



First report of a '*Candidatus Phytoplasma asteris*' associated with little leaf disease of *Ageratum conyzoides* in India

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Received: 02 Feb 2012. Published: 05 Nov 2012. Keywords: weed, direct PCR, nested PCR, BLASTn, 16SrI

Phytoplasmas are cell wall-lacking mollicutes found associated with plant diseases in a wide range of plant species ranging from grasses to trees (Al-Saad & Khan, 2006). *Ageratum conyzoides* (family Asteraceae) commonly known as goat weed, is a globally occurring noxious weed with serious ecological and economic impacts. In India, it is found widely growing on roadsides, railway tracks, fallow fields, arable and other land. Control is often difficult due to its high potential rate of seed production. *A. conyzoides* has been reported as host of pathogens associated with important crop diseases (Pabitra *et al.*, 1997; Raj *et al.*, 2011), demonstrating its potential to harbour different pathogens under natural conditions. During a survey in 2011 in Shahjahnapur district of UP, India, a number of *A. conyzoides* plants growing nearby sugarcane fields were found to be exhibiting little leaf symptoms accompanied with yellowing of leaf lamina, possibly characteristic of phytoplasma-associated disease.

Leaf samples from 10 such symptom-bearing and two apparently symptomless plants were collected and total genomic DNA was extracted from ~100 mg leaf tissues employing a phytoplasma DNA enrichment protocol (Ahrens & Seemüller, 1992). Direct PCR was carried out with universal primers P1/P6 (Deng & Hiruki, 1991) followed by nested PCR with primers R16F2n/R16R2 (Gundersen & Lee, 1996), specific to the 16S rRNA gene. Consequently, DNA fragments of ~1.5 and ~1.2 kb were respectively amplified in all symptom-bearing plants. No PCR products were evident when DNA extracted from symptomless plants was used as template. Three amplicons of ~1.2 kb were sequenced bidirectionally and the nucleotides sequenced. Consensus sequence data were submitted to GenBank (Accession No. JQ446367). BLASTn analysis of the sequence data was compared with sequences available in GenBank, showing a maximum of 97% identity with 16S rRNA gene of Hibiscus yellows and little leaf (FJ939287), Plum little leaf (GU289674), Oilseed rape virescence (HM590625), Gladiolus witches'-broom (HM590619), Diplotaxis virescence (HM590618), American aster yellows (HM590617), and Sesame phyllody (AB558132); all members of the 16SrI '*Candidatus Phytoplasma asteris*' group. In phylogenetic and molecular evolutionary analyses conducted using MEGA version 4.0, the *A. conyzoides* phytoplasma isolate clustered in a clade with the members of 16SrI group and showed only distinct relationships with phytoplasmas belonging to other 16S rRNA groups (Fig. 1).

These findings confirmed that the *A. conyzoides* phytoplasma is an isolate of 16SrI group (aster yellows), and that it is associated with little leaf disease. Since *A. conyzoides* has been reported as a weed host of virus and bacteria that induce diseases in commercial crops in India (Pabitra *et al.*, 1997; Raj *et al.*, 2011), it is possible that it may play a role as a reservoir of phytoplasmas that infect agronomically important species. To the best of our knowledge, this is the first report of a '*Ca. P. asteris*' associated with little leaf disease of *A. conyzoides* in India.

Acknowledgements

The authors are thankful to Dr. M. L. Sharma, the Director of UPCSAR, Shahjahnapur, UP, India, for financial support. The authors are also grateful to KU (Konkuk University) Brain Pool Programme for providing a fellowship to M.S. Khan in 2012.

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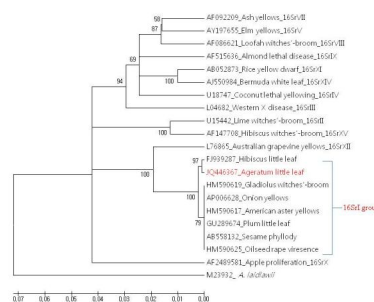


Figure 1

To cite this report: Tiwari AK, Vishwakarma SK, Singh SP, Kumar P, Khan MS, Chun SC, Rao GP, 2012. First report of a '*Candidatus Phytoplasma asteris*' associated with little leaf disease of *Ageratum conyzoides* in India. *New Disease Reports* **26**, 18. [http://dx.doi.org/10.5197/j.2044-0588.2012.026.018]

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