



## *Mussaenda erythrophylla*: A new host of ‘*Candidatus* Phytoplasma asteris’ in India

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### Abstract

Leaf yellowing symptoms were observed in *Mussaenda erythrophylla* at Digiya Park, Jammu during June 2016. Leaf yellowing and flat stem symptoms were also observed on nearby planted *Catharanthus roseus* and *Celosia argentea*, respectively. Amplicons of ~ 1.25 kb were consistently amplified in nested PCR assays with DNA extracted from all the symptomatic *Mussaenda erythrophylla*, *Catharanthus roseus* and *Celosia argentea* samples using primer pairs P1/P7 followed by R16F2n/R2. However, no amplifications were achieved with any of the asymptomatic plant samples. Pair wise sequence comparison of the ~ 1.25 kb products of 16S rDNA sequence of *M. erythrophylla* leaf yellowing, *C. roseus* leaf yellowing and *C. argentea* flat stem phytoplasma isolates revealed 99.8% sequence identity with ‘*Candidatus* Phytoplasma asteris’ related strains. Phylogeny and virtual RFLP analysis of 16S rDNA sequences confirmed the affiliation of ‘*Ca. P. asteris*’ subgroup I-B with all the three symptomatic plant samples. This is the first report of association of ‘*Ca. P. asteris*’ subgroup I-B with *M. erythrophylla* worldwide.

**Keywords** Periwinkle · Plumed cockscomb · Red flag bush · 16SrI-B phytoplasma subgroup

*Mussaenda erythrophylla* Schumach & Thonn. (fam: Rubiaceae), commonly known as red flag bush is an evergreen tropical ornamental shrub valued for its colorful bracts of different shades, including red, rose, white, pale pink or some mixtures of color (Sheat and Schofield 1995). During a survey in 2016, *M. erythrophylla*, *Catharanthus roseus* and *Celosia argentea* plants with leaf yellowing and flat stem symptoms were observed in Digiya Park, Jammu, India. DNA was extracted from leaf midrib of two symptomatic and asymptomatic plants each of *M. erythrophylla*, *C. roseus* and *C. argentea* by CTAB method (Ahrens and Seemüller 1992).

DNA from sesame phyllody phytoplasma (16SrI group) (GenBank accession no. KC920747) was used as positive control. The extracted DNA from the asymptomatic *Mussaenda*, *Catharanthus* and *Celosia* plant samples were taken as negative controls. PCR reactions were performed in mastercycler (Eppendorf Germany) and the cycling protocol was used as described earlier (Panda et al. 2019) using universal primer pair P1/P7 (Deng and Hiruki 1991; Schneider et al. 1995) followed by nested primer pair R16F2n/R2 (Gundersen and Lee 1996). The PCR product was diluted with nuclease free water in 1:20 dilution and 2 µl were used as template in the nested PCR assays (Panda et al. 2019). Nested PCR products was subjected to electrophoresis in 1.0% (w/v) agarose gel, stained with GoodView™ Nucleic Acid stain (BR Biochem Life Sciences Pvt. Ltd., India) and observed under UV transilluminator. The ~ 1.25 kb fragments obtained in nested PCR assay were purified using the Wizard® SV Gel and PCR Clean-up System (Promega, Madison, USA) and sequenced in both directions at Eurofins Genomics India Pvt. Ltd, Bengaluru, Karnataka, India. DNA Baser V.4 (<http://www.dnabaser.com>) online tool was used to assemble the 16Sr DNA sequences obtained from PCR products. Multiple alignment of the sequences along with representative phytoplasma stains obtained from GenBank

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was done by using ClustalW software (Hall 1999) and the consensus phytoplasma sequences were submitted to the GenBank. A phylogenetic tree of 16S rDNA sequences was constructed using the neighbor-joining method 1000 bootstrap replications using MEGA 7.0 (Kumar et al. 2016). The 16S rDNA sequence of *Acholeplasma laidlawii* was used as out group to root the tree. The ~1.25 kb 16S rDNA sequences of phytoplasma isolates subjected to in silico RFLP comparison analysis using the *iPhyClassifier* online tool (Zhao et al. 2009).

An amplification of ~1.25 kb was consistently obtained in all symptomatic *Mussaenda erythrophylla* leaf yellowing (MELY), *Catharanthus roseus* leaf yellowing (CRLY) and *Celosia argentea* flat stem (CAFS) samples along with the positive control in nested PCR assay using primer pairs P1/P7 followed by primer pairs R16F2n/R2, but not in the asymptomatic plant samples (data not shown). All the amplified nested PCR products were sequenced in both directions and consensus regions containing 1248 bp of MELY (Acc.

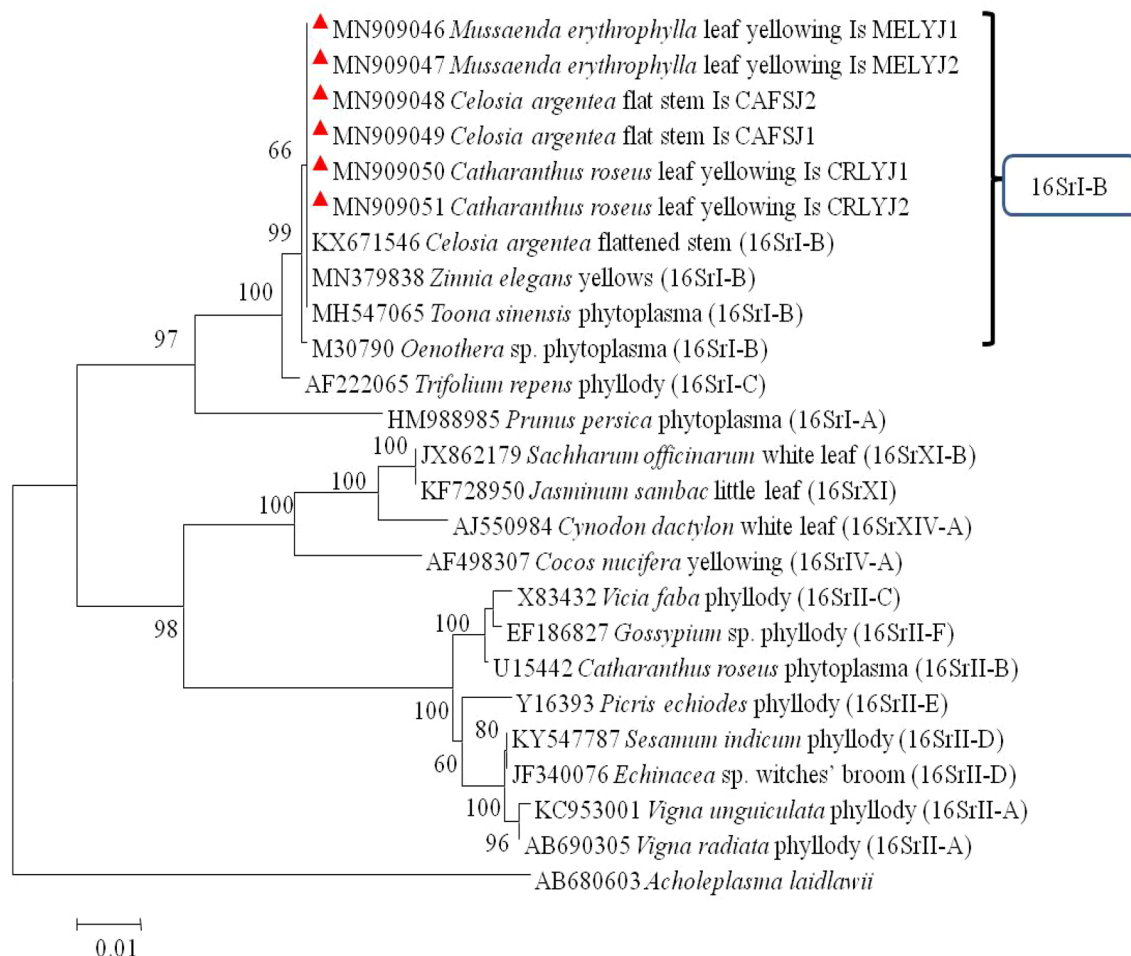
nos. MN909046, MN909047), CRLY (Acc. nos. MN909048, MN909049) and CAFS (Acc. nos. MN909050, MN909051) isolates were deposited in the GenBank (see Fig. 1).

BLAST and pair wise sequence comparison analysis R16F2n/R2 region of 16S rDNA sequence of MELY, CRLY and CAFS phytoplasma isolates showed 100% sequence identity among themselves and 99.8% with '*Candidatus* Phytoplasma asteris' related strains of CAFS (Acc. no. KX671546), *Zinnia elegans* leaf yellows (Acc. no. MN379838) and *Toona sinensis* witches' broom phytoplasma (Acc. no. MH547065).

In phylogenetic analysis, MELY, CRLY and CAFS isolates clustered together with previously identified '*Ca. P. asteris*' related strains of *C. argentea* flat stem, *Z. elegans* yellows and *T. sinensis* witches' broom (Fig. 2). The ~1.25 kb 16S rDNA sequences generated restriction profiles using 17 restriction endonucleases from sequences of MELY, CRLY and CAFS phytoplasma isolates produced identical restriction profiles as of '*Ca. P. asteris*' 16SrI-B subgroup reference

**Fig. 1** Leaf yellowing disease symptom on **a** *Mussaenda erythrophylla*; **b** *Catharanthus roseus* and flat stem disease symptom on **c** *Celosia argentea* plants





**Fig. 2** Phylogenetic relationship of *Mussaenda erythrophylla* leaf yellowing, *Catharanthus roseus* leaf yellowing and *Celosia argentea* flat stem phytoplasma strains (Red triangles) from India and other phy-

toplasmas. Accession numbers are specified in the tree. MEGA 7.0 software was used to construct the tree by neighbor-joining method

strain (GenBank accession number M30790) (Fig. 3) and were therefore classified in the 16SrI-B subgroup.

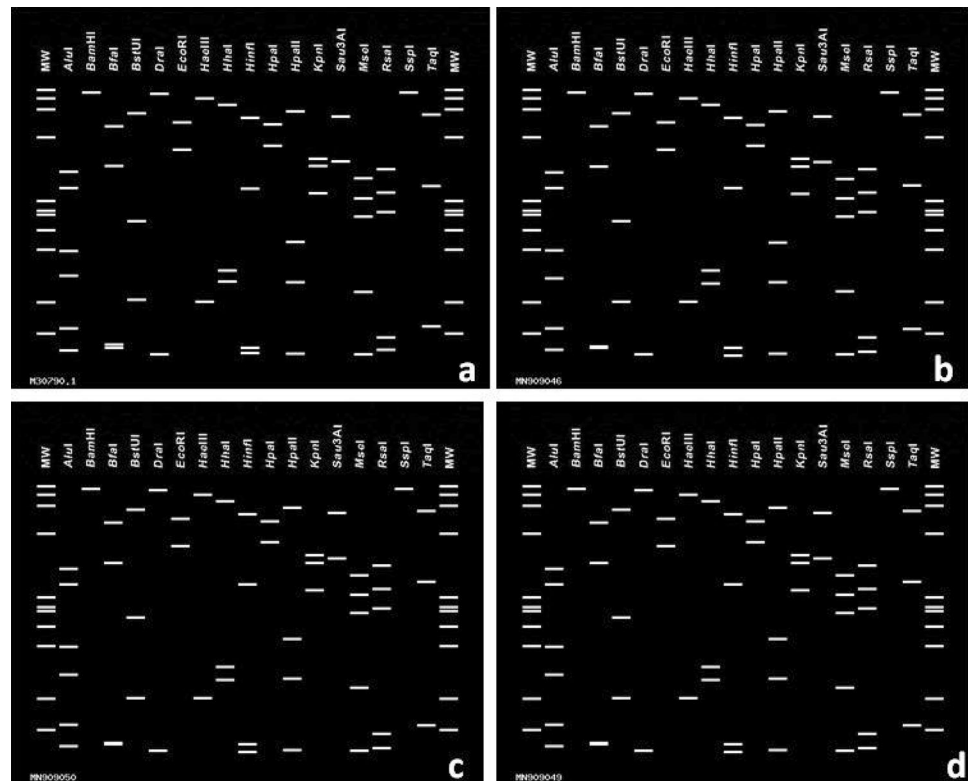
Over 90 ornamental plants species are reported to infect by phytoplasmas strain belong to 14 different 16S ribosomal groups and 30 subgroups causing significant economic losses worldwide (Bellardi et al. 2018). However, six phytoplasma groups (16SrI, II, VI, IX, XI, and XIV) have been identified in 34 ornamental species from seven states of India (Madhupriya and Rao 2017). Phytoplasma strains belonging to 16SrII-C and 16SrII-D subgroups have been reported as widespread infecting ornamental plants in India (Rao et al. 2017). However, 16SrI (aster yellows) is the most wide prevalent group and has been recorded associated with 64 plant species in India (Rao et al. 2017). Earlier reports had confirmed the association of 16SrIII-J, 16SrI-M, 16SrI-B and 16SrII-D subgroup phytoplasma associated with *C. argentea* in Brazil, Iran and India (Eckstein et al. 2012; Aldaghi and Bertaccini 2015; Madhupriya et al. 2017). *C. roseus* is known to be infected by 16SrI group

phytoplasma in Argentina, Egypt, India and Italy (Torres et al. 2004; Omar et al. 2008; Chaturvedi et al. 2009; Parrella et al. 2014). Among the ornamental species, so far phytoplasma have been recorded only from two plant species in Rubiaceae family, such as *Gardenia jasminoides* in China (Sun and Zhao 2012) and *Ixora coccinea* in Puerto Rico. In the present study, we have reported *M. erythrophylla*, belonging to family Rubiceae, as a new host of 16SrI-B phytoplasma subgroup in world.

The identification of two more hosts of ‘*Ca. P. asteris*’ (16SrI-B) as *C. roseus* and *C. argentea* along with *M. erythrophylla* in the study suggested the widespread nature of this phytoplasma strain in study area and indicated the possibility of availability of insect vectors, which would be responsible for transmission of phytoplasma from one source to other, which needs further investigation.

The scenario of natural wider phytoplasma strain spread of 16SrI-B subgroup infecting several crop and weed species and capable of natural transmission through leafhopper

**Fig. 3** Virtual RFLP patterns derived from in silico digestion of ~1.25 kb 16S rDNA sequence of MELY, CAFS, CRLY phytoplasma strains and the phytoplasma reference strain with 17 restriction enzymes using the *iPhyClassifier* program. The patterns are: **a** 16SrI-B reference (*Oenothera* sp.) (acc. no. M30790), **b** *Mussaenda erythrophylla* leaf yellowing phytoplasma isolate 1 (acc. no. MN909046), **c** *Catharanthus roseus* leaf yellowing phytoplasma isolate 1 (acc. no. MN909050), **d** *Celosia argentea* flat stem phytoplasma isolate 1 (MN909049)



vectors in India, poses a serious threat to several agricultural and horticultural crop being grown in Jammu. The addition of a new host of 16SrI-B subgroup of phytoplasma in list from Jammu region of India would be of great significance and needs further study of its sources of spread under natural condition.

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## References

- Ahrens U, Seemüller E (1992) Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. *Phytopathology* 82:828–832
- Aldaghi M, Bertaccini A (2015) Preliminary study on some ornamental plant phytoplasma diseases in north of Iran. *Phytopathog Mollicutes* 5:67–68
- Bellardi MG, Bertaccini A, Madhupriya, Rao GP (2018) Phytoplasma disease in ornamental crops. In: Rao GP, Bertaccini A, Fiore N, Liefting LW (eds) *Phytoplasmas: plant pathogenic bacteria-I*. Springer, Singapore, pp 191–233
- Chaturvedi Y, Tewari AK, Upadhyaya PP, Prabhuji SK, Rao GP (2009) Association of ‘*Candidatus Phytoplasma asteris*’ with little leaf and phyllody disease of *Catharanthus roseus* in Eastern Uttar Pradesh, India. *Med Plants* 1:103–108
- Deng S, Hiruki C (1991) Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. *J Microbiol Methods* 14:53–61
- Eckstein B, Gonçalves Da Silva E, Paulo Bedendo I (2012) Shoot proliferation and leaf malformation of *Celosia argentea* and *Celosia spicata* caused by a phytoplasma of the 16SrIII-J group. *J Phytopathol* 160:206–208
- Gundersen DE, Lee IM (1996) Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathol Mediterr* 35:144–151
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Madhupriya, Rao GP (2017) Phytoplasma diseases of ornamental plants: an Indian overview. *J Ornament Hortic* 20:87–102
- Madhupriya, Yadav A, Thorat V, Rao GP (2017) Molecular detection of 16SrI-B and 16SrII-D subgroups of phytoplasma associated with flat stem and witches’ broom disease of *Celosia argentea* L. *3 Biotech* 7:311
- Omar AF, Emeran AA, Abass JM (2008) Detection of phytoplasma associated with periwinkle virescence in Egypt. *Plant Pathol J* 7:92–97
- Panda P, Nigam A, Rao GP (2019) Multilocus gene characterization of phytoplasmas in 16SrII-D subgroup associated with *Coreopsis grandiflora* little leaf disease in India. *Phytopathogenic Mollicutes* 9(2):295–301
- Parrella G, Paltrinieri S, Contaldo N, Vitale MR, Bertaccini A (2014) Characterization of ‘*Candidatus Phytoplasma asteris*’ strains associated with periwinkle virescence in Southern Italy. *Phytopathogenic Mollicutes* 4:53–58

- Rao GP, Madhupriya, Thorat V, Manimekalai R, Tiwari AK, Yadav A (2017) A century progress of research on phytoplasma diseases in India. *Phytopathogenic Mollicutes* 7:1–38
- Schneider B, Seemüller E, Smart CD, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasma. In: Razin S, Tully JG (eds) *The molecular and diagnostic procedures in mycoplasmaology*, 1. Academic Press, San Diego, pp 369–380
- Sheat B, Schofield G (1995) *Complete gardening in Southern Africa*. Struik Publisher, Cape Town, p 76
- Sun XC, Zhao WJ (2012) First report of a group 16SrI-B phytoplasma associated with *Gardenia jasminoides* in China. *Plant Dis* 96:1576
- Torres L, Galdeano E, Docampo D, Conci L (2004) Characterization of an aster yellows phytoplasma associated with *Catharanthus* little leaf in Argentina. *J Plant Pathol* 86:209–214
- Zhao Y, Wei W, Lee M, Shao J, Suo X, Davis RE (2009) Construction of an interactive online phytoplasma classification tool, *iPhyClassifier*, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *Int J Syst Evol Microbiol* 59:2582–2593

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