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

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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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