Bulletin of Insectology ***64*** *(Supplement): S69-S70, 2011*
ISSN 1721-8861

Diversity among phytoplasmas infecting ornamental plants grown in India

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Abstract

Phytoplasmas were identified in five ornamental species grown in gardens of Uttar Pradesh and Uttarakhand, India and showing suspected phytoplasma symptoms through nested PCR assays. DNA from symptomatic ornamental plants was extracted and amplification of phytoplasma ribosomal DNA was done with the universal phytoplasma primer pairs. The 1.2 kbp amplicons were cloned, sequenced and deposited in GenBank database. Based on sequence identities and phylogenetic relationships, the new phytoplasma strains identified have been classified as related to '*Candidatus* Phytoplasma asteris' (16SrI) group.

Key words: phytoplasma, ornamental plants, nested PCR, phylogenetic analysis, India.

Introduction

Phytoplasmas are mollicutes associated with diseases of several plant species (Al-Saady and Khan 2006; Harrison *et al*., 2008) and cause serious economic losses also in ornamental plants (Chaturvedi *et al*., 2010a). Phytoplasma epidemics have compelled withdrawal of many ornamental varieties from cultivation. General yellowing and stunting of plants, proliferation of shoots, phyllody, virescence and reduced size of flowers and reddening of leaves are the common symptoms observed in ornamental plants (Chaturvedi *et al*., 2010a). So far, 42 phytoplasmas belonging to 9 groups were identified in ornamental plants worldwide (Chaturvedi *et al*., 2010a). Based on the 16Sr sequences identified phytoplasmas in ornamental plants in India mainly belong to 16SrI, 16SrII, 16SrIII, 16SrV, 16SrVI, 16SrVII, 16SrIX, 16SrX, 16SrXII, 16SrXIII and 16SrXIV groups.

Little work has been done on occurrence and identification of phytoplasma in ornamental plants in India. Ajaykumar *et al*. (2007) first time recorded '*Candidatus* Phytoplasma asteris' associated with little leaf disease of *Portulaca grandiflora* and Samad *et al*. (2008) reported little leaf disease of *Portulaca grandiflora* at Lucknow. Raj *et al*. (2007a, 2007b; 2009) observed phytoplasma disease in *Chrysanthemum morifolium, Adenium obesum,* and *Gladiolus* at Lucknow. Chaturvedi *et al*. (2009a, 2009b; 2010b) reported little leaf disease in *Rosa alba, Catharanthus roseus*, and *Hibiscus rosa-sinensis* in Gorakhpur. Occurrence, identification, and characterization of phytoplasmas in five further ornamental species in India is reported.

Materials and methods

Samples from *Alstroemeria hybrids, Duranta erecta, Steblus asper, Petunia hybrida and Zinnia elegans* showing yellowing, phyllody, little leaf, proliferation of axillary buds and witches' broom were collected from ornamental plant from gardens at Mukteswara in Uttarakhand district and from Gorakhpur district of Uttar Pradesh, India. The total DNA was extracted from leaf/stem tissues following a CTAB. The initial PCR was performed using P1/P6 universal primers specific to the 16S rRNA gene (Deng and Hiruki, 1991). Cycle employed was with an initial denaturation at 94°C for 5 min, followed by 35 cycles (94°C for 1 min, 55°C for 60 s, and 72°C for 90 s) and a final extension at 72°C for 5 min. Further, nested PCR was carried out with primer R16F2n/R16R2 (Gundersen and Lee, 1996) as dscribed above except that 2 µL of the 50-fold diluted amplified product from the initial reaction was used as template and the annealing temperature was 50°C. The amplicons obtained were resolved by electrophoresis through a 1.2% agarose gel. Cloning of the second round PCR products was performed. The 16S rRNA sequence generated from the phytoplasma strains identified in ornamental plants were assembled and edited using DNASTAR's Laser Gene software (DNASTAR) and a phylogenetic tree was constructed using Mega 4.2 tool.

Results

Primer P1/P6 amplified a fragment of approximately 1,500 bp, whereas the primer R16F2n/R16R2 amplifies a fragment of about 1.2 kb. Neither by direct nor by nested PCR assays was DNA amplified from template DNA extracted from healthy or non-symptomatic samples. The 1.2 kb obtained amplicons were cloned, sequenced and the nucleotide sequences of the phytoplasma detected in the five ornamental species were deposited in GenBank. Phylogenetic relationships among these phytoplasma sequences are shown in figure 1.

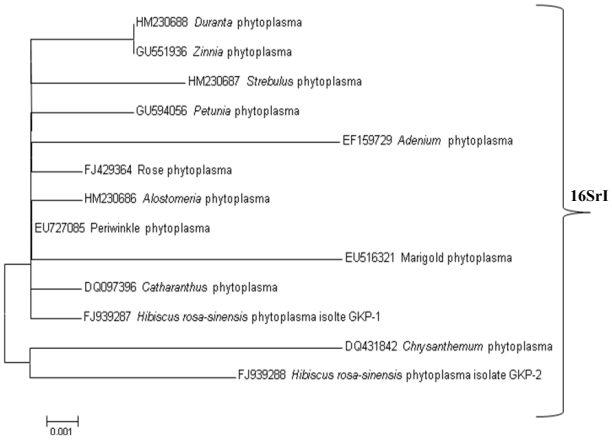

Figure 1. Phylogenetic tree constructed by using Clustal W algorithm and MEGA 4.2 version with 16Sr DNA sequences retrieved from GenBank from different phytoplasmas identified on ornamental plants in India.

The phylogenetic tree showed that *Alstroemeria* phytoplasma (HM230686), *Duranta* phytoplasma (HM230688), *Strebulus* phytoplasma (HM230687), *Petunia* phytoplasma (GU594056) and *Zinnia* phytoplasma (GU551936) shared highest 99% similarity with the 16Sr RNA gene of other phytoplasmas of ornamental plants from India such as *Catharanthus* phytoplasma (DQ097396), periwinkle phytoplasma (EU727085), *Chrysanthemum* phytoplasma (DQ431842), *Adenium* phytoplasma (EF159729), rose phytoplasma (FJ429364), marigold phytoplasma (EU516321) and *Hibiscus* phytoplasma strains 1 and 2 (FJ939287, FJ939288), belonging to 16SrI group. Based on sequence identities and phylogenetic relationships, all the five phytoplasmas have been classified into '*Candidatus* Phytoplasma asteris' (16SrI) group.

Discussion

Phytoplasma detection in symptomatic *Duranta* and *Streblus* is the first report in the world, however all the five ornamental plant species found infected with 16SrI phytoplasmas in Uttarakhand and Uttar Pradesh, are important. Seven other ornamental species were reported to be infected with phytoplasmas mainly belong to 16SrI and 16SrVI groups in India. The result of present study confirms that aster yellows group of phytoplasma predominates in ornamental species cultivated or naturally grown in India. Further identification of phytoplasmas in symptomatic ornamental species will facilitates the devising of control strategy towards management of such diseases in commercial grown ornamental crops in India. The infected ornamental plants may also acts as potential natural reservoir for phytoplasmas to other important agricultural commercial crops and vice-versa. Hence, an immediate attention is required for analyzing more samples for phytoplasma infection and to also look for the factors responsible for secondary spread of these phytoplasmas in nature.

Acknowledgements

The authors express their gratitude to the Director, National Botanical Research Institute (NBRI), Lucknow for facilities and keen interest and to the Council of Scientific Industrial Research (CSIR), New Delhi, India for financial support.

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