

Molecular identification of '*Candidatus Phytoplasma asteris*' associated with little leaf disease of *Chrysanthemum morifolium*

S. K. Raj^{A,B}, M. S. Khan^A and S. Kumar^A

^AMolecular Virology, National Botanical Research Institute, Lucknow 226001, India.

^BCorresponding author. Email: skraj2@rediffmail.com

Abstract. Association of '*Candidatus Phytoplasma asteris*' with little leaf disease of *Chrysanthemum morifolium* was detected by nested PCR and identified by nucleotide sequence analysis of PCR products for the first time from India.

Chrysanthemum morifolium (family Asteraceae) is an important ornamental plant grown in pots and gardens for its beautiful blooms of various colours, sizes and extensive vase life. Various pathogens such as fungi, viruses, viroids and phytoplasma severely affect chrysanthemum cultivation worldwide (Bouwen and van Zaayen 1995). Natural occurrence of little leaf disease on several *C. morifolium* plants was observed in gardens and nurseries at Lucknow, India during winter of 2005–06. The symptoms were excessive proliferation, tiny narrow leaves and shortening of internodes, which altogether gives rise to 'witches'-broom' appearance (Fig. 1). Since chrysanthemums are propagated through suckers or cuttings and the phytoplasma is known to be transmitted by vegetative propagation through cuttings (Bhat *et al.* 2006), the identification of the causal agent of the little leaf disease of *C. morifolium* was attempted.

To investigate the possibility of a phytoplasma causal agent, the total DNA was isolated from leaf tissue of infected and healthy *C. morifolium* plants following the protocol of Ahrens and Seemüller (1992). The initial PCR was performed using P1/P6 universal primers specific to the 16S rRNA gene (Deng and Hiruki 1991). Further, nested PCR was

carried out with primers R16F2n/R16R2 (Gundersen and Lee 1996) employing the initial PCR product as template. Agarose gel electrophoresis of direct and nested PCR products obtained with 16S rRNA-gene-specific primers resulted in the expected size DNA fragments of ~1.5 kb and ~1.4 kb respectively, from infected plant samples but not from healthy samples. The 1.4-kb amplicon obtained from the nested PCR was cloned, sequenced and the data deposited in GenBank (Accession number DQ431842). BLAST search analysis of DQ431842 revealed 99% sequence similarity with the 16S rRNA gene of Ash witches'-broom, Maize bushy stunt, Dogfennel yellows, *Epilobium* phyllody, Onion yellows and Aster yellows phytoplasmas (Accession numbers AY566302, AY265208, DQ381534, AY101386, AP006628 and AY265209), respectively, belonging to the '*Candidatus Phytoplasma asteris*' (16S rI) group.

A literature survey revealed reports of *Chrysanthemum* witches'-broom phytoplasma on *C. coronarium* in Japan (Okuda *et al.* 1997), *Chrysanthemum* yellows phytoplasma on *C. frutescens* in Italy (Bertaccini *et al.* 1990) and Aster yellows phytoplasma on *C. coronarium* in China (Zhong and Shen 2004),



Fig. 1. Naturally infected *Chrysanthemum morifolium* plant showing little leaf symptoms (left), compared with a healthy plant (right).

infecting chrysanthemum cultivars, but to the best of our knowledge, the association of '*Candidatus Phytoplasma asteris*' with little leaf disease of chrysanthemum is the first report of this kind from India.

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