

NEW DISEASE REPORT **OPEN ACCESS**

First Report of a '*Candidatus Phytoplasma trifolii*'-related Strain Infecting *Tridax procumbens*

S. Kumar | S. Mall

Molecular Plant Pathology Laboratory Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India

Correspondence: S. Mall (smriti.bot@ddugu.ac.in)**Received:** 31 December 2025 | **Revised:** 28 March 2026 | **Accepted:** 30 March 2026

Tridax procumbens (family Asteraceae) is a common weed found in and around agricultural fields as well as uncultivated areas in tropical and subtropical regions of the world. Approximately 10–15 *Tridax procumbens* plants grown at the Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, showed little leaf and yellowing symptoms during September 2025 (Figure 1).

Lee 1996). Amplicons of the expected size were obtained from diseased samples, while no amplification was obtained from symptomless samples. The amplicons were purified, sequenced and the consensus 16S rDNA sequence (1232 bp) was deposited in GenBank (Accession No. PX674680). The MEGA software version 12.1.2 (Kumar et al. 2024) was used to generate a phylogenetic tree

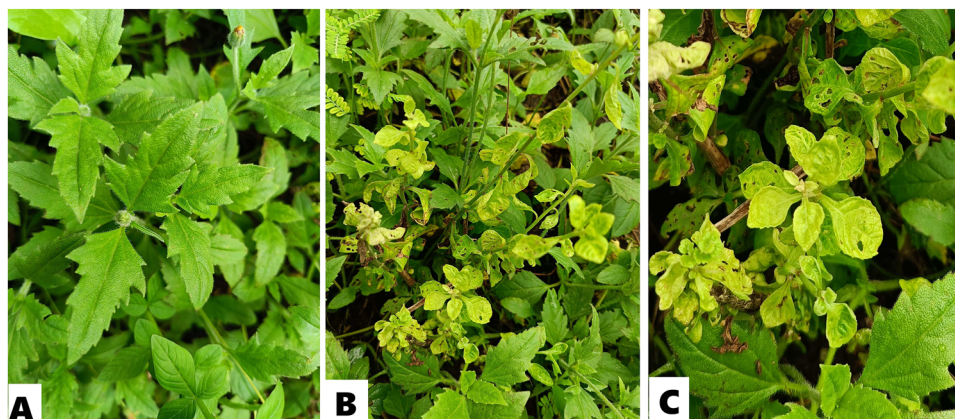


FIGURE 1 | (A) Symptomless plants of *Tridax procumbens*, and (B, C) diseased plants of *Tridax procumbens* showing little leaf and yellowing symptoms.

Three diseased and two symptomless samples were collected. Total genomic DNA was extracted from the leaf midrib tissues using a modified CTAB method (Ahrens and Seemüller 1992). DNA from symptomless *T. procumbens* plants served as a negative control. Phytoplasma detection was done by direct PCR amplification of the phytoplasma 16S rRNA gene with the universal primer pair P1/P7 (Seemüller et al. 1998), followed by a nested PCR using primers R16F2n/R16R2 (Gundersen and

using the neighbour joining method and *Acholeplasma laidlawii* as an outgroup (Figure 2A). BLAST analysis revealed 98.7% to 98.9% sequence identity with '*Candidatus Phytoplasma trifolii*' reference strains group 16SrVI, formerly the Clover proliferation group. In addition, virtual RFLP analysis performed on the 16S rDNA sequence of the *T. procumbens* phytoplasma and 16SrVI strains using iPhyClassifier showed restriction profiles consistent with the 16SrVI-D group, corresponding to a similarity coefficient

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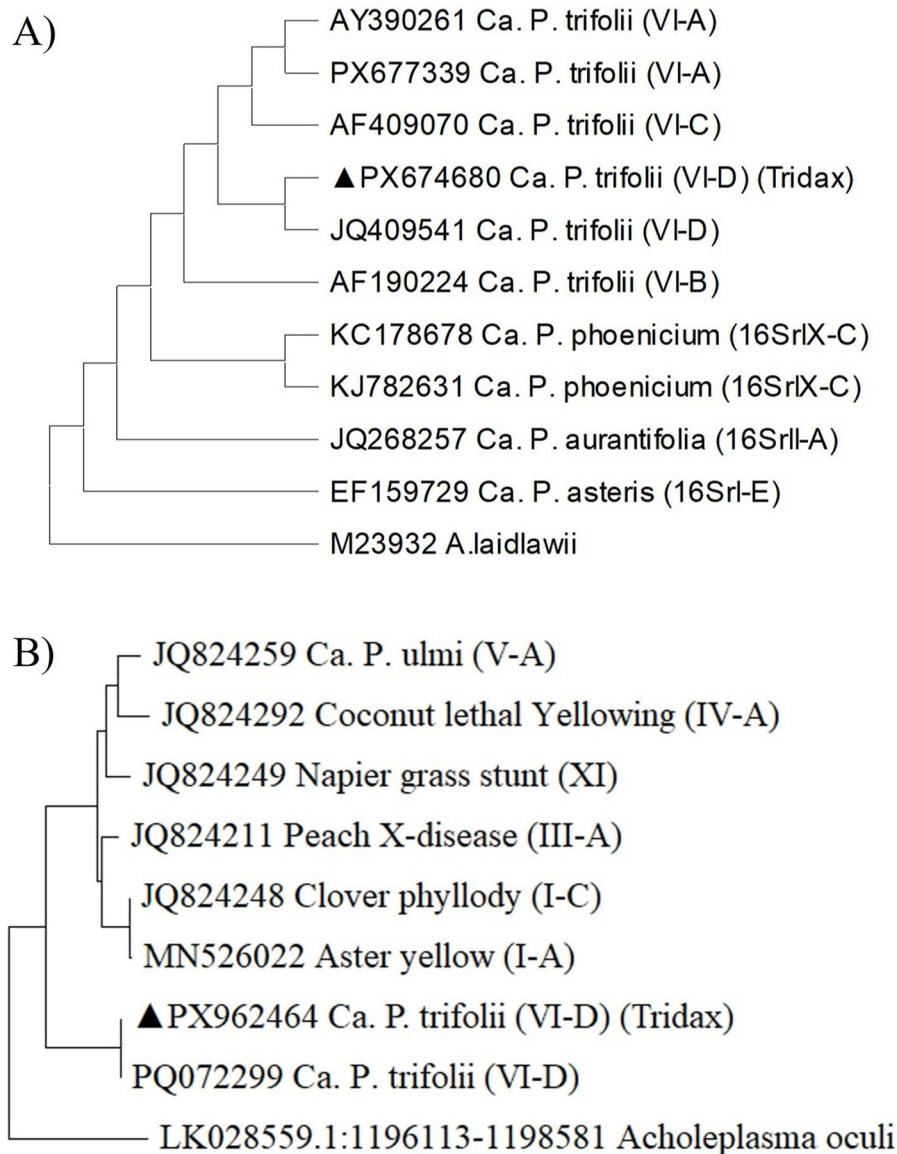


FIGURE 2 | Phylogenetic tree based on (A) 16S rRNA and (B) *tuf* gene sequences of the *Tridax procumbens* phytoplasma and reference phytoplasmas.

of 0.98. To further support this identification, the *tuf* gene was amplified from diseased samples using the fTufl/rTufl primer pair (Schneider, and Gibb, 1997), sequenced and deposited in GenBank (PX962464). The resulting *tuf* sequence had 100% identity with '*Ca. P. trifolii*' sequences available in GenBank, and a phylogenetic analysis confirmed assignment to the 16SrVI-D subgroup (Figure 2B).

The 16SrVI-D phytoplasma subgroup, mainly transmitted by the leafhopper *Hishimonas phycitis*, is widespread throughout India causing diseases in several weed, ornamental and vegetable crops, including *Capsicum chinense*, *Daucus carota*, *Lactuca sativa*, *Portulaca oleracea* and *Solanum melongena* (Tiwari et al. 2023). The detection of a *Ca. P. trifolii*-related strain in *T. procumbens*, a pantropical weed species, suggests its potential epidemiological role in phytoplasma disease cycles.

To our knowledge, this study represents the first association of a '*Ca. P. trifolii*'-related strain infecting *T. procumbens* worldwide. Further studies are required to identify insect vectors involved in transmission and to assess the role of *T. procumbens* as a natural reservoir contributing to the spread of phytoplasma-associated diseases.

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