Britain and Bougainville in Papua New Guinea (PNG). A fragment of the mitochondrial genome cytochrome b (cytb) region was amplified from these DNAs using the polymerase chain reaction (PCR) and compared to cytochrome b sequences from Phytophthora palmivora and other Phytophthora (two isolates previously isolated from cocoa lesions and two P. palmivora isolates obtained from culture collection-Australia). All isolates were identical in their cytb gene sequence and similar to P. palmivora. Additionally, we sequenced the mitochondrial genomes of four isolates from PNG. The syntenic arrangement of genes in one complete assembly was compared with other published mitochondrial genomes. The sequences of four mitochondrial genes (COII, nad2, rps10 and SecY) from the four PNG isolates were aligned with orthologues from accessions of P. palmivora and other Phytophthora species available in the NCBI Genbank reference database. A concatenated data matrix was produced with 2,295 homologous sequence positions. 34 accessions of Phytophthora (including 14 P. palmivora) were used to construct a maximum likelihood tree of phylogenetic relationships. This reconstruction recovered all 10 major clades of Phytophthora previously reported. In this phylogenetic reconstruction, the four PNG isolates were clearly identifiable as P. palmivora and these were closely related to the Clade 4 Phytophthora species P. megakarya and P. quercetora. Of the genes analysed, COII showed greatest variability, resolving P. palmivora into three sub groups. COII was sequenced in all P. palmivora isolates from PNG and used to reconstruct an ML tree. The phylogenetic analyses suggested a potential origin for the PNG strain of P. palmivora in Samoa. Syntenic comparisons of P. palmivora and other clade 4 species identified a potential target for developing a Loop Mediated Amplification (LAMP) assay for P. palmivora near the atpH gene region. DNA amplification primers were designed for this region using PrimerExplorer, V4, Eiken Chemical CO.Ltd) and validated against available DNAs for Clade 4 and other Phytophthora species.
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