Molecular characterization of ‘Clover proliferation’ phytoplasma subgroup-D (16SrVI-D) associated with vegetables crops in India

Gopala¹ · Ekta Khasa¹ · Ashutosh Rao¹ · Madhupriya¹ · G. P. Rao¹

Received: 4 April 2017 / Revised: 29 November 2017 / Accepted: 26 December 2017 / Published online: 22 January 2018
© Prof. H.S. Srivastava Foundation for Science and Society 2018

Abstract Nine vegetable plants species exhibiting phytoplasma suspected symptoms of white/purple leaf, little leaf, flat stem, witches’ broom, phyllody and leaf yellowing were observed in experimental fields at Indian Agricultural Research Institute, New Delhi from December 2015 to July 2016. Total DNA extracted from the three healthy and three symptomatic leaves of all the nine vegetables were subjected to PCR assays using phytoplasma specific primers P1/P7 followed by R16F2n/R16R2 and 3Far/3Rev to amplify the 16S rDNA fragments. No amplifications of DNA were observed in first round PCR assays with primer pair P1/P7 from any of the symptomatic samples. However, phytoplasma DNA specific fragments of ~ 1.3 kb were amplified from *Apium graveolens* L. (two isolates), *Brassica oleracea* var. *capitata* L. (one isolate) and *Solanum melongena* L. (one isolate) by using 3Far/3Rev primer pair and 1.2 kb fragment was amplified from *Lactuca sativa* L. (one isolate) by using R16F2n/R16R2 primer pair. No DNA amplification was seen in other symptomatic vegetable samples of tomato, carrot, cucurbit, bitter gourd and *Amaranthus* species utilizing either P1/P7 primer pair followed by 3Far/3Rev or R16F2n/R16R2 primer pairs. Out of three leafhopper species collected from the symptomatic vegetable fields, only *Hishimonus phycitis* was found positive for association of phytoplasma. No DNA amplifications were observed in healthy plant samples and insects collected from non-symptomatic fields. Comparative sequence comparison analyses of 16S rDNA of positive found vegetable phytoplasma strains revealed 100%

sequence identities among each other and with phytoplasma strains of ‘clover proliferation’ (16SrVI) group. Phytoplasma sequences, virtual RFLPs and phylogenetic analyses of 16S rDNA sequence comparison confirmed the identification of 16SrVI subgroup D strain of phytoplasmas in four vegetables and one leafhopper (HP) species. Further virtual RFLP analysis of 16S rDNA sequence of the vegetables phytoplasma strains confirmed their taxonomic classification with strains of ‘clover proliferation’ subgroup D. Since, *H. phycitis* feeding on symptomatic vegetable species in the study was also tested positive for the 16SrVI phytoplasma subgroup-D as of vegetables; it may act as potent natural reservoir of 16SrVI-D subgroup of phytoplasmas infecting vegetable and other important agricultural crops.

Keywords Phytoplasma · 16SrVI group · Identification · Lettuce · Cabbage · Celery · Brinjal · *Hishimonas phycitis*

Introduction

Vegetables play a major role in Indian economy by providing high returns in terms of food, nutrition and economic security. In India, vegetables provide greater income leading to improved livelihoods. Major factors that limit vegetable production besides its narrow genetic base are extreme susceptibility of the crops to biotic and abiotic stresses. Among the biotic stress, phytoplasmas are causing severe losses to many economical vegetable species all around the world (Bertaccini et al. 2014). Phytoplasma are small phytopathogenic bacteria that inhabit phloem sieve elements of infected plants with genome size ranging between 530 kb and 1650 kb. Phytoplasma caused more than 600 plant diseases and cause serious damage with

✉ G. P. Rao
gpao_gor@rediffmail.com

¹ Division of Plant Pathology, Indian Agricultural Research Institute, Pusa Campus, New Delhi 110012, India

significant impact on agriculture economics (Bertaccini et al. 2014). There is no authorized curative treatment for phytoplasma disease in vegetables except using genetic resistant sources (Jaurausch et al. 1999; Rao and Kumar 2017). Management of phytoplasma can also be effectively monitored by controlling the insect vectors and roughing alternate/collateral hosts, elimination of diseases through meristem tip culture and applying antibiotics, which are the best approaches to minimize the incidence of phytoplasmas (Bertaccini et al. 2014).

Many different vegetable crops are seriously affected by different groups of phytoplasmas worldwide (Sichani et al. 2014). The most important phytoplasma diseases, infecting vegetables are brinjal little leaf, potato witches' broom, potato purple top, tomato big bud, chilli leaf curl and cucurbit phyllody, which cause significant economic losses (Lee et al. 2000; Girsova et al. 2008; Hosseini et al. 2011; Ivanović et al. 2011; Tran-Nguyen et al. 2003; Mejia et al. 2011; Du et al. 2013; Dehghan et al. 2014; Esmailzadeh Hosseini et al. 2015). Considering the economic importance of the vegetables and the little existing knowledge of phytoplasma strains association available with vegetable crops in India (Rao et al. 2010), the present study was planned to identify the phytoplasmas in nine vegetable crops showing phytoplasma suspected symptoms grown at IARI, New Delhi, India in the year 2015 and 2016.

Materials and methods

Plant samples and leafhopper survey

During survey of vegetable crops in 2015 and 2016, phytoplasma suspected symptoms and incidence in celery (*Apium graveolens* L.), carrot (*Daucus carota*), cabbage (*Brassica oleracea* var. *capitata* L.), lettuce (*Lactuca sativa* L.), brinjal (*Solanum melongena* L.), cucurbit (*Cucurbita pepo* L.), bitter gourd (*Momordica charantia* L.), amaranth (*Amaranthus* spp.) and tomato (*Solanum lycopersicum* L.) was recorded. Leaf leafhopper (LH) species from the symptomatic and non symptomatic vegetable fields were also collected in the morning hours by sweeping net method at Indian Agricultural Research Institute New Delhi, India. All the symptomatic, non-symptomatic vegetable plants and leafhopper samples were collected from the fields and stored at -20°C for PCR assays. The present study was planned to identify the pathogens associated with nine vegetable crops showing phytoplasma suspected symptoms. The collected leafhopper specimens were identified from Division of Entomology, IARI, New Delhi.

DNA extraction and PCR assays

DNA was extracted from leaf vein tissues of nine symptomatic and asymptomatic vegetables plant species and salivary glands from ten individuals of identified leafhopper species by CTAB method (Ahrens and Seemuller 1992). The salivary gland of leafhopper species was isolated as method described by Marzachi et al. (1998). Extracted DNA from all the vegetable plants and leafhoppers species were used as template in nested PCR assays by primer pair P1/P7 in first round (Schneider et al. 1995) followed by nested primer pair 3Far/3Rev (Manimekalai et al. 2010) and R16F2n/R16R2 (Gundersen and Lee 1996) in second round nested PCR assays. PCR reactions were performed in a Master cycler (Eppendorf, Germany) and the cycling protocol was followed as described earlier (Rao et al. 2014). The DNA of toria phyllody phytoplasma (16SrIX group) maintained on periwinkle was used as positive control. The DNA extracted from healthy and asymptomatic vegetable plants and leafhoppers collected from distant non-symptomatic vegetable fields were used as negative controls. Twenty-five microlitres of PCR product was subjected to electrophoresis through a 1.0% (w/v) agarose gel followed by staining with Good View dye and visualization of DNA bands with UV transilluminator. Nested PCR products (1.2 and 1.3 kb amplicons) were purified using the Wizard[®] SV Gel and PCR Clean-up System (Promega, USA).

Sequencing analysis

The ~ 1.2 and ~ 1.3 kb nested PCR products of vegetable phytoplasma isolates were sequenced directly using 3Far/3Rev and R16F2n/R16R2 primers by Macrogen Company (South Korea) for both the strands. The sequences were assembled using DNA baser V.4 program and were further aligned using CLUSTAL W program of Bio-Edit software. Aligned and edited sequences were deposited in NCBI GenBank. Samples identification was completed by comparison of the consensus 16S rDNA sequences available in Genbank by BLAST protocol (<http://blast.ncbi.nlm.nih.gov>). The sequence identity percentages provided in results are over entire length of the query sequences in GenBank.

Phylogenetic and RFLP analysis

The six 16S rDNA sequence generated from the vegetables and leafhopper phytoplasma strains from this study and eight selected reference phytoplasma strains' sequences belonging to different 16SrVI subgroups representatives retrieved from GenBank were used to compute phylogenetic tree by neighbor joining method with 1000

Table 1 Survey, symptoms, locations and PCR results for phytoplasma detection on vegetable plants

Sr. no.	Plant species common name/family	Survey period	Disease symptoms	PCR assay	GenBank Acc. No	Group & sub-group
1	<i>Apium graveolens</i> L. Carrot (Apiaceae)	May 2016	WL	+	KX671551, KX671552	16Sr VI-D
2	<i>Solanum melongena</i> L. Brinjal (Solanaceae)	May 2016	LL	+	KX421256	16Sr VI-D
3	<i>Cucurbita pepo</i> L. Pumpkin (Cucurbitaceae)	February 2016	FV	–	–	–
4	<i>Lactuca sativa</i> L. Lettuce (Asteraceae)	May 2016	FS	+	KX671554	16Sr VI-D
5	<i>Momordica charantia</i> L. Bitter gourd (Cucurbitaceae)	June 2016	LY	–	–	–
6	<i>Amaranthus</i> spp (Amaranthaceae)	June 2016	LY	–	–	–
7	<i>Solanum lycopersicum</i> L. Tomato (Solanaceae)	July 2016	LY	–	–	–
8	<i>Brassica oleracea</i> var. <i>capitata</i> L. Cabbage (Brassicaceae)	May 2016	WB	+	KX6971553	16Sr VI-D
9	<i>Daucus carota</i> L. Carrot (Apiaceae)	July 2016	VL	–	–	–

IARI = Indian Agricultural Research Institute, New Delhi

WL white leaf, WB witches' broom, LL little leaf, FV floral virescence and phyllody, FS flattened stem, LY leaf yellowing, VL violet leaves

replications for each bootstrap value using MEGA 5.0 software version (Tamura et al. 2011). The 16S rRNA sequence of *Acholeplasma laidlawii* (Acc. No. M 23932) was employed as outroot the phylogenetic tree.

In silico RFLP analyses of 3Far/3Rev and R16F2n/R16R2 amplicons of vegetables and leafhopper were digested with 17 restriction enzymes (*AluI*, *BfaI*, *TaqI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *BamHI*, *RsaI*, *BstUI* and *SspI*) using pDRAW32 program (<http://www.acaclone.com>) and compared with representative sequences of the brinjal little leaf phytoplasma strain (Acc. No KP027505) and *Catharanthus* phyllody phytoplasma strain (Acc. No KP027531) for assigning 16Sr sub-groups status of vegetable phytoplasma strains analysed by the same restriction mapping utilizing AcaClone software generated RFLP profiles.

Results

Survey and symptomatology

Phytoplasma suspected symptoms of white leaf in celery (*Apium graveolens*), purple leaf in carrot (*Daucus carota*), witches' broom in cabbage (*Brassica oleracea* var. *capitata*), flat stem in lettuce (*Lactuca sativa*), little leaf in brinjal

(*Solanum melongena*), phyllody in cucurbit (*Cucurbita pepo*) and leaf yellowing in bitter gourd (*Momordica charantia*), Amaranth (*Amaranthus* spp) and tomato (*Solanum lycopersicum*) were recorded during survey of vegetable fields at IARI, New Delhi in 2015 and 2016 (Table 1, Fig. 1a–i). The disease incidence varied from 2 to 6% in different surveyed fields (Table 1).

Identification of the leafhoppers specimens

Empoasca prima (Distant), *Exitianus indicus* (Distant) and *Hishimonus phycitis* (Distant) were identified as the major leafhopper species feeding on symptomatic vegetable plants in the fields of IARI, New Delhi during December 2015 to July 2016.

Detection of phytoplasma by PCR Assays

First round PCR amplification did not yield the expected 1.8 kb product of the 16S rRNA gene region from any of the symptomatic vegetable test samples with primer pair P1/P7. However, 1.3 kb DNA products were amplified from *A. graveolens* (two isolates), *B. oleracea* var. *capitata* (one isolate), *S. melongena* (one isolate) and one leafhopper species (*H. phycitis*) by using 3Far/3Rev primer pair, however, 1.2 kb product was amplified from *L. sativa* by

Fig. 1 Phytoplasma suspected symptoms on vegetable plant species **a** white leaf in celery (*Apium graveolens*); **b** witches' broom in cabbage (*Brassica oleracea* vr. *capitata*); **c** flat stem in lettuce (*Lactuca sativa*); **d** little leaf in brinjal (*Solanum melongena*);

e phyllody in cucurbit (*Cucurbita pepo*); **f** leaf yellowing in bitter melon (*Momordica charantia*); **g** leaf yellowing in amaranth (*Amaranthus* spp); **h** leaf yellowing in tomato (*Solanum lycopersicum*); **i** violet coloured leaf in carrot (*Daucus carota* sub sp. *sativus*)

using R16F2n/R16R2 primer pair and the positive control (toria phyllody phytoplasma, Azadvar and Baranwal 2012) (Table 1). No amplifications were observed in any of the rest five symptomatic vegetable samples of tomato, carrot, cucurbit, bittergourd and *Amaranthus* sp along with the non-symptomatic samples (Table 1). There were also no amplification observed from DNA isolated from salivary glands of two leafhopper species, *E. prima* and *E. indicus*.

Sequence analysis and phylogenetic relationships

Pair wise sequence comparison of the 16S rRNA gene sequences of the five positive vegetable phytoplasma strains of *A. graveolens* (two isolates), *B. oleracea* vr. *capitata*, *L. sativa*, *S. melongena* (one isolate) and one phytoplasma strain of *H. phycitis* revealed 100% genetic identity among themselves and with brinjal little leaf (Acc. No. KP027505), *Catharanthus* phyllody (Acc. No.

KP027531) and *Mollicutes* sp phytoplasma strain (Acc. No. X83431) sequences, which belonged to clover proliferation (16SrVI) group. Phylogenetic analysis also revealed the clustering of all the five vegetable phytoplasma strains and one leafhopper (HP) phytoplasma strain in the present study with strains of 16SrVI-D (Fig. 2).

The results of virtual RFLP gel plotting using the pDRAW32 program revealed that the all the five positive phytoplasma strains from vegetables and one leafhopper associated strain produced a identical virtual RFLP profiles to phytoplasma reference strains of brinjal little leaf (KP027505) and *Catharanthus* phyllody (KP027531) phytoplasmas belonging to 16SrVI-D subgroups (Fig. 3). Therefore, five phytoplasma strains from the four vegetable species and the *H. phycitis* (leafhopper) in the present study were identified as strains of 16Sr VI-D subgroup.

Fig. 2 Phylogenetic tree constructed by neighbor-joining method showing the relationships among celery white leaf, lettuce flattened stem, cabbage witches' broom and brinjal little leaf phytoplasma, and reference phytoplasma strains. Accession numbers are specified in the

tree. 'Ca. P.' stands for 'Candidatus Phytoplasma sp.' *A. laidlawii* was used as an out group. Mega 7.0 software was used to construct the tree

Discussion

Phytoplasma-associated vegetable diseases are distributed worldwide, and in several cases were associated with severe epidemics. So far, five different groups (16SrI, 16SrII, 16SrVI, 16SrIX, 16SrXII) of phytoplasmas were identified in vegetables all over the world (Lee et al. 2000; Verdin et al. 2003; Zhang et al. 2004; Delic et al. 2016; Salehi et al. 2007; Rao et al. 2010; Ivanovic et al. 2011; Bertaccini et al. 2014). Among them, the aster yellows phytoplasma group (16SrI) is the widest distributed and dominant group recorded on vegetables species from all over the world (Kumar 2015).

In India, different groups of phytoplasmas were reported to be associated with several vegetable plants like 16SrI group in potato (Tiwari et al. 2013), chilli (Khan and Raj 2006), ladies' finger (Kumar et al. 2012a, b) and 16SrII group in lettuce, carrot and beans (Ghosh et al. 1999; Ghanekar et al. 1988; Arocha et al. 2008). Besides, three different phytoplasma groups (16Sr I, -II and -VI) were identified associated with brinjal little leaf disease from different states of India (Rao and Kumar 2017). In the present study we have reported occurrence of 16Sr VI subgroup D phytoplasma strain in brinjal, lettuce, celery and cabbage.

Earlier infection of 16SrI-B subgroup phytoplasma associated with stunting and witches' broom in cabbage (*B. oleracea* vr. *capitata*) was reported from Greece (Gkavaleka et al. 2012), China (Mou et al. 2012) and USA (Lee

et al. 2001). Salehi et al. (2007) reported 16SrVI group of phytoplasma on cabbage (*B. oleracea* vr. *capitata*) from Iran. No report of phytoplasma on cabbage (*B. oleracea* vr. *capitata*) is available from India, hence, occurrence of 16Sr VI-D subgroup phytoplasma strain on cabbage is new report from India.

Lee et al. (2000) reported phytoplasma of 16SrI-B subgroup infecting celery crops in North America and Europe. Later, other groups of phytoplasmas were reported on celery from other parts of the world, like tomato big bud phytoplasma (16SrII-E) from Australia (Tran-Nguyen et al. 2003) and stolbur phytoplasma (16SrXII-A) from Italy, Serbia and Romania (Carraro et al. 2008; Ivanović et al. 2011; Chireceanu et al. 2016), 16SrVII and IX-A groups from New Zealand (Liefteing et al. 2011) and 16SrI-C from the Czech Republic (Fránová and Špak 2013). No report of 16SrVI group of phytoplasmas incidence has been recorded earlier on celery from India and abroad, and hence, is the new report.

In lettuce, there are three phytoplasma reports available as 16SrIX and 16 SrI-B groups from Iran (Salehi et al. 2006; Sichani et al. 2014), 16SrI-B subgroup from China (Zhang et al. 2004) and 16SrII group from India (Arocha et al. 2008). The report of 16SrVI-D subgroup of phytoplasma on lettuce from India in the present study is also the new report.

Brinjal little leaf phytoplasmas belonging to four groups, viz. 16SrI from Japan, Bangladesh and India (Okuda et al. 1997; Kelly et al. 2009; Kumar et al.

Fig. 3 Comparison of virtual RFLP patterns derived from in silico digestions of 1.2 and 1.3 kb 16S rDNA sequences of vegetables in the present study, along with reference standard strains of 16SrVI-D

2012a, 2012b), 16SrII-D from Egypt (Omar and Foissac 2012), 16SrIII-J and 16SrIII-U from Brazil (Mello et al. 2011) and 16SrVI-C, -D and -E from China, India and USA (Hiruki and Wang. 2004,2008; Wei et al. 2008; Kumar et al. 2017) were reported to infect brinjal. In the present study, we have reported again the occurrence of 16SrVI-D subgroup phytoplasma associated with brinjal which was earlier recorded as the most wide spread group of phytoplasmas infecting brinjal crops in India (Kumar et al. 2017). This confirm the regular recurrence of the disease which is responsible for serious economic losses.

In addition to vegetable crops, we have also identified 16SrVI-D subgroup phytoplasma in the salivary gland of the leafhopper, *H. phycitis* from the phytoplasma infected vegetable fields in the study area indicating its possible role as a vector of this group of phytoplasmas. Earlier, Kumar

et al. (2017) established the role of *H. phycitis* as a natural vector in transmitting brinjal little leaf phytoplasma strain belonging to 16SrVI-D group from India. The 16SrVI-D phytoplasma subgroup, have already been reported earlier as a major group of phytoplasma infecting several crops viz. brinjal (Rao and Kumar 2017), ornamental plants (Khasa et al. 2016), medicinal plants (Madhupriya et al. 2015) and sesame (Madhupriya et al. 2015) in India.

In India, many vegetables are being cultivated parallel with many other agricultural crops in different seasons. The reports of wider spread, broad host range and capability of transmission through insect vectors and weeds, 16SrVI-D phytoplasma strains pose a serious threat and thus this phytoplasma strain is of great epidemiological significance in India. Hence, further studies are required to work out the role of epidemiological significance of this strain

(16SrVI-D subgroup) in different parts of India for planning a better management approach.

Compliance with ethical standards

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

References

- Ahrens U, Seemuller 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. *J Phytopathol* 82:828–832
- Arocha Y, Singh A, Pandey M, Tripathi AN, Chandra B, Shukla SK, Arif M (2008) New plant hosts for group 16SrII, '*Candidatus Phytoplasma aurantifolia*' in India. *New Dis Rept* 17:36
- Azadvar M, Baranwal VK (2012) Multilocus sequence analysis of phytoplasma associated with brinjal little leaf disease and its detection in *Hishimonas phycitis* in India. *Phytopathogenic Mollicutes* 2:15–21
- Bertaccini A, Duduk B, Paltrinieri S, Contaldo N (2014) Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. *Am J Plant Sci* 5(12):46–62
- Carraro L, Ferrini F, Martini M, Ermacora P, Loi N (2008) A serious epidemic of stolbur on celery. *J Plant Pathol* 90(1):131–135
- Chireceanu C, Chiriloiu A, Teodoru A (2016) Molecular detection of "stolbur" phytoplasma in celery plants in Romania. *Phytopathogenic Mollicutes* 6(1):46–49
- Dehghan H, Salehi M, Khanchezar A, Afshar H (2014) Biological and molecular characterization of a phytoplasma associated with greenhouse cucumber phyllody in Fars province. *Iran J Plant Pathol* 50:393–401
- Delic N, Contaldo B, Lolic D, Moravčević, Bertaccini A (2016) first report of '*Candidatus Phytoplasma solani*' in pepper and celery in Bosnia and Herzegovina. *J Plant Pathol* 98(1):171–185
- Du Y, Mou H, Shi B, Xu X, Xiang B (2013) Molecular detection and identification of a 16SrVI group phytoplasma associated with tomato big bud disease in Xinjiang, China. *J Phytopathol* 161:870–873
- Esmailzadeh Hosseini SA, Salehi M, Mirchenari SM, Tarizeh D, Gholampoor H (2015) First report of a 16SrVI group related phytoplasma associated with cucumber phyllody in a greenhouse in Iran. *New Dis Rep* 32:5
- Fránová J, Špak J (2013) First report of a 16SrI-C phytoplasma infecting celery (*Apium graveolens*) with stunting, bushy top and phyllody in the Czech Republic. *J Phytopathol* 161:666–670
- Ghanekar AM, Manohar SK, Reddy SV, Nene YL (1988) Association of a mycoplasma-like organism with chickpea phyllody. *Indian Phytopathol* 41:462–464
- Ghosh DK, Das AK, Shayam S, Singh SJ, Ahlawat YS, Singh S (1999) Occurrence of witches' broom, a new phytoplasma disease of acid lime (*Citrus aurantifolia*) in India. *Plant Dis* 83:302
- Girsova N, Bottner KD, Mozhaeva KA, Kastalyeva TB, Owens RA, Lee IM (2008) Molecular detection and identification of group 16SrI and 16SrXII phytoplasmas associated with diseased potatoes in Russia. *Plant Dis* 92:654
- Gkavaleka S, Efthimiou K, Lotos L, Katis NI (2012) First report of a '*Candidatus Phytoplasma asteris*' related strain associated with cabbage stunting in Greece. *J Plant Pathol* 94(4):4–6
- Gundersen DE, Lee IM (1996) Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathol Mediter* 1:144–151
- Hiruki C, Wang K (2004) Clover proliferation phytoplasma: '*Candidatus Phytoplasma trifolii*'. *Int J Syst Evol Microbiol* 54:1349–1353
- Hiruki C, Wang K (2008) Clover proliferation phytoplasmas and their subgroup members. In: Harrison NA, Rao GP, Marcone C (eds) *Characterization, diagnosis and management of phytoplasmas*. Studium Press LLC, Texas, pp 325–351
- Hosseini P, Bahar M, Madani G, Zarak L (2011) Molecular characterization of phytoplasmas associated with potato purple top disease in Iran. *J Phytopathol* 159:241–246
- Ivanović Z, Nenad T, Svetlana Ž, Erika PD, Nenad D, Jelena J, Milana M (2011) First report of stolbur phytoplasma infecting celery in Serbia. *Bull Insectol* 64:S239–S240
- Jaurausch W, Eyquard J, Mazy K, Lannac M, Dosba F (1999) High level of resistance of sweet cherry (*Prunus avium* L.) towards European stone fruit yellows phytoplasmas. *Adv Hort Sci* 13(3):108–112
- Kelly PL, Arocha Y, Dider SZ (2009) First report of a 16SrI, '*Candidatus Phytoplasma asteris*' isolate affecting eggplant and *Mikania* sp. in Bangladesh. *New Dis Rep* 18:52
- Khan MS, Raj SK (2006) First report of molecular detection of an aster yellows phytoplasma ('*Candidatus Phytoplasma asteris*') isolate infecting chilli (*Capsicum annuum*) in India. *Plant Pathol* 155:822
- Khasa E, Gopala Taloh A, Prabha T, Rao GP (2016) Molecular characterization of phytoplasmas of 'Clover proliferation' group associated with three ornamental plant species in India. *3 Biotech* 6(2):237
- Kumar M (2015) Genetic diversity and natural spread sources of brinjal little leaf phytoplasma. M.Sc. Thesis, IARI, New Delhi, p 153
- Kumar J, Gunapati S, Singh SP, Lalit A, Sharma NC, Tuli R (2012a) First report of a '*Candidatus Phytoplasma asteris*' (16SrI group) associated with little leaf disease of *Solanum melongena* (brinjal) in India. *New Disease Rep* 26:21
- Kumar S, Singh V, Lakhanpaul S (2012b) Molecular characterization and phylogeny of phytoplasma associated with bunchy top disease in its new host Okra (*Abelmoschus esculentus*) in India reveal a novel lineage within the 16SrI group. *Eur J Plant Pathol* 133(2):371–378
- Kumar M, Priya M, Rao GP (2017) Molecular characterization, vector identification and sources of phytoplasmas associated with brinjal little leaf disease in India. *3 Biotech* 7(1):1–11
- Lee IM, Hammond RW, Davis RE, Gundersen DE (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma-like organisms. *Phytopathology* 83:834–842
- Lee IM, Davis RE, Gundersen-Rindal DE (2000) Phytoplasma: phytopathogenic mollicutes. *Ann Rev Microbiol* 54:221–255
- Lee IM, Dane RA, Black MC, Troxclair N (2001) First report of an aster yellows phytoplasma associated with cabbage in southern Texas. *Plant Dis* 85(4):447
- Liefting LW, Stella V, Gerard RG, Clover (2011) New hosts of '*Candidatus Phytoplasma australiense*' in New Zealand. *Aust Plant Pathol* 40(3):238
- Madhupriya Rao GP, Kumar A, Baranwal VK (2015) Classification of the sesame phytoplasma strains in India at the 16Sr subgroup level. *J Plant Pathol* 97:523–528
- Manimekalai R, Soumya VP, Kumar RS, Selvarajan R, Reddy K, Thomas GV, Sasikala M, Rajeev G, Baranwal VK (2010) Molecular detection of 16SrXI group phytoplasma associated with root (wilt) disease of coconut (*Cocos nucifera*) in India. *Plant Dis* 94:636
- Marzachi C, Verati F, Bosco D (1998) Direct PCR detection of phytoplasmas in experimentally infected insects. *Ann Appl Biol* 133:45–54

- Mejia JF, Contaldo N, Paltrinieri S, Pardo JM, Rios CA, Alvarez A, Bertaccini A (2011) Molecular detection and identification of group 16SrV and 16SrXII phytoplasmas associated with potatoes in Colombia. *Bull Insectol* 64:S97–S98
- Mello AP, Eckstein B, Flores D, Kreyci PF, Bedendo IP (2011) Identification by computer-simulated RFLP of phytoplasmas associated with eggplant giant calyx representative of two subgroups, a lineage of 16SrIII-J and the new subgroup 16SrIII-U. *Int J Syst Evol Microbiol* 61:1454–1461
- Mou HQ, Zhang YJ, LiH X, Zhu SF, Li ZH, Zhao WJ (2012) Molecular identification of a '*Candidatus Phytoplasma asteris*' associated with cabbage witches-broom in china. *J Phytopathol* 1160:304–307
- Okuda S, Prince JP, Davis RE, Dally EL, Lee IM, Mogen B (1997) Two groups of phytoplasma from Japan distinguished on the basis of amplification and restriction analysis of 16S rDNA. *Plant Dis* 81:301–305
- Omar AF, Foissac X (2012) Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crop in Egypt. *Eur J Plant Pathol* 133:353–360
- Rao GP, Kumar M (2017) World status of phytoplasma diseases associated with eggplant. *Crop Protection* 96:22–29
- Rao GP, Mall S, Raj Snehi SK (2010) Phytoplasma diseases affecting various plant species in India. *Acta Phytopathol et Entomol Hungarica* 46:59–99
- Rao GP, Madhupriya Tiwari AK, Kumar S, Baranwal VK (2014) Identification of sugarcane grassy shoot-associated phytoplasma and one of its putative vectors in India. *Phytoparasitica* 42:349–354
- Salehi M, Izadpanah K, Nejat N (2006) A new phytoplasma infecting lettuce in Iran. *Plant Dis* 90(2):247
- Salehi M, Izadpanah K, Siampour M (2007) Characterization of a phytoplasma associated with cabbage yellows in Iran. *Plant Dis* 91:625–630
- Schneider B, Seemueller E, Smart CD, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S, Tully JG (eds) *Molecular and diagnostic procedures in mycoplasmaology*, vol 1. Academic Press, San Diego, pp 369–380
- Sichani VF, Bahar M, Zirak L (2014) Characterization of phytoplasmas related to aster yellows group infecting annual plants in Iran, based on the studies of 16S rRNA and *rp* genes. *J Plant Prot Res* 54(1):1–8
- Tamura K, Dudley J, Kumar S (2011) MEGA5, molecular evolutionary genetics analysis (MEGA) software version 5.0. *Mol Biol Evol* 24:1596–1599
- Tiwari AK, Khan MS, Iqbal A, Chun SC, Priya M (2013) Molecular identification of '*Candidatus Phytoplasma asteris*' (16SrI-B) associated with the little leaf disease of potato in India. *J Plant Pathol* 95(3):659–668
- Tran-Nguyen LTT, Persley DM, Gibb KS (2003) First report of phytoplasma disease in capsicum, celery and chicory in Queensland, Australia. *Aust Plant Pathol* 32:559
- Verdin E, Salar P, Danet JL, Choueiri E, Jreijiri F, El Zammar S, Gèlie B, Bové J, Garnier M (2003) '*Candidatus Phytoplasma phoeniceum*', a new phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. *Intl J Syst Evol Microbiol* 53:833–838
- Wei W, Lee IM, Davis RE, Zhao Y (2008) Automated RFLP pattern comparison and similarity coefficient calculation for rapid delineation of new and distinct phytoplasma 16Sr subgroup lineages. *Inter J Syst Evol Microbiol* 58:2368–2377
- Zhang J, Hogenhout SA, Nault LR, Hoy CW, Miller SA (2004) Molecular and symptom analyses of phytoplasma strains from lettuce reveal a diverse population. *Phytopathology* 94:842–849