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ORIGINAL ARTICLE

Molecular characterization, vector identification and sources of phytoplasmas associated with brinjal little leaf disease in India

Manish Kumar[1] · Madhupriya[1] · Govind Pratap Rao[1]

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Abstract Brinjal little leaf (BLL) is a widespread disease of phytoplasma etiology in India that induces severe economic losses. Surveys were conducted in eight brinjal-growing states of India during July 2014 to September 2015 and eighteen BLL samples showing little leaf, phyllody and witches' broom symptoms were collected for phytoplasma identification. Presence of phytoplasmas was confirmed in all the eighteen BLL samples using polymerase chain reaction with phytoplasma-specific primer pairs (P1/P6, R16F2n/R16R2). Pair wise sequence comparison and phylogenetic relationship of *16S rRNA* gene sequences of BLL phytoplasma strains confirmed that sixteen out of eighteen BLL strains belonged to clover proliferation phytoplasma (16SrVI) group and two BLL strains (GKP-A and GKP-B) from Gorakhpur, Uttar Pradesh, were classified under 16SrII group. Further virtual RFLP analysis of 16S rDNA sequences allowed finer classification of BLL strains into 16SrII-D and 16SrVI-D subgroups. BLL phytoplasma strains belonging to 16SrVI-D subgroup were found as the most widespread phytoplasma strains associated with BLL disease in India. 16SrVI-D subgroup phytoplasma association with two symptomatic weed species viz. C*annabis sativa* subsp. *sativa* at Noida, Uttar Pradesh and *Portulaca oleracea* at IARI fields, New Delhi was also confirmed by nested PCR assays with similar set of phytoplasma-specific primers, pairwise 16S rDNA sequence comparison, phylogeny and virtual RFLP analysis. Out of five identified leafhopper species from BLL-infected fields at Noida, Uttar Pradesh and Delhi, only *Hishimonas phycitis* was identified as carrier and natural vector of 16SrVI-D subgroup of phytoplasmas by nested PCR assays, sequence comparison, phylogeny, virtual RFLP analysis and transmission assays.

Keywords 16SrII-D subgroup · 16SrVI-D subgroup · Phytoplasma · *Hishimonas phycitis* · *Cannabis sativa* subsp. *sativa* · *Portulaca oleracea*

Introduction

Eggplant (*Solanum melongena* L.) which is known in South Asia, Southeast Asia and South Africa as brinjal, is an important vegetable crop cultivated all over the world, particularly in the tropics and sub-tropics. In India, it is one of the most popular and principal vegetable crops grown throughout the country. India is the second largest producer of brinjal in the world next to China and produces 12.2 M mt over an area of 0.7 M ha and with an average productivity of 17.42 mt/ha (Anonymous 2013). Brinjal fruit is rich source of minerals, vitamins, total water-soluble sugars, free reducing sugars and amide proteins (Gopalan et al. 2007). Brinjal fruits have also medicinal properties and have been found good for diabetic patients and those suffering with liver diseases, cough due to allergy, rheumatism, colilithiasis, leucorrhea and intestinal worms (Shukla and Naik 1993).

Major factor that limits brinjal productivity besides its narrow genetic base is extreme susceptibility to biotic and abiotic stresses. It is affected by several diseases of which little leaf disease caused by phytoplasma is one of the most important factors causing considerable economic losses (Mitra 1993; Rao et al. 2010). The infected plants are

✉ Govind Pratap Rao
gprao_gor@rediffmail.com

[1] Plant Virology Unit, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

characterized by little leaves, proliferation of shoots, phyllody and stunting (Rao et al. 2010). The disease was first reported by Thomas and Krishnaswami (1939) in India, and later, several biological aspects of the disease have been described (Varma et al. 1969; Mitra 1993; Mello et al. 2011). So far, phytoplasmas belonging to six groups, viz. 16SrI from Japan, Bangladesh and India (Okuda et al. 1997; Kelly et al. 2009; Kumar et al. 2012), 16SrII-D from Egypt (Omar and Foissac 2012), 16SrIII-J and 16SrIII-U from Brazil (Mello et al. 2011), 16SrVI-A and -D from Turkey and India (Sertkaya et al. 2007; Azadvar and Baranwal 2012), 16SrIX-C from Iran (Tohidi et al. 2015) and 16SrXII-A from Russia (Ember et al. 2011) were reported to infect brinjal worldwide.

Phytoplasma etiology of BLL disease in India has been confirmed on the basis of symptoms, electron microscopy and PCR assays (Varma et al. 1975; Verma and Dubey 1978; Shantha and Lakshmanan 1984; Azadvar and Baranwal 2012). *Datura stramonium* was reported as natural weed host for BLL phytoplasma in India (Singh et al. 2012) and the leafhopper *Hishimonas phycitis* was identified as putative vector (Bindra and Singh 1969; Azadvar and Baranwal 2012). No transmission assays were performed by earlier workers to confirm any of the leafhoppers as natural vector of BLL disease in India. So far, only two phytoplasma groups (16SrI and 16SrVI) were reported to be associated with BLL disease in India (Azadvar and Baranwal 2012; Kumar et al. 2012). Genetic diversity and subgroup-level taxonomic classification of phytoplasma strains infecting BLL disease phytoplasma strains from different brinjal-growing states of India is not attempted till date.

BLL is a very serious disease causing severe losses to brinjal crops and is widespread in brinjal-growing regions of India (Mitra 1993; Azadvar and Baranwal 2012). Limited information is available on genetic diversity of BLL phytoplasma strains in India; hence, an attempt was made to characterize the BLL-associated phytoplasma strains up to subgroup-level taxonomic assignments along with identification of natural insect vectors and weed hosts by 16S *rRNA* sequence comparison, phylogeny clustering, virtual RFLP analysis and transmission assays.

Materials and methods

Survey and collection of plant samples

Leaf samples (in triplicate) from symptomatic brinjal plants were collected by quadrat method of sampling from fields of eight states of India (Uttar Pradesh, Haryana, Maharashtra, Odisha, Chhattisgarh, Assam, Bihar and Delhi) during July 2014–September 2015 between 30 and 60 days age of the crop. At least three to four symptomatic brinjal samples from each state were collected along with two healthy samples from each field. Leaf samples of symptomatic weed species were also collected from Delhi and Uttar Pradesh. Incidence of disease (visual observation) and types of symptoms on brinjal plants and weed species were also recorded.

DNA extraction and PCR assays

The total genomic DNA was extracted by CTAB method (Ahrens and Seemüller 1992) from leaf veins of symptomatic brinjal, weeds and the whole body of ten individuals of each leafhopper species from different surveyed locations and used as a template for PCR assays. DNA extracted from non-symptomatic brinjal and weed species, and leafhoppers collected from healthy brinjal fields were used as negative control. The DNA isolated from symptomatic periwinkle infected with toria phyllody phytoplasma (16SrIX, pigeon pea witches' broom phytoplasma; Azadvar et al. 2009) maintained in the greenhouse was used as positive control. Amplification of phytoplasma ribosomal DNA was performed with phytoplasma universal primer pairs P1/P6 (Deng and Hiruki 1991) followed by nested primer pairs R16F2n/R16R2 (Smart et al. 1996). The product of the first round of PCR assay was diluted 1:4 with sterile water and 2 µl was used as template in nested PCR assay. Five microlitres of nested PCR product was subjected to electrophoresis in a 1.0% (w/v) agarose gel, stained with ethidium bromide and observed under UV transilluminator. The amplified 16S rDNA phytoplasma fragments were purified using the Wizard^{R} SV Gel and PCR Clean-up System (Promega, Madison, USA).

Sequencing, pairwise sequence comparison and phylogenetic analysis

Purified PCR products of R16F2n/R16R2 were outsourced and sequenced in both directions at Xcelris Genomics, India. The sequences of PCR products were 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 (Hall 1999) and the consensus sequences were submitted to GenBank (Table 1). The phylogenetic tree was constructed using the neighbor-joining method with MEGA 5.0 (Tamura et al. 2011) using 1000 bootstrap replications. *Acholeplasma laidlawii* phytoplasma 16S rDNA sequence (Ac. No. NR074448) was used to root the trees in phylogeny.

Virtual RFLP analysis

The phytoplasma sequences corresponding to the R16F2n/R16R2 fragments associated with BLL strains were

Table 1 Distribution and incidence of brinjal little leaf disease in different states of India along with GenBank submission and phytoplasma identification in brinjal, weeds and insect

S. no	States and BLL Isolates	Average incidence (%)*	GenBank 16S rDNA sequences for BLL phytoplasma strains	Group/subgroup identified
1	Delhi (IARI)			
	IARI-A	22	KX689234	16SrVI-D
	IARI-B	21	KX689235	16SrVI-D
2	Uttar Pradesh GKP (Jhanga bazaar)			
	GKP-A	11.5	KX689253	16SrII-D
	GKP-B	12.5	KX689254	16SrII-D
	Noida			
	Noida-A	18	KX689238	16SrVI-D
	Noida-B	15.5	KX689239	16SrVI-D
3	Haryana (Karnal)			
	HR-A	16.5	KX689241	16SrVI-D
	HR-B	15	KX689242	16SrVI-D
4	Odisha (Bhubaneswar)			
	OD-A	15	KX689243	16SrVI-D
	OD-B	27	KX689244	16SrVI-D
5	Chhattisgarh (Raipur)			
	CG-A	16	KX689245	16SrVI-D
	CG-B	19.5	KX689246	16SrVI-D
6	Assam (Biswanath)			
	Assam-A	12.5	KX689251	16SrVI-D
	Assam-B	14.5	KX689252	16SrVI-D
7	Maharashtra (Warora)			
	MS-A	10.5	KX689247	16SrVI-D
	MS-B	8	KX689248	16SrVI-D
8	Bihar (Gopalganj)			
	BR-A	18	KX689249	16SrVI-D
	BR-B	15	KX689250	16SrVI-D
9	Insect			
	Hishimonas phycitis (Delhi, IARI)		KX689237	16SrVI-D
10	Weed species			
	Cannabis sativa subsp. *sativa* (Noida)		KX689240	16SrVI-D
11	*Portulaca oleracea* (Delhi, IARI)		KX689236	16SrVI-D

NA not attempted

* Average incidence of four plots was calculated for each location

subjected to in silico RFLP comparison using the iPhyClassifier online tool (http://www.plantpathology.ba.ars.usda.gov/cgibin/resource/iphyclassifier.cgi) and compared with representative sequences of phytoplasma strains of 16SrII-D (Ac. No. JQ868448) and 16SrVI-D (Ac. No. AF228052) subgroups (Zhao et al. 2009).

Insect sampling, identification and population dynamics

The major leafhopper (LH) species feeding on brinjal crops were collected at ten days interval using sweeping net method from July to October 2014 at Delhi, Noida and Gorakhpur locations. The collected insects were stored in plastic vials and stored at 4 °C in 70% ethanol for further studies. The collected insects were taxonomically identified by Network Project on Insect Biosystematics, Department of Entomology, Bangalore, India. For population sampling, yellow sticky trap cards were used in the brinjal fields (placed at North, South, East and West side of the field plots one meter inside border row) at a height of 30 cm and population sampling of leafhopper species on yellow sticky traps (5 × 5 cm) was calculated at ten days intervals from July to October 2014. The leafhopper population was

Fig. 1 Different types of symptoms on brinjal plants showing (**a**) little leaf at Delhi; (**b**) shortening of internodes and little leaf at Maharashtra; (**c**) witches' broom at Uttar Pradesh; (**d**) phyllody at Chhattisgarh; (**e**) stunting and yellowing of entire plant at Odisha; and (**f**) necrosis at Haryana

counted and correlated with brinjal little leaf disease incidence in later months in the same field (Un Nabi et al. 2015a).

Transmission trials

Eggs, nymphs and adults of *H. phycitis* (Distant) were collected from the disease-free fields and their colonies were established on healthy greenhouse-grown brinjal plants in pots until the emergence of first generation (3 weeks later). Ten individuals LH from established colony were tested by PCR assays for the presence of phytoplasmas, to ensure that leafhopper colonies were phytoplasma-free.

For leafhopper transmission assays, seeds of brinjal plants were sown in pots and placed in insect-free greenhouse inside the cages covered with plastic cylinder of 13″ diameter tightened on the top with muslin cloth. A total of 20 brinjal plants were grown in four pots in cages with five healthy brinjal plants in each pot. A total of 25 adults of *H. phycitis* (from established colonies in greenhouse) after acquisition access feeding of 72 h in BLL-infected pots were transferred to three of the pots (at four leaf stage) containing five healthy brinjal plants followed by an inoculation access period of 7 days. One cage with five healthy brinjal plants was used as control where no leafhopper was released. The leafhoppers in the treated pots were killed after 72 h using insecticide, imidacloprid (1 ml/3 l water) and the plants in each cage were continuously monitored for symptom expression up to 60 days of post inoculation. The killed insects and the symptomatic brinjal plants in experimental cages after transmission test were collected and analyzed for the presence of phytoplasma using PCR assays as described above.

Results

Survey, incidence of disease and symptomatology

During survey of brinjal fields at Uttar Pradesh, Haryana, Maharashtra, Odisha, Chhattisgarh, Assam, Bihar and

Delhi states of India during June 2014 to September 2015, an incidence of 3–34% (on the basis of visual observation) of phytoplasma suspected symptoms like little leaf, witches' broom, phyllody, necrosis and stunting of plants were recorded on brinjal plants (Table 1; Fig. 1a–f). Witches' broom and leaf yellowing symptoms were also observed on two weed species, *Cannabis sativa* subsp. *sativa* (Noida, Uttar Pradesh) and *Portulaca oleracea* (IARI, New Delhi) wildly grown in brinjal fields (Fig. 2a, b).

Identification of the leafhoppers

Five major leafhopper species feeding on symptomatic brinjal plants collected from brinjal fields of Noida, Gorakhpur and Delhi in the months of July–October 2014, were identified as *Empoasca prima* (Distant), *Amrasca biguttula biguttula* (Ishida) (ABB), *Oliarus* spp., *Hecalus* spp. and *Hishimonas phycitis* (Distant) (HP) (Table 2). The HP leafhopper was recorded as the major leafhopper species present in brinjal fields at Delhi and Noida (Uttar Pradesh) during August–October, 2014 followed by ABB at Delhi and Noida in August–October, 2014 on the basis of number of insects trapped on yellow sticky card. The high populations of HP in brinjal fields at Delhi (455) and Noida (275) were also found to be positively correlated with the increase in incidence of BLL symptoms in the respective brinjal fields in later months (Table 2; Fig. 6).

Phytoplasma detection by PCR assays

Eighteen symptomatic brinjal plants collected from different fields of eight states of India and phytoplasma control sample of toria phyllody phytoplasma (positive control) yielded approximately ~1.5 and 1.25 kb amplicons of 16S rDNA in direct and nested PCR assays, respectively, with primer pair P1/P6 and R16F2n/R16R2 (Table 1) (data not shown). However, no DNA amplification was achieved with any of five leafhopper species and two symptomatic weed species (*C. sativa* subsp. *sativa* and *P. oleracea*) analyzed in the first-round PCR assays with primer pair P1/P6. In nested PCR analysis, however, ~1.25 kb amplified products were obtained only with leafhopper *H. phycitis* from Delhi and both the symptomatic test weed species from Noida and Delhi (Table 1). No DNA amplification products were observed in direct and nested PCR assays with the same set of specific primers to 16S *rRNA* genes when DNA from asymptomatic brinjal, asymptomatic weed plants and *H. phycitis* collected from healthy brinjal fields was used. The 16S rDNA sequences of eighteen BLL strains, two weeds and the

Fig. 2 **a** Little leaf symptoms on *Cannabis sativa* subsp. *sativa* plants in BLL-infected field at Noida; **b** leaf yellowing and little leaf symptoms on *Portulaca oleracea* plants in BLL-infected field at IARI, New Delhi

Table 2 Detection of brinjal little leaf phytoplasma from different leafhopper samples in brinjal ecosystems

Insect vector	Location of collection of insects					
	Noida		Delhi		Gorakhpur	
	No. of insects collected (90 days)	PCR-positive samples/all tested samples	No. of insects collected (90 days)	PCR positive samples/all tested samples	No. of insects collected (90 days)	PCR-positive samples/all tested samples
Hishimonas phycitis	275	7/10	455	5/10	Nil	–
Amrasca biguttula biguttula	206	0/20	245	0/20	Nil	–
Empoasca prima	151	0/20	172	0/20	115	0/20
Hecalus spp.	121	0/20	95	0/20	Nil	–
Oliarus spp.	101	0/20	120	0/20	Nil	–

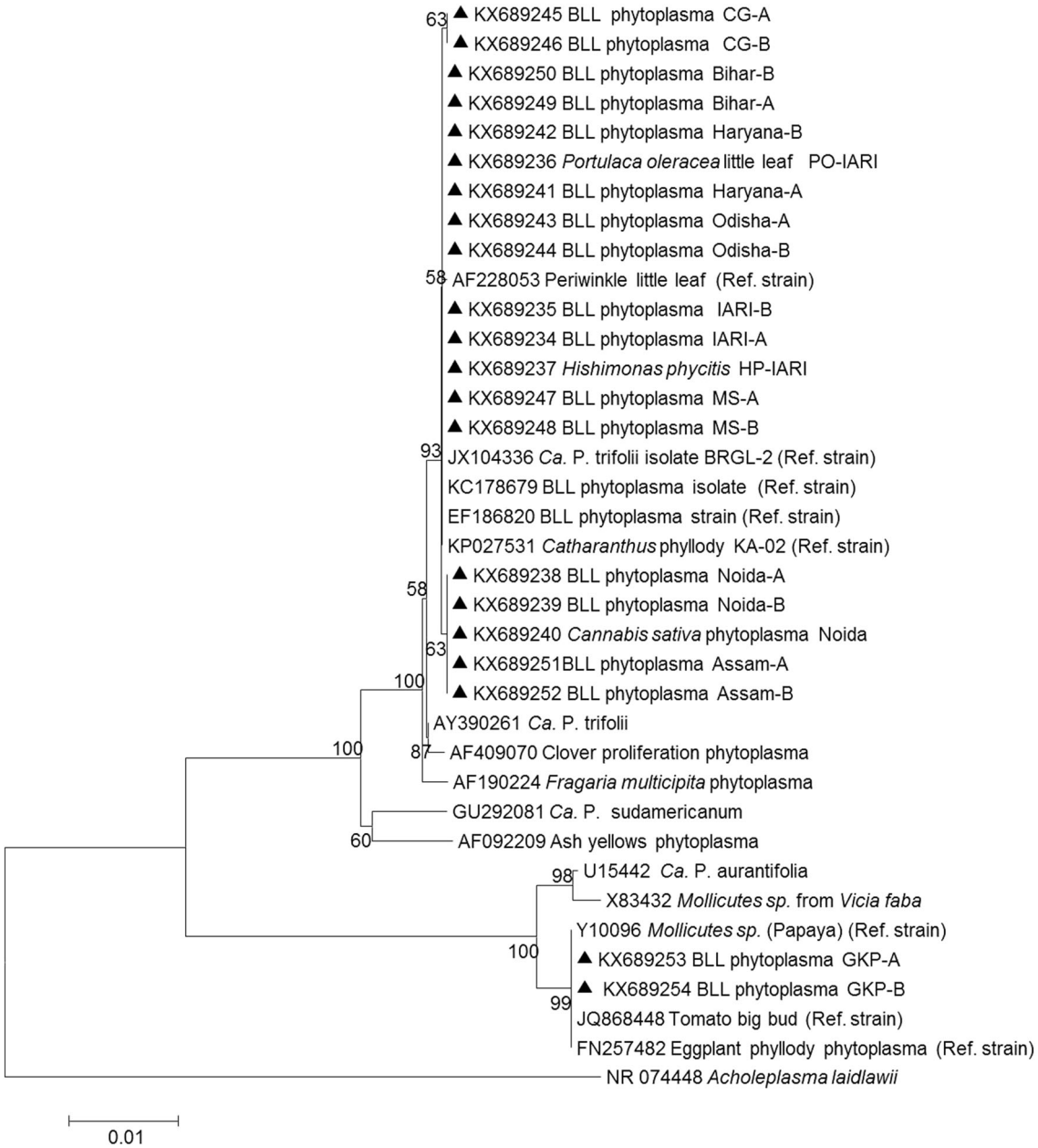

Fig. 3 Phylogenetic tree based on 16S rDNA constructed by neighbor-joining method showing the relationships among brinjal phyllody phytoplasma isolates, and reference phytoplasma strains. Accession numbers are specified in the tree. *Acholeplasma laidlawii* was used as an outgroup. MEGA 5.0 software was used to construct the tree. *Numbers* on branches are bootstrap values obtained for 1000 replicates (only values above 80% are shown)

leafhopper (*H. phycitis*) were submitted to the GenBank (Table 1).

Sequence analysis of phytoplasmas strains identified in brinjal, weeds and leafhopper

In pairwise sequence comparison, *16S rRNA* partial gene sequences of sixteen BLL phytoplasma strains associated with brinjal plants from eight states, *H. phycitis* (Distant) (Delhi), *C. sativa* subsp. *sativa* (Noida, Uttar Pradesh) and *P. oleracea* (IARI, Delhi) showed maximum 16S rDNA sequence identity of 99% with phytoplasma strains of clover proliferation group (16SrVI). However, the two identified BLL phytoplasma strains from Gorakhpur (GKP-A and GKP-B) revealed 99% sequence identity with phytoplasma strains of '*Ca. P. australasia*' group (16SrII) (Table 1). The phylogenetic analysis of 16S rDNA sequences confirmed that all the eighteen BLL strains, two weeds and leafhopper phytoplasma strains clustered together with the corresponding

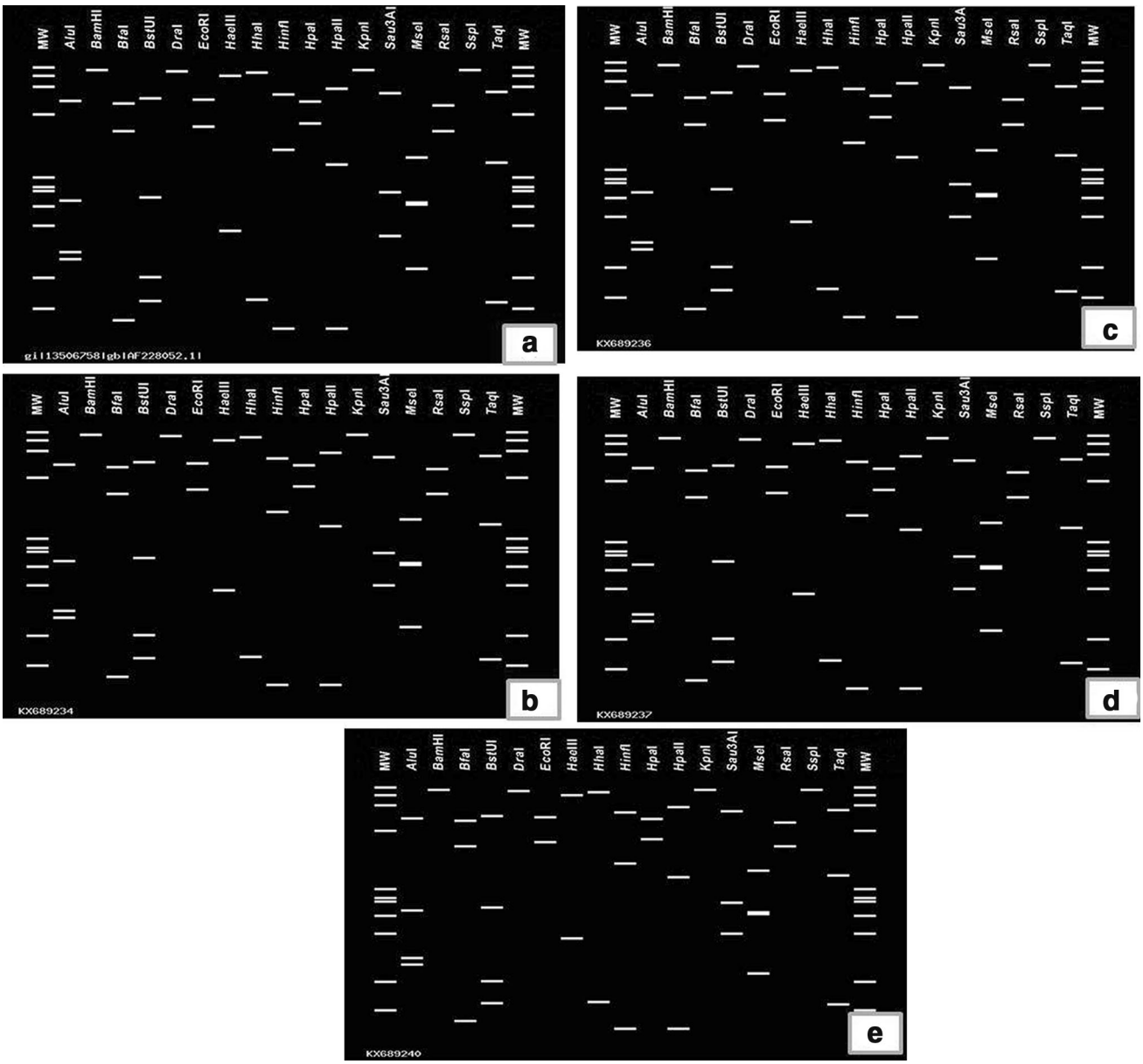

Fig. 4 Virtual RFLP patterns from in silico digestion of 16S rDNA R16F2n/R2 fragments of phytoplasmas strains infecting brinjal plants in India and the phytoplasma reference strains with 17 restriction enzymes (*Alu*I, *BamH*I, *Bfa*I, *BstU*I, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hinf* I, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I (*Sau3A*I), *Mse*I, *Rsa*I, *Ssp*I, and *Taq*I) using iPhyClassifier program. The patterns are compared for, 16SrVI-D reference strain (GenBank accession number AF228052 (**a**)) with IARI-A strain [Ac. No. KX689234 (**b**)], weeds viz. *C. sativa* subsp. *sativa* Noida strain [Ac. No. KX689240 (**e**)], *P. oleracea* IARI, Delhi strain [Ac. no. KX689236 (**c**)] and leafhopper *H. phycitis* strain [Ac. No. KX689237 (**d**)]

phytoplasma strains of subgroups 16SrII-D and 16SrVI-D (Fig. 3).

Virtual RFLP analysis

The virtual RFLP analysis of the F2nR2 region using 17 restriction enzymes for the representative BLL phytoplasma strains of both the 16SrII (Ac. No. KX689253) and 16SrVI (Ac. No. KX689234) groups, weed phytoplasma strains (*C. sativa* subsp. *sativa*, Ac. No. KX689240; *P. oleracea*, Ac. No. KX689236) and the leafhopper phytoplasma strain (Ac. No. KX689237) was performed for 16Sr subgroup assignments. Virtual RFLP analysis results revealed that BLL phytoplasma Delhi strain (Ac. No. KX689234), both the weed phytoplasma strains (Ac. No. KX689240, KX689236) and the HP IARI strain (Ac. No. KX689237) produced RFLP profiles identical to those of phytoplasma reference strain belonging to 16SrVI-D

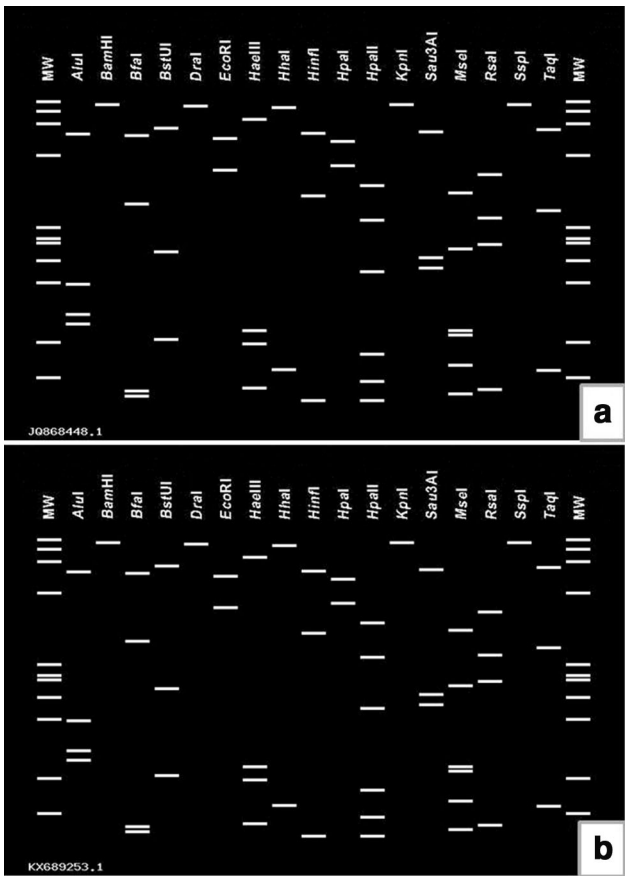

Fig. 5 Virtual RFLP patterns from in silico digestion of 16S rDNA R16F2n/R2 fragments of phytoplasmas strains infecting brinjal plants in India and the phytoplasma reference strains with 17 restriction enzymes (*Alu*I, *BamH*I, *Bfa*I, *BstU*I, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hinf* I, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I (*Sau3A*I), *Mse*I, *Rsa*I, *Ssp*I, and *Taq*I) using iPhyClassifier program. The patterns are compared for, 16SrII-D reference strain Tomato big bud phytoplasma [Ac. No. JQ868448 (**a**)] with GKP-A strain [Ac. No. KX689253 (**b**)]

subgroup (Ac. No. AF228052) (Fig. 4). However, predicted digestion fragments of the BLL phytoplasma strains GKP-A (Ac. No. KX689253) were found to be similar to the restriction profiles of the reference strain of tomato big bud phytoplasma classified under 16SrII-D subgroup (Ac. No. JQ868448) (Fig. 5).

Transmission of BLL by leafhopper

Since the leafhopper, *H. phycitis* was only found positive for the phytoplasma in our study, transmission assays were performed with HP under greenhouse conditions. In the transmission assays, 40–60% brinjal plants in all the three cages inoculated with *H. phycitis* showed typical little leaf disease symptoms after 60 days of leafhopper inoculation feeding and were tested positive for the presence of phytoplasma in the PCR assays by utilizing nested primer pairs (P1/P6 followed by R16F2n/R16R2). Five plants in the control cage without leafhopper inoculation did not display any disease symptoms and were also tested negative for the phytoplasma in PCR test. Sequence analysis of PCR amplicons from experimental caged insects and brinjal plants after transmission test indicated the presence of 16SrVI-D phytoplasma both in plants and insects (data not shown). The high population densities of HP leafhopper in the present study at Delhi and Noida which were positively correlated with high incidence of BLL symptoms in brinjal fields indicated its potentiality for secondary spread of BLL phytoplasma strain 16SrVI-D in nature (Fig. 6).

Discussion

The brinjal little leaf disease has a history of more than 75 years (Rao et al. 2010). Among the major biotic constraints in the production of brinjal, little leaf and phyllody disease is a serious disease capable of inflicting up to 34 per cent loss in yield (Abraham et al. 1977). No comprehensive genetic diversity study has been worked out on phytoplasma strains associated with BLL disease in India. So far, phytoplasmas belonging to six groups, viz. 16Sr I, 16Sr II-D, 16Sr III-J, III-U, 16Sr VI-A and -D, 16SrIX-C and 16SrXII-A were reported to infect brinjal worldwide. However, in India, so far, only group-level identification of BLL phytoplasma strains by utilizing 16S rDNA and *secA* gene was reported (Khan et al. 2007; Kumar et al. 2012; Manjunatha et al. 2012; Azadvar and Baranwal, 2012). In the present study, association of two phytoplasma subgroups, 16Sr VI-D and II-D, was confirmed with BLL strains infecting brinjal plants in eight states of India on the basis of sequence comparison, phylogeny and RFLP analysis of 16S rDNA sequences. Our results also confirmed that 16SrVI-D phytoplasma is the most widespread infecting brinjal crops in India and the 16Sr II-D subgroup was only reported from Uttar Pradesh. However, the 16SrI group earlier reported from Bihar associated with BLL was not confirmed in the present study indicating the absence of 16SrI group phytoplasmas in surveyed fields during the study.

In India, 16SrVI group of phytoplasmas has been reported to be associated with several diseases of plants, viz. *Araucaria* little leaf (Gupta et al. 2009), brinjal little leaf (Azadvar and Baranwal 2012), *Withania* little leaf (Zaim and Samad 1995; Samad et al. 2006), *Portulaca* little leaf (Samad et al. 2008), *Datura* little leaf (Raj et al. 2009; Singh et al. 2012) and *Calotropis gigantea* leaf yellows (Madhupriya et al. 2010). However, occurrence of 16SrVI-D subgroup phytoplasma was reported only on anantmool, lemongrass, sesame, *Hibiscus rosa-sinensis*, *Saponaria officinalis* and *Allamanda cathartica* (Madhupriya et al. 2014, 2015; Khasa et al. 2016). Hence, in our

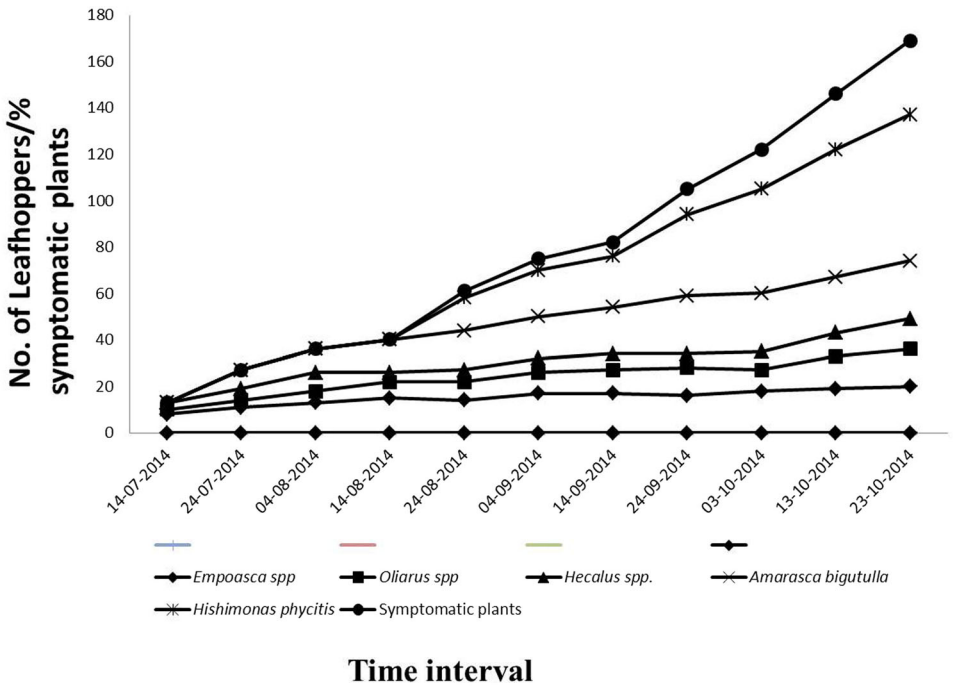

Fig. 6 Population density of leafhopper species (ES, HP, ABB, HS and OS) feeding on brinjal plants and brinjal little leaf incidence in the fields at Delhi

study, brinjal is reported to be an additional host of '*Ca. P. australasia*' subgroup D in India.

In India, BLL transmission was demonstrated by *H. phycitis* earlier, but only on the basis of presence and identification of leafhoppers in infected fields and presence of phytoplasma in insects by PCR assays (Bindra and Singh 1969; Azadvar and Baranwal 2012). No proper transmission assays were performed earlier to confirm any leafhopper species as natural vector of BLL disease in India. In the present study, we confirmed that *H. phycitis* is a natural vector of BLL phytoplasma strain belonging to 16SrVI-D subgroup. *H. phycitis* leafhopper was also reported as natural vector of phytoplasma associated with sesame phyllody in India (Un Nabi et al. 2015b) and lime witches' broom in Iran (Siampour et al. 2006; Salehi et al. 2007). The high population densities of *H. phycitis* (tested positive for phytoplasma) in the present study from July to October, 2014 followed by high incidence of BLL symptoms in brinjal fields in later months at Delhi indicates its potentiality as natural vector of BLL phytoplasma of 16SrVI group.

Several weeds are reported as reservoirs of important phytoplasmas and play an important role in natural spread of phytoplasmas (Blanche et al. 2003; Pasquini et al. 2007; Mall et al. 2011). Chaube et al. (2015) reported the association of 16SrXIV group phytoplasma in *Cannabis sativa* subsp. *sativa* at Shahjahanpur, Uttar Pradesh, India. Association of '*Ca. P. trifolii*' (16SrVI-D) was also identified and characterized in two weed hosts, *C. sativa* subsp. *sativa* and *P. oleracea* in and around brinjal fields by 16S rDNA sequence comparison, RFLP and phylogenetic analysis. Early detection of phytoplasmas in weed plants is very important to check the possibility of further spread of phytoplasma diseases to other commercial crops.

In India, brinjal is cultivated in the same season with many other agricultural crops. The scenario of natural phytoplasma spread from brinjal to other plant species and vice versa, through an efficient vector species, is of major concern. Hence, it would be important to evaluate the role of different epidemiological factors involved in the natural spread of phytoplasmas associated with BLL disease in India.

The results of the present study confirmed about a new natural vector and new weed host species of 16SrVI-D phytoplasma strain. BLL disease is a major problem in all brinjal-growing regions of India. The resistant cultivars, management of insect vectors and natural hosts would be the most efficient control measures of the disease in the field (Rao et al. 2010). Hence, the new BLL phytoplasma vector and the possible weed reservoirs of phytoplasma reported in this study will provide further research prospective on formulating management strategies of BLL disease in India.

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Compliance with ethical standards

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

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