Molecular characterization of phytoplasma associated with four important ornamental plant species in India and identification of natural potential spread sources

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Abstract

Phytoplasma suspected symptoms of phyllody, witches' broom, leaf yellowing, stunting and little leaf were observed in *Chrysanthemum morifolium*, *Bougainvillea glabra*, *Jasminum sambac* and *Callistephus chinensis* during survey of flower nurseries and experimental ornamental fields at Delhi, Maharashtra, Tamil Nadu and Karnataka from 2014 to 2016. Pleomorphic bodies typical to phytoplasma structures were observed in the phloem sieve elements of ultrathin sections of all the four symptomatic ornamental plants (stem tissue) in transmission electron microscope. Amplification of 1.8 and 1.2 kb phytoplasma DNA products was observed in all the four test plants in PCR assays using universal primer pairs P1/P7 followed by nested primer pair R16F2n/R16R2, respectively. Pairwise sequence comparison, phylogeny and virtual RFLP analysis of 16S rDNA sequences confirmed the association of two phytoplasma subgroups (16SrI-B and 16SrII-D) in four ornamental plant species. '*Ca. P. aurantifolia*' subgroup D (16SrII-D) was found associated with chrysanthemum phyllody and leaf yellowing at Delhi and Tamil Nadu, bougainvillea little leaf and yellowing at Delhi and Chinese aster phyllody at Bengaluru, Karnataka. However, jasmine little leaf and yellowing at Bengaluru, Karnataka and chrysanthemum stunting at Pune were found to be associated with '*Ca. P. asteris*' subgroup B-related strains (16SrI-B). The identification of 16SrII-D subgroup phytoplasma infecting bougainvillea and 16SrI-B subgroup infecting jasmine are the new reports to the world. Besides weed species, *Cannabis sativa* showing witches' broom in jasmine fields at Bengaluru and *Parthenium hysterophorus* showing witches' broom symptoms in chrysanthemum fields at Delhi were identified to be caused by phytoplasma strains classified under subgroups 16SrI-B and 16SrII-D, respectively, by PCR assays and 16Sr DNA sequence comparison analysis. Among the three major leafhopper species identified, only *Hishimonas phycitis* was identified positive for 16SrI-B and 16SrII-D subgroups of phytoplasmas from chrysanthemum fields at Delhi and jasmine fields at Bengaluru, respectively. The identity of similar phytoplasma strains infecting ornamental species in leafhopper and the weed species in the present study suggested that *H. phycitis* and weeds may act as potential natural sources for secondary spread of the identified phytoplasma strains.

Keywords Chrysanthemum · Bougainvillea · Jasmine · Chinese aster · Phytoplasma · Weeds · Leafhopper · India

Introduction

Phytoplasmas are fastidious prokaryotes, which can survive and multiply only in hypotonic habitats such as plant phloem or insect hemolymph. Therefore, they are strictly host dependent. They are known to be pathogenic to more than a three hundred plant species (Bertaccini et al. 2014).

Comprehensive phylogenetic studies based on 16S rRNA gene sequencing and other housekeeping genes provided evidence that these wall-less prokaryotes inhabiting plant phloem cells and insects constitute a large monophyletic group within the class Mollicutes, a branch of the Gram-positive eubacteria (Mitrovic and Contaldo 2011; Bertaccini et al. 2014).

Flower cultivation has been practiced in India since times immemorial, but it is only in the recent years that floriculture has blossomed into a viable business sector. Total area under flower crops in the country was 0.249 Mha with production of 1659 and 484 million tons (MT) of loose and cut flowers, respectively. The country has exported 22,947.23 MT of

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floriculture products to the world for the worth of Rs. 460.75 crores (70.8 million USD) in 2014–2015 that included dry flowers, cut flowers, foliage and branches, plants, seeds and planting material and other flower products. The age-old domestic market dominated by loose flowers has also grown approximately from 18 million USD in the 1990s to 153 million USD in 2015 (APEDA, 2015). All the segments of floriculture encounter significant manifestations of various diseases. Phytoplasma diseases are one of the major constraints in commercial ornamental plants production by lowering the quantum and quality gaining international importance. Phytoplasma causes different symptoms of yellowing and stunting of plants, proliferation of shoots, phyllody, virescence and reduced size of flowers in many ornamental plants which affect their economic value (Chaturvedi et al. 2010; Bertaccini 2015). So far, fourteen different groups (16SrI, 16SrII, 16SrIII, 16SrIV, 16SrV, 16SrVI, 16SrVII, 16SrIX, 16SrX, 16SrXII, 16SrXIII and 16SrXIV, 16SrXV, 16SrXXXII) of phytoplasmas were identified in ornamental plants worldwide (Madhupriya 2016). Out of these fourteen groups, aster yellows, 16SrI; peanut witches' broom, 16SrII; clover proliferation, 16SrVI; pigeon pea witches' broom, 16SrIX; rice yellow dwarf, 16SrXI and Bermuda grass white leaf, 16SrXIV have been identified in 34 ornamental species from India (Rao et al. 2017). Among them, the 16SrI phytoplasma group was reported to have the widest host range followed by 16SrII and 16SrVI groups (Rao et al. 2017). In India chrysanthemum, bougainvillea, jasmine and Chinese aster share the major commercial market business. Limited information is known so far on characterization and natural spread of phytoplasma strains infecting these ornamental species in India (Rao et al. 2017). In the present study, an attempt was made to identify and characterize the identification of pathogen associated with four symptomatic ornamental plants species (chrysanthemum, bougainvillea, jasmine and Chinese aster) along with weeds using electron microscopy, PCR assays and *16S rRNA* sequence comparison analysis. Attempt was also made to identify the potential vectors feeding on ornamental plants for their possible role in natural transmission of the identified phytoplasma strains.

Materials and methods

Plant sample survey and symptomatology

A survey was made to record the symptoms and incidence of phytoplasma disease on four ornamental species, viz. chrysanthemum (*Chrysanthemum morifolium*) at Delhi and Maharashtra, bougainvillea (*Bougainvillea glabra*) at Delhi, jasmine (*Jasminum sambac*) and Chinese aster (*Callistephus chinensis*) at Karnataka and weed species during 2014–2016. Symptomatic plants were collected from different surveyed

fields and kept in the deep freezer (− 80 °C) for further PCR assays.

Leafhopper sample survey

The predominant leafhopper (LH) species feeding in flower nurseries and ornamental fields were collected at blooming stage from July to December using sweeping net method and yellow sticky traps. Collected insects were stored in plastic vials at 4 °C in 70% ethanol for further analysis. The major LH species collected from all the survey locations were taxonomically identified by Division of Entomology, Indian Agricultural Research Institute, New Delhi, India, and Department of Entomology, Gandhi Krishi Vigyan Kendra (GKVK), Bangalore, India.

Microtomy and electron microscopy

The fresh samples were processed for microtomy to observe the phytoplasma bodies under electron microscope. Small pieces of infected and healthy shoots of chrysanthemum, jasmine, Chinese aster and bougainvillea were fixed in 2% glutaraldehyde in 0.05 M phosphate buffer at 4 °C overnight and then washed three times for 20 min in 0.05 M phosphate buffer. Tissues were then post-fixed in 2% osmium tetroxide (O_8O_4) in 0.05 M phosphate buffer at 4 °C for overnight and then washed three times for 20 min in ddh₂O. Tissues were then dehydrated in a graded acetone series (25, 50, 70, 96 and 100%, all 15 min each) and in Spurr's low-viscosity embedding medium (Spurr/ETOH 100%) as, 1 part spurr to 3 parts ETOH for overnight, 1 part spurr to 1 parts ETOH for overnight, 3 part spurr to 1 parts ETOH for overnight and 100% spurr for overnight followed by polymerization at 70 °C for 12 h. Ultrathin 60-nm sections were cut with a diamond knife (type 35°, Datome, Biel, Switzerland) on a Leica EM UC7 microtome (Leica Microsystems, Wetzlar, Germany). Sections were post-stained for 10–15 min with 2% uranyl acetate in H₂O for 5 min in lead citrate (Reynolds 1963). Samples were examined in a JEOL JEM-1011 (Japan) electron microscope operated at 80 kV. Micrograph was taken using an Olympus SIS Megaview camera (Olympus SIS, Muenster, Germany).

DNA extraction and PCR assays

DNA was extracted from the young shoot tissues of three symptomatic and non-symptomatic of all the five ornamental plants, weed species and salivary gland tissues isolated from ten individuals of identified leafhopper species from all the surveyed locations by CTAB method (Ahrens and Seemuller 1992). The DNA concentrations were quantified with gel and Nanodrop spectrophotometer (ND-1000 UV/VIS, USA). Extracted DNA from the ornamental plants,

weeds and leafhoppers species were used as template in PCR assays by universal primer pair P1/P7 in first round (Deng and Hiruki 1991; Schneider et al. 1995) followed by R16F2n/R16R2 in second round nested PCR assays (Gundersen and Lee 1996) derived from conserved regions of the *16S rRNA* gene. PCRs were performed in a Mastercycler (Eppendorf Germany) and the cycling protocol used for the first and nested round PCR was similar as described (Rao et al. 2014). The DNA isolated from brinjal infected with little leaf of phytoplasma (16SrVI-D) was used as positive control (Kumar 2015). The DNA extracted from non-symptomatic ornamentals, weeds and leafhoppers collected from non-symptomatic ornamental fields were used as negative controls. Twenty-five microliters of each PCR product was subjected to electrophoresis in a 1.0% (w/v) agarose gel, stained with ethidium bromide and observed under UV transilluminator. Nested PCR product (1.25 kb amplicon) was purified using the Wizard SV Gel and PCR Clean-up System (Promega, USA).

Sequence analysis

Purified amplified PCR products of R16F2n/R16 (from ornamentals, weeds, leafhopper) were outsourced for sequencing in both directions at AgriGenome, India. The sequence of PCR products was assembled using DNA Base V.4 (<http://www.dnabaser.com>). The 16S rDNA sequences were aligned with phytoplasma group/subgroup representatives available in GenBank using ClustalW software (Table 1). The 16S rDNA sequence generated from the present study and the reference phytoplasma strain sequences retrieved from GenBank were used to construct phylogeny by the neighbor-joining method with 1000 replications for each bootstrap value using MEGA 6.0 software version for ‘*Candidatus* Phytoplasma species’

assignment (Tamura et al. 2013). *Acholeplasma laidlawii* 16S rDNA sequence was used as out group to root the phylogenetic tree.

Virtual RFLP analysis

The phytoplasma sequences of ornamentals (chrysanthemum, bougainvillea, jasmine and Chinese aster), weeds (*C. sativa* and *P. hysterophorus*) and leafhopper (*H. phycitis*) corresponding to the R16F2n/R16R2 region was subjected to in silico RFLP analysis using *iPhyClassifier* online tool (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>) (Zhao et al. 2009) and compared with representative sequences of the *Ca. Phytoplasma asteris* strain 16SrI-B (Acc. No. KY565572) and *Ca. Phytoplasma aurantifolia* strain 16SrII-D (Acc. No. KX013260) subgroup.

Results

Survey, incidence of disease and symptomatology

The phytoplasma suspected symptoms of phyllody were observed in chrysanthemum cultivar (cv). DFR-203 (Delhi), cv. Sel-5 (Tamil Nadu), yellowing in cv. Bagi (Tamil Nadu) and stunting symptoms from chrysanthemum at Pune, Maharashtra, and little leaf and yellowing in bougainvillea at Delhi, phyllody in Chinese aster at Bengaluru, Karnataka, and little leaf and yellowing in jasmine at Bengaluru (Table 1; Fig. 1a–i). *C. sativa* showing witches’ broom was recorded from jasmine fields at Bengaluru and *P. hysterophorus* showing witches’ broom symptoms from chrysanthemum fields at IARI, Delhi (Fig. 2a, b).

Table 1 Survey and symptoms of ornamental species in different states of India along with GenBank submission and phytoplasma identification in ornamentals, weeds and insects

S. No	Host plant	Location	Symptoms	Incidence (%)	EMBL/GenBank/ DDBJ accession No	Group/ subgroup identified
1	Chrysanthemum (cv.DFR-203)	IARI (Delhi)	Phyllody	25–35	KY689731	16SrII-D
2	Chrysanthemum	Pune (Maharashtra)	Stunting	15–24.5	KY689733	16SrI-B
3	Chrysanthemum (cv.Bagi)	Tamil Nadu	Leaf yellowing	20–25	KY689735	16SrII-D
4	Chrysanthemum (cv.Sel-5)	Tamil Nadu	Phyllody	15–23	MF583741	16SrII-D
5	Bougainvillea	IARI (Delhi)	Little leaf and yellowing	21.5–25	MF509776	16SrII-D
6	Jasmine	Bengaluru (Karnataka)	Little leaf	20–25	MF775667	16SrI-B
8	Chinese aster	IIHR (Karnataka)	Phyllody	32–35	MF771266	16SrII-D
9	<i>Hishimonus phycitis</i>	Bengaluru (Karnataka)	–	–	MG255125	16SrI-B
10	<i>Hishimonus phycitis</i>	IARI (Delhi)	–	–	MG062732	16SrII-D
11	<i>Cannabis sativa</i>	Bengaluru(Karnataka)	Witches’ broom	30–55	MF775666	16SrI-B
12	<i>Parthenium hysterophorus</i>	IARI (Delhi)	Witches’ broom	35–50	MG255126	16SrII-D

Fig. 1 Phytoplasma disease symptoms in different ornamental species: **a** chrysanthemum phyllody cultivar cv. DFR-203, Delhi; **b** chrysanthemum phyllody cv. Sel-5, Tamil Nadu; **c** chrysanthemum leaf yellowing cv. Bagi, Tamil Nadu; **d**: chrysanthemum stunting, Pune; **e**

bougainvillea healthy plant, Delhi; **f** bougainvillea little leaf and yellowing, Delhi; **g** jasmine healthy plant, Bengaluru; **h** jasmine little leaf, Bengaluru; **i** Chinese aster phyllody, Bengaluru

Identification of the leafhoppers

Seven different leafhoppers species, viz. *Empoasca prima* (Distant), *E. motti* (Singh-Pruthi), *Hishimonus phycitis* (Distant) (HP), *Hecalus porrectus* (Walker, F.), *Psammotettix* sp., *Oliarus* spp. and *Amrasca biguttula biguttula* (Ishida), feeding on ornamental plants were identified from different surveyed fields during the course of study (Table 2). The *Empoasca prima* (Distant), *Hishimonus phycitis* (Distant) (HP) and *Amrasca biguttula biguttula* (Ishida) were recorded as the major leafhopper species present at one and/or more surveyed locations in ornamental fields on the basis of number of insects trapped by sweeping net method and on yellow sticky traps (Table 2).

Electron microscopy

Examination of ultrathin sections of the shoots of all the four symptomatic ornamental plants revealed a large number

of pleomorphic phytoplasma bodies bounded by a poorly defined membrane in the sieve tube cells of the infected tissues of ornamental plants in the present study (Fig. 3a–g). These bodies were surrounded by membranes, lacked cell walls and had round or oblong shapes. No phytoplasma-like structures were seen in healthy shoot tissues of any ornamental plant under electron microscope. Size of the phytoplasma bodies measured from 200 to 800 nm (0.2–0.8 μ m) in diameter.

Phytoplasma detection by PCR assays

In direct PCR assays, P1/P7 primer amplified 1.8 kb fragments, whereas in nested PCR assay with R16F2n/R16R2 amplified 1.2 kb fragments of 16S rDNA region from all the symptomatic ornamental plants (chrysanthemum, bougainvillea, jasmine and Chinese aster) along with phytoplasma positive control samples (data not shown). A 1.2-kb-amplified product was also obtained in two symptomatic weeds

Fig. 2 Phytoplasma disease symptoms in weed species; **a** *Cannabis sativa* witches' broom at Bengaluru; **b** *Parthenium hysterophorus* witches' broom at Delhi

Table 2 Detection of phytoplasma in leafhopper samples collected from the ornamental fields in the present study

Insect vector	Location of collection of insects							
	Bengaluru (Karnataka) (jasmine fields)		Delhi (chrysanthemum fields)		Tamil Nadu (chrysanthemum fields)		Pune (Maharashtra) (chrysanthemum fields)	
	No. of insects collected for (180 days)	PCR results	No. of insects collected for (180 days)	PCR results	No. of insects collected for (180 days)	PCR results	No. of insects collected for (180 days)	PCR results
<i>Hishimonus phycitis</i>	454	+ve	356	+ve	Nil	–	82	–ve
<i>Amrasca biguttula biguttula</i>	195	–ve	188	–ve	255	–ve	163	–ve
<i>Empoasca prima</i>	Nil	–	142	–ve	106	–ve	Nil	–
<i>Empoasca motti</i>	105	–ve	82	–ve	74	–ve	38	–ve
<i>Hecalus porrectus</i>	78	–ve	34	–ve	Nil	–	Nil	–
<i>Psammotettix</i> spp.	Nil	–	26	–ve	Nil	–	Nil	–
<i>Oliarus</i> spp.	Nil	–	14	–ve	55	–ve	22	–ve

^aSalivary glands of ten individual leaf hoppers were assayed for phytoplasma presence with the help of P1/P7 and R16F2n/R2 primer pair

(*C. sativa* and *P. hysterophorus*) and the leafhopper (HP) samples from the states of Delhi and Karnataka (data not shown) in nested PCR assays using primer pair R16F2n/R16R2. No DNA amplification products were observed in

direct and nested PCR assays with the same set of primers to *16S rRNA* genes when DNA from asymptomatic ornamental plants, weeds and *H. phycitis* collected from non-symptomatic ornamental fields were used. The *16S rRNA* sequences

Fig. 3 Ultrathin sections of phytoplasma-infected phloem tissue; **a** chrysanthemum phyllody cv. DFR-203 (Delhi); **b** chrysanthemum phyllody cv. Sel-5 (Tamil Nadu); **c** chrysanthemum leaf yellowing cv. Bagi (Tamil Nadu); **d** chrysanthemum stunting (Pune); **e** Bougainvillea little leaf and yellowing (Delhi); **f** Chinese aster phyllody (Bengaluru); **g** jasmine little leaf (Bengaluru); ultrathin section showed pleomorphic phytoplasma bodies (arrows) fill a phloem sieve element with a large number

of four ornamental plants, two weeds and one leafhopper (*H. phycitis*) were submitted to the GenBank (Table 1).

Sequence analysis

In pairwise sequence comparison, *16S rRNA* partial gene sequences of four ornamental species, viz. chrysanthemum phyllody cv. DFR-203 Delhi strain and cv. Sel-5 Tamil Nadu

strain, leaf yellowing cv. Bagi Tamil Nadu strain, bougainvillea little leaf and yellowing Delhi strain, Chinese aster phyllody Bengaluru strain, leafhopper (*H. phycitis*) Delhi strain and *P. hystrophorus* Delhi strain showed maximum sequence identity of 99% with phytoplasma strains of *Ca. P. aurantifolia* (16SrII) group. However, *16S rDNA* sequences of jasmine little leaf and leafhopper (*H. phycitis*) Bengaluru strain, chrysanthemum stunting Pune strain and *C. sativa*

Bengaluru strain had 99% sequence identities to ‘*Ca. P. asteris*’ (16SrI)-related strains (Table 1). The phylogenetic analysis of 16S rDNA sequences revealed that all the identified phytoplasma strains from four ornamentals, two weeds and one leafhopper (HP) clustered together with the corresponding phytoplasma strains belonging to subgroups 16SrII-D and 16SrI-B (Fig. 4).

In silico RFLP analysis

Virtual RFLP analysis results also confirmed that chrysanthemum phytoplasma strains from Delhi and Tamil Nadu (Acc. No. KY689731, KY689735, MF583741), bougainvillea phytoplasma Delhi strain (Acc. No. MF509776), Chinese aster phytoplasma Bengaluru strain (Acc. No. MF771265), *P. hysterophorus* phytoplasma Delhi strain (Acc. No. MG255126) and *H. phycitis* Delhi strain (Acc. No. MG062732) produced RFLP profiles identical to those of phytoplasma reference strain of *Ca. P. aurantifolia* belonging to 16SrII-D subgroup (*Crotalaria witches’ broom* Acc. No. KX013260) (Fig. 5). However, predicted similar digestion RFLP fragments of the chrysanthemum phytoplasma Pune strain (Acc. No. KY689733), *C. sativa* phytoplasma Bengaluru strain (Acc. No. MF775666), jasmine little leaf and *H. phycitis* phytoplasma Bengaluru strain (Acc. No. MF775667, MG255125) were found similar to the restriction profiles of the reference strain of *Ca. P. asteris* classified under 16SrI-B subgroup (Aster yellows phytoplasma; Acc. No. KY565572) (Fig. 6).

Discussion

The phytoplasmas are important group of plant pathogens which drastically damage growth and marketing parameters of ornamental plants and affect their commercial value (Chaturvedi et al. 2010). Knowledge of the importance of phytoplasmas as plant disease agents has advanced rapidly over the last decade with increased significant economic losses (Bertaccini et al. 2014). So far, 74 phytoplasmas belonging to 14 groups were identified in ornamental plants worldwide and six groups in India (16SrI, 16SrII, 16SrVI, 16SrIX, 16SrXI and 16SrXIV groups) (Rao et al. 2017). In the present study, two phytoplasma subgroups, 16SrI-B and 16Sr II-D, were identified and characterized with four ornamental species, two weed species and one leafhopper from four states of India on the basis of symptoms, electron microscopy observation and analysis of 16S rDNA sequence analysis.

The chrysanthemum phyllody cv. DFR-203 Delhi strain, cv. Sel-5 Tamil Nadu strain and leaf yellowing in cv. Bagi Tamil Nadu strain, bougainvillea little leaf and yellowing Delhi strain and Chinese aster phyllody Bengaluru strain were identified to be associated with ‘*Ca. P. aurantifolia*’ subgroup D. However, jasmine little leaf Bengaluru strain and chrysanthemum stunting Pune strain were identified to be caused by ‘*Ca. P. asteris*’ subgroup B. So far, three different groups of phytoplasmas were identified worldwide in chrysanthemum crops, viz. 16SrI from Korea, China

Fig. 4 Phylogenetic tree based on 16S rDNA gene sequences of ornamentals, weeds and insects with other selected phytoplasma strains from GenBank. Accession numbers are specified in the tree. *A. laidlawii* was used as an out group. Mega 5.0 software was used to construct the tree. Numbers on branches are bootstrap values obtained for 1000 replicates. The bar represents a phylogenetic distance of 1%

Fig. 5 Virtual RFLP patterns from in silico digestion of 16S rDNA R16F2n/R2 fragments of phytoplasma strains infecting ornamental plants in India and the phytoplasma reference strains with 17 restriction enzymes (*AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *MboI*, *MseI*, *RsaI*, *SspI* and *TaqI*) using *iPhyClassifier* program. The patterns are compared with 16SrII-D reference strain (GenBank Acc. No. KX013260 (**h**)), with chrysanthemum phyllody cv. DFR-203 Delhi strain [Acc. No. KY689731 (**a**)], chrysanthemum leaf yellowing cv. Bagi Tamil Nadu strain [Acc. No. KY689735 (**b**)], chrysanthemum phyllody cv. Sel-5 Tamil Nadu strain [Acc. No. MF583741 (**c**)], bougainvillea little leaf and yellowing Delhi strain [Acc. No. MF509776 (**d**)], Chinese aster phyllody Bengaluru strain [Acc. No. MF771266 (**e**)], *Parthenium hysterophorus* witches' broom Delhi strain [Acc. No. MG255126 (**f**)], *Hishimo-phycitis* Delhi strain [Acc. No. MG062732 (**g**)]

and India (Bongnam et al. 2007; Chung 2008; Min and Hu 2009; Kumar and Byadgi 2012; Raj et al. 2007), 16SrII from India (Yadav et al. 2015) and 16SrIII from China (Chung 2008) were identified in chrysanthemum crops (Two different groups of phytoplasmas were reported from jasmine, 16SrI from Italy (Marzachi et al. 1999) and 16SrII group from Oman (Al-Zadjali et al. 2007); however, in India only one report is available on jasmine belonging to 16SrXI group from Jaipur, Rajasthan (Madhupriya et al. 2015). In bougainvillea, two phytoplasma groups (16SrI-B and 16SrIII) were reported from Brazil (Silva et al. 2014); however, no reports are available on occurrence of phytoplasma infection on bougainvillea from India. In Chinese aster, two groups belonging to 16SrI and 16SrII were reported from Korea (Win et al. 2011), Germany (Marcone et al. 2000) and USA (Zhang et al. 2004; Lee et al. 2004). In India, only 16SrII group phytoplasma was reported on Chinese aster (Mahadevakumar et al. 2017). In the present study, report of '*Ca. P. aurantifolia*' (16SrII-D) infecting bougainvillea and '*Ca. P. asteris*' (16SrI-B) infecting jasmine are the new records to the world.

Two weeds species, *C. sativa* showing witches' broom at Bengaluru, Karnataka and *P. hysterophorus* showing witches' broom symptoms at Delhi, were also identified and characterized under phytoplasma subgroups 16SrI-B and 16SrII-D, respectively. Several weeds are reported as natural reservoirs of important phytoplasmas which play an important role in natural spread and serve as natural alternative hosts for different groups of phytoplasmas (Blanche et al. 2003; Pasquini et al. 2007; Mall et al. 2011; Rao et al. 2017). More than 34 weed species are reported to be the hosts of phytoplasmas belonging to five different groups (16SrI, 16SrII, 16SrV, 16SrVI and 16SrXIV) throughout India (Rao et al. 2017). In India, *C. sativa* has already been reported as natural hosts of 16SrI, 16SrII, 16SrVI and 16SrXIV groups of phytoplasma (Raj et al. 2008a, b; Mall 2009; Chaube et al. 2015; Kumar et al. 2017). However, *P. hysterophorus* was confirmed as

natural host of 16SrI and 16SrII group phytoplasmas in India (Raj et al. 2008b; Thorat et al. 2016). In the present study, these two weed species *C. sativa* and *P. hysterophorus* were again confirmed as host of 16SrI-B and 16SrII-D phytoplasma subgroups, which were also identified on ornamentals (jasmine and chrysanthemum) in the present study. This shows the potential of these weed species to harbor different groups of phytoplasmas. Hence, these weed species may play a key role as natural reservoir of identified phytoplasma strains and may help in natural spread of phytoplasmas to other ornamental and agricultural crops with the help of potential leafhopper vectors (Pasquini et al. 2007; Rao et al. 2017).

Out of three most dominant identified leafhopper species, viz. *E. prima*, *H. phycitis* and *A. biguttula biguttula* from the four surveyed locations in the present study, only *H. phycitis* was found positive for the presence of 16SrI-B and 16SrII-D subgroups of phytoplasma strains from jasmine fields in Karnataka and from chrysanthemum fields in Delhi, respectively. Many leafhopper species, viz. *Empoasca variegates*, *E. decipiens*, *E. motti*, *Macrosteles quadripunctulatus*, *Euscelis incisus*, *Amarasca biguttula*, *Oliarus spp*, *Hecalus porrectus* and *H. phycitis* have been identified as vectors of different phytoplasmas infecting ornamentals from Italy, Saudi Arabia, Lebanon and USA (Galletto et al. 2011a, b; Pastore et al. 2004; Arocha et al. 2005, 2007; Parrella et al. 2008; Alhudaib et al. 2009; Pérez et al. 2010; Abou-Jawdah et al. 2011; Feeley et al. 2001; Bosco et al. 2007). But in India no report is available on the identification of any vector of phytoplasma strain associated with any ornamentals. Earlier, un Nabi et al. (2015) and Kumar et al. (2017) reported *H. phycitis* as a natural and potential vector to transmit the sesame phyllody and brinjal little leaf phytoplasma strains belonging to 16SrI and 16SrII groups in India. HP, identified as putative vector in the present study for two different groups of phytoplasmas associated with jasmine little leaf disease at Karnataka and chrysanthemum phyllody at Delhi may play an important role in natural transmission of phytoplasma strains from ornamentals to ornamentals or weeds to ornamentals and vice versa in respective areas of their existence. Results of this study will also facilitate further work on epidemiology, diversity and management of phytoplasmas infecting ornamental crops in India.

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Fig. 6 Virtual RFLP patterns from in silico digestion of 16S rDNA R16F2n/R2 fragments of phytoplasma strains infecting ornamental plants in India and the phytoplasma reference strains with 17 restriction enzymes (*AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *MboI*, *MseI*, *RsaI*, *SspI* and *TaqI*) using *iPhyClassifier* program. The patterns are compared for 16SrI-B reference strain (GenBank Acc. No. KY565572 (**d**)), with chrysanthemum stunting Pune strain [Acc. No. KY689733 (**a**)], jasmine little leaf and yellowing Bengaluru strain [Acc. No. MF77567 (**b**)], *Hishimonus phycitis* Bengaluru strain [Acc. No. MG255125 (**c**)], *Cannabis sativa* witches' broom Bengaluru strain [Acc. No. MF775666 (**d**)]

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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