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First Report of the Association of a Clover Proliferation Phytoplasma Strain With Shoot Proliferation and Little Leaf Disease of Mangosteen (*Garcinia mangostana*) in Kerala, India

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Mangosteen (*Garcinia mangostana*) is an important fruit crop, cultivated for its unique sweet-sour fruits which are rich in metabolites. It is predominantly grown in Southeast Asian countries, among which Thailand is the main exporter (Palakawong and Delaquis 2018). In India, mangosteen is also used as a medicinally important plant, valued for its pharmacological properties (Saraswathy et al. 2022). Symptoms of shoot proliferation, little leaf, leaf yellowing and the absence of flowers and fruits (Figure 1) were observed in 60 of 312 mangosteen cv. K100 observed during September 2024 at the Pangode village (8°45′23.22″N 76°58′48.68″E), Thiruvananthapuram, Kerala, India.



FIGURE 1 | (a) Healthy mangosteen tree and (b) phytoplasma-associated symptoms, shoot proliferation and little leaf, in diseased mangosteen in Pangode village, Thiruvananthapuram, Kerala, India.

Leaf samples from 10 diseased and 2 asymptomatic trees were collected and total DNA was extracted (QIAamp Plant Genomic DNA Kit, Germany). The extracted DNA was amplified by conventional PCR using *16S rRNA* gene primers (P1/Tint and 3 For/3Rev) (Manimekalai et al. 2010) and primers (fTufl/rTufl) that amplify the elongation factor, *tuf* gene (Schneider and Gibb 1997). Amplicons of c. 1.3 and c. 1.1 kb of *16S rRNA* and *tuf* genes, respectively, were obtained from all the diseased samples, but not from the asymptomatic samples. The PCR products were directly sequenced at Biologia Research India Pvt. Ltd, Karnal, Haryana, and the sequences were submitted to GenBank with Accession Nos. PQ725444 (*16S rRNA*) and PQ812405 (*tuf* gene). BLASTn analysis based on the *16S rRNA* and *tuf* gene sequences revealed that the mangosteen Kerela (MSK) strains displayed 99.86% and 100% sequence identities, respectively, with those of the reference phytoplasma strains of the former clover proliferation (16SrVI)

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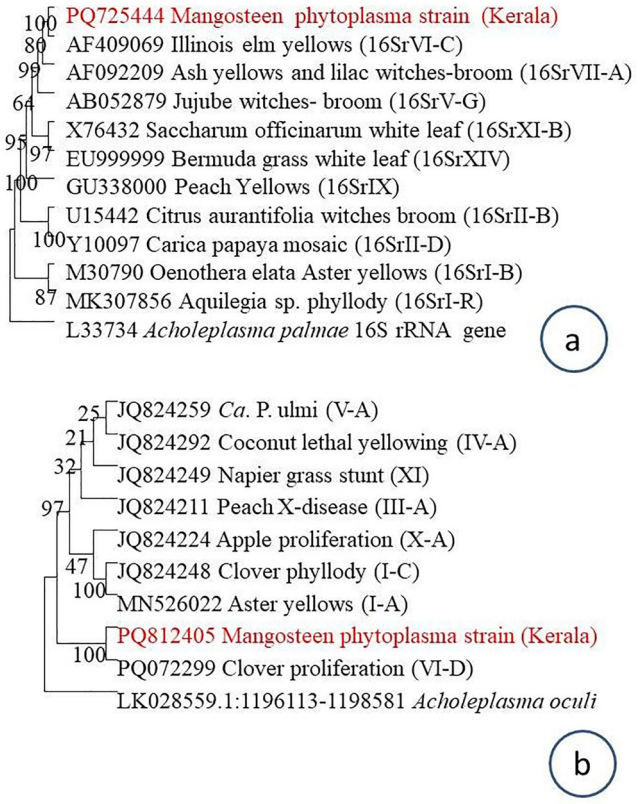


FIGURE 2 | Phylogenetic tree constructed by maximum likelihood method based on *16S rRNA* gene (a) and *tuf* gene (b) sequences of the mangosteen shoot proliferation and little leaf phytoplasma strain with selected phytoplasma strains from GenBank. Accession numbers are specified and *Acholeplasma palmae* and *Acholeplasma oculi* were used as outgroups. Numbers on branches are bootstrap values obtained for 1000 replicates.

group. A phylogenetic tree (MEGA 11, version 11.0.13) revealed that the MSK phytoplasma strains cluster within the subgroups 16SrVI-E (*16S rRNA* gene) and 16SrVI-D (*tuf* gene) (Figure 2). The virtual RFLP pattern generated for MSK *16S rRNA* phytoplasma strain through pDRAW analysis was identical to the reference pattern of 16VI-E subgroup (AY270156). The gene sequence comparison analysis of MSK strains confirmed the association with the 16VI-E subgroup.

This is the first report of a clover proliferation phytoplasma strain (16SrVI-E subgroup) infecting mangosteen in India and globally. Earlier, 16SrVI-A and 16SrVI-D subgroup related strains were reported on important agricultural and fruit crops in India (Rao 2021). The present finding suggests that mangosteen is a new natural host of the 16SrVI-E phytoplasma strain and may potentially contribute to the phytoplasma pathogen spread to other crops. The association of the MSK phytoplasma with this fruit tree is very important as it may affect yield and lead to epidemiological implications, which require further investigation in order to improve disease and crop management.

Data Availability Statement

The data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov/nucleotide/, reference number PQ725444, PQ812405.

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