



# First report of the molecular identification of a phytoplasma associated with dieback disease of papaya in Sri Lanka

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Papaya (*Carica papaya*) is a very popular fruit crop in Sri Lanka, grown as a home garden and commercial crop for local and export markets. Papaya is commercially cultivated in Sri Lanka in about 8,000 ha with production of about 32,000 tonnes/year. As elsewhere, phytoplasma diseases have become a threat to the papaya crop in Sri Lanka. During November 2012, dieback-like symptoms were observed in papaya fields at Monaragala, Uva Province, in Sri Lanka. Symptoms in the field initially included a bright yellowing of the upper young leaves (Fig. 1a) that progressively evolved to tip necrosis in the tender leaves (Fig. 1b), followed by drying of the upper leaves and eventual death of the whole plant (Fig. 1c). Moreover, diseased plants exhibited discolouration of the vascular tissues, abnormal growth and shapes of fruits and premature fruit fall.

Total genomic DNA was extracted from the midribs of fresh leaf samples (1 g) from four plants with symptoms and two symptomless plants using the DNeasy Plant Mini Kit (QIAGEN, UK) according to the manufacturer's protocol. Phytoplasma rDNA was amplified with the universal phytoplasma primer pairs P1/P7 in the first round of polymerase chain reaction (PCR), and R16F2n/R16R2 in a nested PCR (Dickinson *et al.*, 2013). Amplifications of the 16S rDNA were performed in 25 µl reactions using Ready-To-Go PCR beads<sup>TM</sup> (Amersham Pharmacia Biotech, UK) in a TECHNE TC-3000 thermocycler, and resulted in PCR bands of approximately 1.2 kb from symptom-bearing plants but not from the symptomless papaya plants (Fig. 2). Three representative nested PCR products were purified (PCR Cleanup kit, Sigma, UK) and directly sequenced. A phylogenetic tree (Fig. 3) was constructed based on the 16S rDNA consensus sequence using the neighbour-joining method with default values and 1000 replicates for bootstrap analysis (MEGA version 3.1). The partial 16S rDNA sequences of the three representative phytoplasma isolates shared 100% sequence identity with each other. The BLAST analysis of the consensus sequence of the Monaragala papaya dieback phytoplasma (GenBank Accession No. KJ027722) revealed 99% sequence identity with that of phytoplasmas of group 16SrI (*Candidatus Phytoplasma asteris*). Acosta *et al.* (2011) previously reported a phytoplasma from group 16SrI associated bunchy top-like symptoms of papaya in Cuba. Moreover, phytoplasmas belonging to different 16Sr groups have been identified in papaya, particularly in the Asian continent including group 16SrII (*Candidatus Phytoplasma aurantifolia*) in India associated with similar dieback symptoms (Rao *et al.*, 2011) and axillary

shoot proliferation (Verma *et al.*, 2012); and group 16SrXII 'Stolbur' associated with a dieback-like disease of papaya in Israel (Gera *et al.*, 2005), and yellows disease in Taiwan (Bau *et al.*, 2011). To our knowledge, this is the first record of the identification of a 16SrI 'Ca. Phytoplasma asteris' isolate associated with a dieback-like disease of papaya in Sri Lanka. This finding demonstrates significant phytosanitary impact for the papaya industry in Sri Lanka, and the region, considering that 16SrI is the group that possesses the widest plant host range and the most complex epidemiology worldwide.

## Acknowledgements

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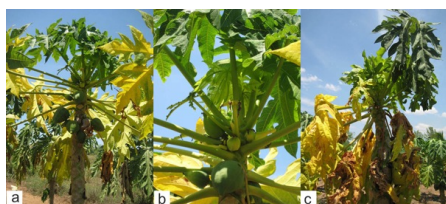


Figure 1

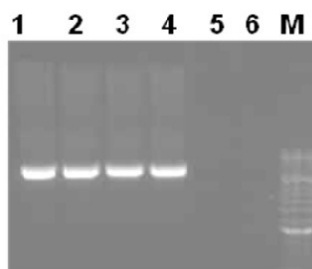


Figure 2

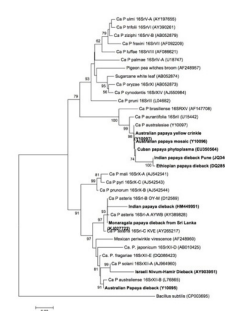


Figure 3

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