New Disease Reports

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NEW DISEASE REPORT OPEN ACCESS

First Report of '*Candidatus* Phytoplasma trifolii' Associated With Leaf Yellowing Disease of *Aegle marmelos* in India

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Received: 29 August 2025 | **Accepted:** 26 October 2025

Keywords: bael | 16S rRNA | *secY* gene

Aeglemarmelos (commonly known as bael) is a perennial fruit tree widely distributed across Southeast Asia (R. Singh et al. 2022). Bright leaf yellowing and reduced fruit yield symptoms were recorded in two *A. marmelos* varieties (NB-5 and NB-9) in October 2024 in fruit orchards on the university campus of Jammu, Jammu and Kashmir, India (Figure 1). Genomic DNA was extracted from three leaf samples each from diseased and asymptomatic trees of both varieties using a Plant DNA extraction kit (Qiagen, Germany). The genomic DNA samples were tested for the presence of the 16S rRNA gene using phytoplasma-specific primer pairs, P1/Tint and3Fwd/3Rev (Smart et al. 1996; Manimekalai et al. 2010), and the *secY* gene using primer pairs

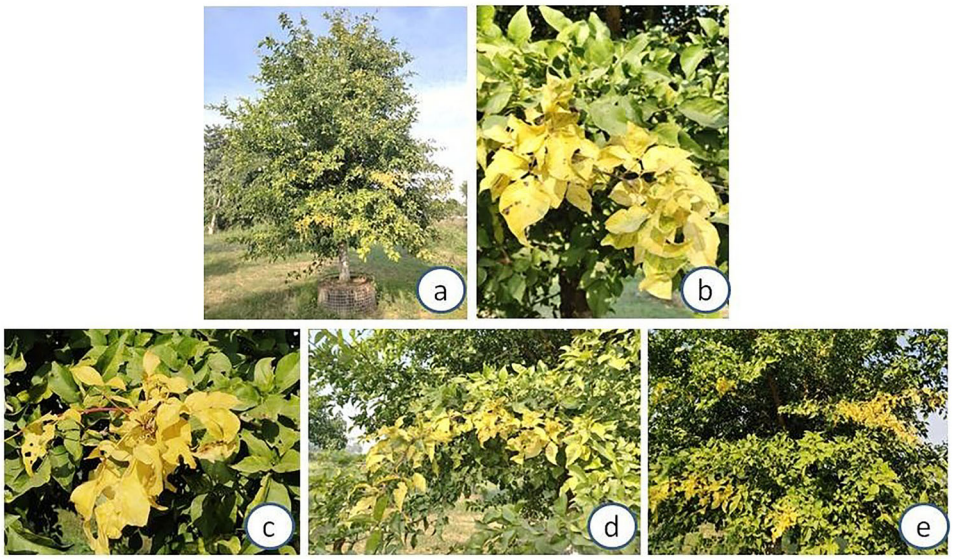


FIGURE 1 | Phytoplasma symptoms on *Aegle marmelos*: (a) branch of variety NB-5 showing leaf yellowing; (b) bright leaf yellows symptoms on variety NB-9 (close up); (c) leaf yellowing symptoms on variety NB-9; (d) side branch of variety NB-5 showing complete yellowing of leaves; and (e) several branches of variety NB-9 showing complete or partial yellowing of leaves.

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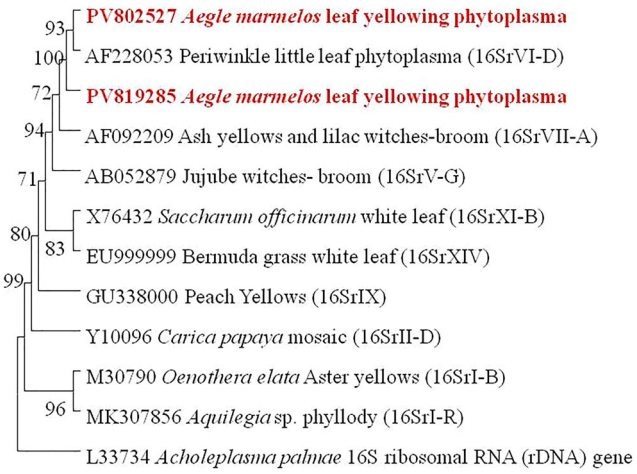


FIGURE 2 | Phylogenetic tree constructed by the maximum likelihood method based on 16S rRNA gene sequences of the *Aegle marmelos* leaf yellowing phytoplasma strain with selected phytoplasma strains from GenBank. Accession numbers are specified in the tree and *A. palmae* was used as an outgroup. Numbers on branches are bootstrap values obtained for 1000 replicates.

rRNA gene of brinjal little leaf phytoplasma (ON181948) and *secA* gene of *Prunus avium* witches' broom phytoplasma (OM037110) in the16SrVI group. The taxonomy of the *Amarmelos* phytoplasma isolate was supported by a phylogenetic tree analysis, which clustered the 16S rRNA and *secY* gene sequences of *A. marmelos* isolates with 'Ca. Ptrifolii'-related strains (16SrVI-D subgroup) from India and abroad (Figures 2 and 3).

Fruit crops in India have been reported to host six phytoplasma groups: 16SrI, 16SrII, 16SrV, 16SrVI, 16SrXI and 16SrXIV in apricot, almond, peach, papaya, sapota, sweet cherry, litchi, mango, plum and grapevines (Rao 2021). Previously, 'Ca. Paustralasiaticum', a 16SrII-D subgroup strain was reported to be associated with *A. marmelos* from Uttar Pradesh, India (S. K. Singh et al. 2024). This is the first report globally of *A. marmelos* as a natural host of a phytoplasma in the 16SrVI-D subgroup. The widespread occurrence of 16SrVI phytoplasma strain, across the diverse agro-climatic zones of India, raises immediate concern about this strain spreading further via infected vegetative saplings or through potential vectors to other economically important crops.



FIGURE 3 | Phylogenetic tree constructed by the maximum likelihood method based on *secY* gene sequences of *Aegle marmelos* leaf yellowing phytoplasma strain with selected phytoplasma strains from GenBank. Accession numbers are specified in the tree and *Acholeplasma oculi* was used as an outgroup. Numbers on branches are bootstraps values obtained for 1000 replicates.

*SecY*F1(VI)/*SecY*F2(VI)/*SecY*R1(VI) (Lee et al. 2010) in nested PCR assays. Nested PCR products, c. 1300 bp for 16S rRNA and 1700 bp for the *secY* gene, were amplified in all diseased samples. No amplifications were observed in any of the healthy and asymptomatic leaf samples from any of the tested varieties.

All three amplified 16S rRNA and *secY* gene fragments were purified, cloned and sequenced. The samples had identical sequences for both genes and two representative sequences of the 16S rRNA (GenBank Acc. Nos. PV819285 and PV802527), and *secY* genes (PV870205 and PX406233) were submitted to GenBank. BLASTn analyses of the 16S rRNA and *secY* gene sequences demonstrated 100% nucleotide identity with the 16S

Acknowledgements

The authors wish to express sincere thanks to the Head of Division of Plant Pathology and Director of the Indian Agricultural Research Institute for providing laboratory facilities.

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