



First report of phytoplasma '*Candidatus Phytoplasma trifolii*' (16Sr VI) group associated with leaf yellows of *Calotropis gigantea* in India

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Calotropis gigantea (family Asclepiadaceae), commonly known as milkweed, is a common wasteland weed. It is traditionally used in India to treat fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhoea. Symptoms of severe leaf yellowing were noticed on *C. gigantea* plants (Fig. 1) growing widely along the roadsides at Gorakhpur, Uttar Pradesh, India during January 2010. To investigate the possibility of phytoplasma infection, total DNA was isolated from leaf tissues of infected and healthy *C. gigantea* plants using the phytoplasma enrichment procedure (Ahrens & Seemüller, 1992). Nested PCR was performed using universal primers specific to the phytoplasma 16S rRNA gene: P1/P6 (Deng & Hiruki, 1991) and R16F2n/R16R2 (Gundersen & Lee, 1996). Expected size amplicons of ~1.5 kb and ~1.2 kb, respectively, were obtained from infected samples (2/2) but not from healthy plants. The amplicon of ~1.2 kb was eluted through the PCR Clean-up System (Promega, USA) and ligated into pGEM-T Easy Vector System (Promega, USA). Two clones were sequenced and the consensus sequence data of both clones were deposited in GenBank (Accession No. HM485690). BLAST analysis of the partial 16S rDNA sequence of the *C. gigantea* phytoplasma (HM485690) revealed the highest sequence identity (99%) with that of the *Datura innoxia* phytoplasma (EU573925), a member of the '*Ca. Phytoplasma trifolii*' (16SrVI) group (Raj *et al.*, 2009). Phylogenetic analysis of the *C. gigantea* phytoplasma 16S rDNA sequence with those of 16SrVI phytoplasma isolates and other phytoplasma groups (MEGA version 4.0) revealed its closest phylogenetic relationship with members of the 16SrVI group (Fig. 2). The *C. gigantea* phytoplasma was identified as a '*Ca. Phytoplasma trifolii*' (16SrVI) isolate.

The results suggest that *C. gigantea* may be a reservoir for '*Ca. P. trifolii*'. The fact that very closely related '*Ca. Phytoplasma trifolii*' isolates have been identified in *C. gigantea* and previously in *D. innoxia*, suggests that in nature, these two hosts could have possible epidemiological implications for natural spread of '*Ca. P. trifolii*' by suitable vectors, particularly when growing nearby cropping areas. '*Ca. P. trifolii*' group has also been reported from India in *Withania somnifera* (Samad *et al.*, 2006) and

Portulaca grandiflora (Samad *et al.*, 2008). However, the association of '*Ca. P. trifolii*' with leaf yellows of *C. gigantea* found in Gorakhpur, Uttar Pradesh, India is a first report.

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Figure 1

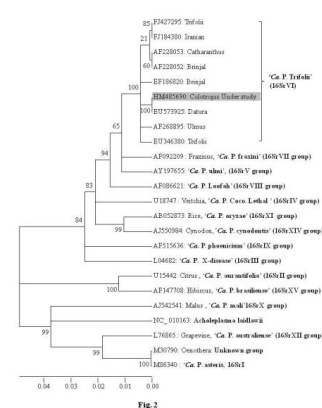


Figure 2

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