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First Report of a '*Candidatus Phytoplasma Asteris*' Strain Associated With Mint Little Leaf Disease in India

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Mint (*Mentha arvensis*) is an aromatic and medicinal herb belonging to the family Lamiaceae which is commercially grown in India. During a field survey in Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India, in January 2026, we found mint plants with typical phytoplasma symptoms growing outdoors (Figure 1). These plants had characteristic little leaf

symptoms, including shortened internodes and stunted growth. The plants were approximately 2–3 months' old at the time of sampling, and had been sourced locally. Disease prevalence ranged from 3% to 5%.

Three diseased and two asymptomatic mint plants were collected and the CTAB method (Ahrens and Seemuller 1992) was used to



FIGURE 1 | Diseased *Mentha arvensis* (mint) plant showing symptoms of little leaf disease (left) and an asymptomatic plant (right).

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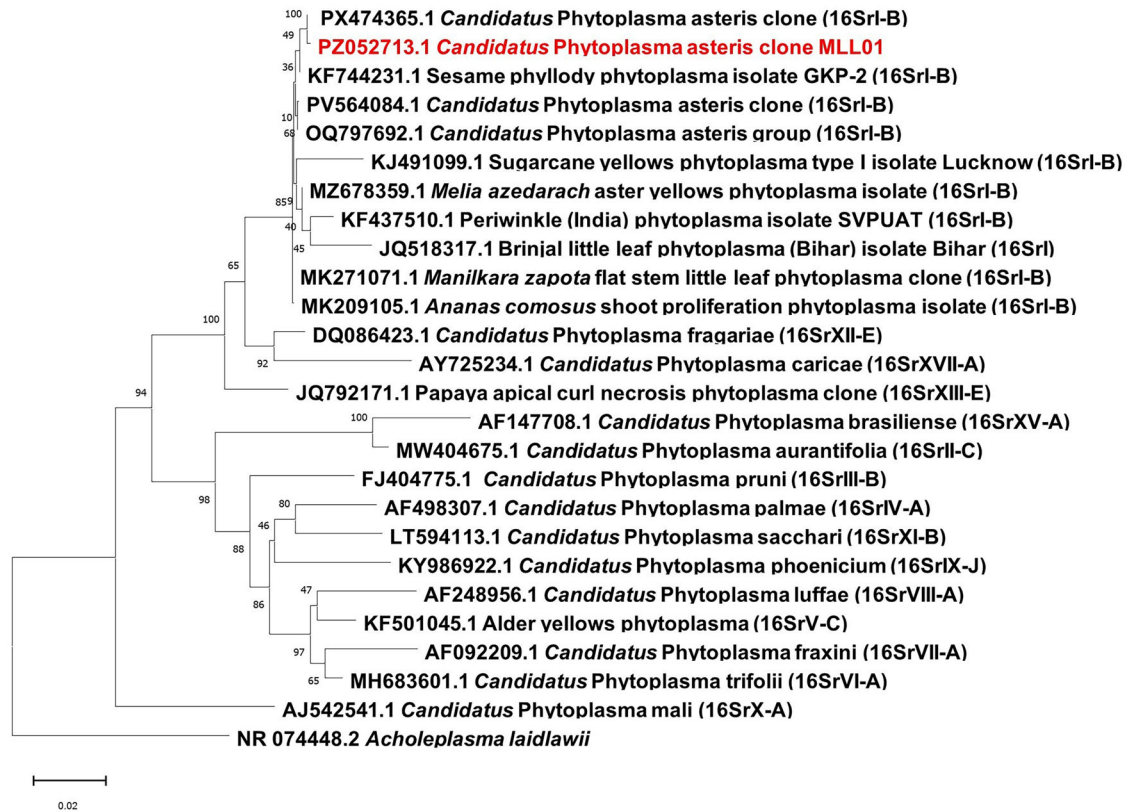


FIGURE 2 | Phylogenetic tree built using the maximum likelihood method from partial 16S rRNA gene sequences of the mint little leaf-associated phytoplasma isolate (MLL 01) and selected phytoplasma strains from GenBank. *Acholeplasma laidlawii* served as an outgroup for rooting the tree. The phytoplasma sequence from this study is marked in red. The bar indicates the number of nucleotide substitutions per site.

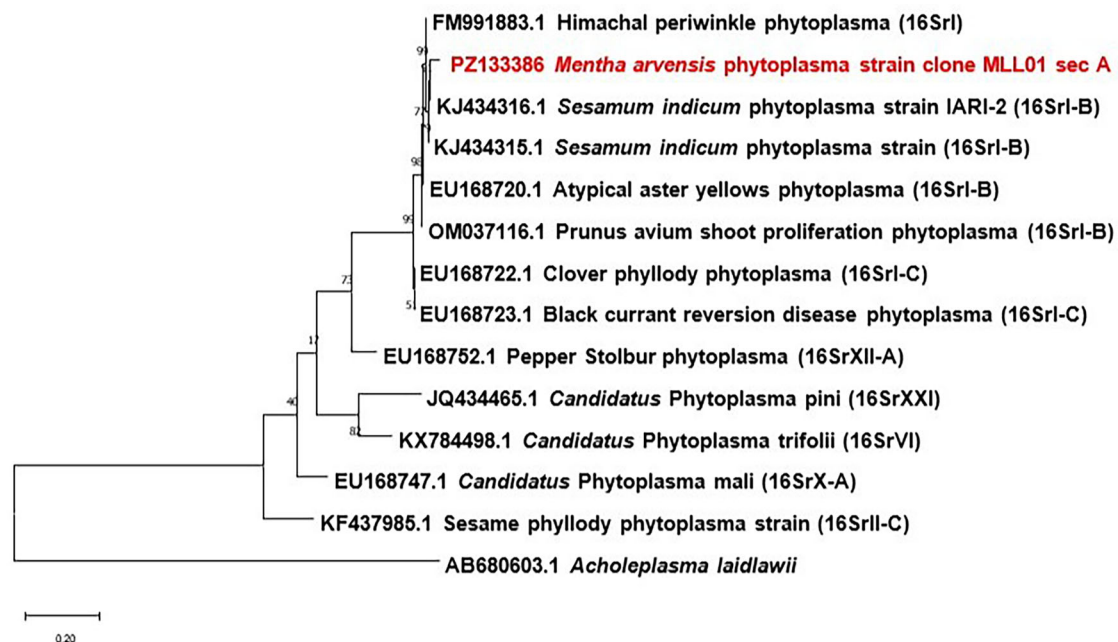


FIGURE 3 | Phylogenetic tree built using the maximum likelihood method from partial *secA* gene sequence of the mint little leaf-associated phytoplasma isolate and selected *secA* gene phytoplasma isolates from GenBank. *Acholeplasma laidlawii* served as an outgroup for rooting the tree. The phytoplasma sequence from this study is marked in red. The bar indicates the number of nucleotide substitutions per site.

harvest DNA from leaf midrib tissues. The 16S rRNA gene was amplified initially using a nested PCR technique with universal phytoplasma primer pairs P1/P7 and R16F2n/R2 (Lee et al. 1998) and the *sec A* gene was amplified using secfor1/secArev3 and secAfor2/secArev3 primer pairs in semi-nested PCR (Hodgetts et al. 2008). PCR amplicons of both 16S rRNA (c. 1.25 kb) and *sec A* (c. 450 bp) genes were obtained from all diseased plants but not asymptomatic plants. PCR products from one representative sample (MLL-01) were sequenced directly in both directions using the respective amplification primers without prior cloning (Barcode Biosciences, India). The sequences were assembled and deposited in GenBank under Accession Nos. PZ052713 (16S rRNA) and PZ133386 (*secA*). A BLASTn search showed > 99% nucleotide identity of the 16SrRNA gene with multiple strains of 'Ca. *P. asteris*', including 99.1% identity to the reference strain of 'Ca. *P. asteris*' belonging to 16SrI subgroup B (M30790). Further confirmation was obtained through *secA* gene analysis, which also supported the association of 16SrI-B phytoplasma with mint little leaf disease in India. Phylogenetic analysis using the maximum likelihood method based on partial 16S rRNA gene and *secA* gene sequences of the mint little leaf-associated phytoplasma along with selected phytoplasma strains from GenBank showed that isolate MLL 01 clustered closely with 'Ca. *P. asteris*' strains within the 16SrI-B subgroup (Figures 2 and 3).

Mentha piperita was previously reported to be infected with 'Ca. *P. solani*' (stolbur) phytoplasma in Serbia (Mitrović et al. 2016). 'Ca. *P. asteris*' (16SrI group; aster yellows phytoplasma) is one of the most widely distributed phytoplasmas in India, infecting diverse species, including economically important crops, ornamentals and weeds. In India, it has been associated with diseases in plants such as sesame (*Sesamum indicum*), brinjal (*Solanum melongena*), periwinkle (*Catharanthus roseus*), and sugarcane (*Saccharum officinarum*). Several reports have also documented its occurrence in northern regions of India, including Uttar Pradesh and adjoining states, indicating its widespread distribution in similar agro-climatic zones. The present finding further expands the host range of 'Ca. *P. asteris*' to *Mentha arvensis* in India. To our knowledge, this is the first report of phytoplasma disease caused by 'Ca. *P. asteris*' (16SrI-B) on mint (*Mentha arvensis*) worldwide.

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