First report of *Mirabilis* and *Chrysanthemum* little leaf associated with ‘*Candidatus* Phytoplasma aurantifolia’ in India

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Abstract *Mirabilis jalapa* and *Chrysanthemum* sp. plants showing little leaf symptoms were observed during 2010 in New Delhi, India. Nested-PCR assays using phytoplasma specific 16S ribosomal DNA primers resulted in amplicons with sequence similarity to members of 16SrII group. Phylogenetic analysis confirmed this finding by showing the relatedness of both the little leaf phytoplasmas from the current study with previously reported 16SrII members. This is first report of *M. jalapa* and *Chystanthemum* sp. as hosts of a 16SrII (Peanut witches' broom) group phytoplasma from India.

Keywords Phytoplasma · Mollicutes · Nested-PCR · Phylogeny

Mirabilis jalapa (4o' clock plant), a member of Nyctagina-ceae, is valued as an ornamental and used as laxative, antispasmodic (Aoki et al. 2008). Ethanolic extracts of *M. jalapa* plant, proteins from roots and seeds are endowed with antimicrobial properties. *Chrysanthemum* sp. are especially valued for their ornamental flowers. Both the *M. jalapa* and *Chystanthemum* plants showing symptoms of little leaf disease (Fig. 1a, b) were observed in gardens during August 2010 at New Delhi, India. Additional symptoms were observed on *M. jalapa* such as crowding of leaves in the apical region, shortening of internodes, small sized leaves and flowers accompanied by stunting of plants. These symptoms are commonly associated with phytoplasmas, which are a group of wall-less bacteria (the Order

Mollicutes) that may be transmitted by phloem sap feeding insects of Order Hemiptera. To test the possibility of a phytoplasma association with the phenotypes of the observed diseases, DNA was isolated from the stem tissues

Fig. 1 Healthy (left) and diseased (right) *Mirabilis jalapa* (a) and *Chrysanthemum* sp. (b) plants showing little leaf symptoms

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of five affected plants and two symptomless ones each of *M. jalapa* and *Chrysanthemum* sp. as per Saghai-Maroo et al. (1984). The samples were subjected to nested-PCR using phytoplasma specific universal 16S rDNA primer pair P1/P7 (Schneider et al. 1995) for the first round of PCR followed by the nested primer pair R16F2/rU3 for the second round of PCR. PCR products from first round were diluted in 1:30 ratio and used as the template in the second round. Amplicons were resolved on 1.2 % agarose gel. The characteristic band of desired size (~1.2 kb) was obtained only with the symptomatic samples. On the other hand, symptomless plants gave no amplification. The amplicons were gel extracted (QIAquick gel extraction kit, QIAGEN, USA) and sequenced bidirectionally using BigDye terminator v3.1 kit, ABI Prism 3130XL (PE Applied Biosystems, Foster City, CA, USA). Sequence comparisons via BlastN (Altschul et al. 1997) searches revealed 99 % sequence similarity with members of 16SrII (Peanut witches'-broom group) phytoplasmas viz. eggplant phyllody (HQ423156.1), pelargonium phytoplasma (HM583340.1), sesame phyllody

(HM449957.1) and chickpea phyllody phytoplasma (FJ870549.1). The partial sequence for the 16S rDNA of *Mirabilis jalapa* little leaf (MJLL) phytoplasma and *Chrysanthemum* little leaf (CLL) phytoplasma was submitted to GenBank (Accession No. JQ268255 and JQ268257, respectively). The isolates were identical to each other. Phylogeny was performed by construction of evolutionary tree (MEGA version 4.01) using sequences from earlier reported phytoplasmas (Fig. 2). Sequences were aligned by CLUSTAL X and trimmed to the length of the two phytoplasma sequences being analyzed in the current study. Maximum parsimony method was used for tracing evolutionary relationships. The outgroup chosen for rooting was *Acholeplasma palmae* (L33734). Both MJLL and CLL phytoplasma grouped closest to the 16SrII phytoplasma cluster, depicting their relatedness, however showed a divergent clade with 87 % bootstrap consensus. Further studies like restriction profiling and use of other loci are required to strengthen this finding.

Earlier reports have shown phytoplasma of 16SrII group to be associated with yellowing and internode shortening of *M.*

Fig. 2 Dendrogram depicting evolutionary relationships of *Mirabilis jalapa* little leaf phytoplasma and *Chrysanthemum* little leaf phytoplasma with respect to other reference phytoplasmas downloaded from GenBank

jalapa in Northern Israel (Sobolev et al. 2007). *Chrysanthemum* virescence has been associated with ‘*Candidatus* Phytoplasma aurantifolia’ (Takashi et al. 2007), while yellowing disease in this plant was commonly shown to be linked with 16SrI group phytoplasmas (Lee et al. 2004; Min et al. 2009). Such variations observed in symptoms could be due to different phytoplasma strains affecting the host plant. Additionally, due to the widespread plantation of these ornamental plants, they can act as reservoirs of phytoplasma and spread them to still un-invaded territories. So far, this is first record of *M. jalapa* and *Chrysanthemum* sp. as hosts of phytoplasma from the Indian region.

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