View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by GSSRR.ORG: International Journals: Publishing Research Papers in all Fields

International Journal of Sciences:
Basic and Applied Research
(IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Detection of *Candidatus* Phytoplasma Asteris' (16srI) Associated with Bitter Gourd Leaf and Floral Malformations in Malaysia

Tennakoon Mudiyanselage Nadika Darshanie Tennakoon[a], Khairulmazmi Ahmad[b], Kong Lih Ling[c], Ganesan Vadamalai[d]*

[a,b,d]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Malaysia*

[c]*Institute of Plantation Studies, Universiti Putra Malaysia, 43400, Serdang, Malaysia*

[a]*Email: nadika74@yahoo.com*

[b]*Email: khairulmazmi@upm.edu.my*

[c]*Email: lihling@upm.edu.my*

[d]*Email: ganesanv@upm.edu.my*

Abstract

Bitter gourd vines (*Momordica charantia)* exhibiting symptoms of leaf and floral malformations including reduced leaf and flower size and shortened internodes were observed in farmer's fields in Selangor, Malaysia. The causal agent was detected by nested and semi nested Polymerase Chain Reaction (PCR) using phytoplasma universal primers based on 16SrRNA and *SecA* gene sequences. Sequence analysis of 1.2 kb and 480 bp amplicons of the 16SrRNA and *SecA* gene respectively confirmed the presence of phytoplasma DNA associated with *Candidatus* phytoplasma asteris (Group16SrI) in the symptomatic bitter gourd samples. Phylogenetic analysis of the 16SrDNA and *SecA* sequences placed the bitter gourd phytoplasma in the 16SrI phytoplasma group. This is the first report of phytoplasma infection in bitter gourd in Malaysia.

Key words: phytoplasma; bitter gourd; molecular identification; Malaysia.

---

* Corresponding author.

1. Introduction

Bitter gourd (*Momordica charantia)* also known as bitter melon, belongs to the Family Cucurbitaceae. It is cultivated for use as vegetables and medicine throughout the world including Malaysia. Bitter gourd is very low in calories but rich in dietary fibre, minerals, vitamins and antioxidants [1]. It has been reported that bitter gourd is a host plant for different types of pathogens including viruses, bacteria, fungi and phytoplasma [2,3]. These pathogens affect the yield of the crop irrespective of the area grown. Leaf and flower malformations were observed in bitter gourd farmer fields in the state of Selangor, Malaysia during a survey conducted in October, 2019. The leaves and flowers were reduced in size and the vines were stunted due to shortened internodes (Figure 1). These symptoms are associated with phytoplasma infections.

Figure 1: Bitter gourd vines, a- symptoms with little leaf and malformation, b - Healthy vine

Phytoplasma is a phloem-limited cell wall-less pathogen that causes plant diseases [4]. They are associated with numerous plant diseases worldwide. Symptoms of phytoplasma diseases include, virescence, phyllody, stunting, witches' broom, yellowing, leaf and flower malformations [5]. These symptoms are dependent on the host, environmental factors and the phytoplasma strain infecting the host. Phytoplasma can be visualized by microscope, but is not ideal as a routine and rapid diagnostic method. Few attempts have been made to identify the pathogen using antibody–based detection systems but it would be specific for a particular phytoplasma rather than generic. Nucleic acid based detection and diagnostic systems are widely used for the detection of pathogens including phytoplasma since it is rapid and can be used as a generic tool [5]. The most common and simplest diagnostic method for the detection of phytoplasma is PCR using specific primers to amplify various regions of rRNA operon. Diagnostic and phylogenetic analysis of phytoplasma are mostly based on the 16SrRNA gene due to the availability of universal primers to detect this region [6]. However, the standard universal primers can amplify closely related organisms (bacteria) making false positives and diagnostic errors [7]. In addition, *SecA* gene, which encodes SecA, the ATP dependent force generator in the bacterial precursor protein translocation cascade system can also be used for identification and classification of phytoplasma [5]. Based on the analysis of 16SrRNA sequences there are 34 different groups of phytoplasmas and more than 100 sub-groups in the world. From Malaysia 16SrI, 16sXIVand 16SrXXXII groups have been reported [8,9,10] but there are no reports of phytoplasma in bitter gourd in Malaysia.

2. Materials and Methods

2.1 Total nucleic acid extraction and Polymerase Chain Reaction (PCR) amplification

Samples of bitter gourd showing leaf and flower malformation were collected from naturally infected bitter gourd plants grown in vegetable fields in state of Selangor, Malaysia in 2019. Total DNA was extracted from 450 mg of leaf samples using the Cetyl trimethylammonium Bromide (CTAB) extraction method [11]. The DNA was amplified by Nested PCR for 16SrRNA and a semi nested PCR for *SecA* gene. The amplification of the 16SrRNA was done using P1 /P7 [12] primers that amplifies 16S-23S rDNA intergenic spacer region of 16srRNA gene. Phytoplasma specific R16F2n and R16R2 [13] primers were used for the second round PCR. Amplification was performed in 25 µl of final volume consist of 12.5µl Gotaq master mix, 1 of each primer (10 mmol) and 1µl of DNA template (concentration was 10-100 nmol/µl) and the final volume was made up with nuclease free water. The primer pair P1/P7 amplification was performed in 35 cycles with 95^0C for 2 minutes for initial denaturation, in an automated thermal cycler (Bio-Rad) and each cycle consisted with denaturation 95^0C for 2 min, annealing 55^0C for 1 min and extension step at 72^0C for 2 minute. Final extension was at 72^0C for 10 minutes. Resulting PCR product of 1 µl was used for the second round amplification. PCR conditions for second round with primer pair R16F2n and R16R2 were 95^0C for 2 minutes initial denaturation followed by 35 cycles of 95^0C for 1 minute denaturation, 57^0C for 1 minute annealing and 72^0C denaturation with 2 minutes. Final extension was done 72^0C for 10 minutes. The final PCR product is expected to be around 1.2 kb in size. Amplification of the non- ribosomal *SecA* gene was performed by a semi nested PCR assay employing primers of SecAFor1/ SecARev3 [14] followed by SecAFor2 / SecARev3 [14]. Amplification was performed in 25 µl of final volume consist of 12.5µl Gotaq master mix, 1 of each primer (10 mmol) and 1µl of DNA template (concentration was 10-100 nmol/µl) and the final volume was made up with nuclease free water. The PCR conditions for the first round of the semi nested PCR (SecAFor1/ SecARev3) were 95^0C for 2 minutes followed by 35 cycles of 95^0C for 1 minute, 53^0C for 1 minute and 72^0C for 90 seconds and final extension step of 72^0C for 10 minutes. One microliter of the first round PCR product was used for the second round. The PCR condition for SecAFor2 and SecARev3 was the same as used for the SecAFor1/ SecARev3 except the annealing temperature, which was 51^0C instead of 53^0C. Periwinkle DNA was used as a positive control. The final PCR product is expected to be around 480 bp in size. The PCR products were analysed on 1.5% agarose gel stained with Florasafe® and visualized under UV trans-illuminator. The PCR positive products were purified using Minelute gel extraction kit (Qiagen). Non symptomatic bitter gourd samples were used as a negative control.

2.2 Sequencing analysis

PCR products were sequenced at MyTACG Bioscience Sdn Bhd (Malaysia) on both strands of the PCR products using R16F2n/ R16R2 and SecAfor2/ SecARev3 primers. Sequence editing was done using sequence scanner v1.0 and assembly of forward and reverse sequences were done using BioEdit 7.2. The gene sequences obtained from 16SrRNA and *SecA* genes were deposited to the GenBank under the accession numbers of MT422719 (16SrDNA) and MN478168 (*SecA*). The gene sequences obtained in this study were compared with other reported phytoplasmas in the National Center for Biotechnology Information (NCBI) using BLAST searches (http://ncbi.nlm.gov/ BLAST).

2.3 Phylogenetic analysis

Table 1: Phytoplasma strains employed to construct the phylogenetic trees based on 16SrDNA and *SecA* gene sequences.

Named '*Candidatus* Phytoplasma' species	Gen Banks Acession number	16Sr group	origin
Ca. Phytoplasma asteris'	M30790	16SrI	NA
Ca. Phytoplasma asteris'	MN585898	16SrI	Malaysia
Ca. Phytoplasma asteris'	MT192345	16SrI	Malaysia
Ca. Phytoplasma asteris'	MN877917	16SrI	Iran
Ca. Phytoplasma asteris'	KF803561	16SrI	Malaysia
Ca. Phytoplasma asteris'	KF728953	16SrI	India
Ca. Phytoplasma asteris'	FJ008869	16SrI	Malaysia
Ca. Phytoplasma asteris'	KC924727	16SrI	Malaysia
Ca. Phytoplasma asteris'	KC924728	16SrI	Malaysia
Ca. Phytoplasma asteris'	KX179474	16SrI	India
Ca. Phytoplasma asteris	EU168722	16SrI-C	UK
Ca. Phytoplasma asteris.	EU168723	16SrI-C	Czech Rep
Ca. Phytoplasma asteris	EU168724	16SrI-F	Spain
Ca. Phytoplasma asteris	EU168721	16SrI-B	USA
Ca. Phytoplasma asteris	JN977034	16SrI- B	Myanmar
Ca. Phytoplasma aurantifolia	U15442	16SrII	France
Ca. Phytoplasma pruni'	JQ044393	16SrIII-A	USA
Ca. Phytoplasma pruni	EU168734	16SrIII-B	Italy
Ca. Phytoplasma pruni	EU168732	16SrIII-A	USA
Ca. Phytoplasma palmae	AF498307	16SrIV-A	Caribbean region
Ca. Phytoplasma palmae	EU168737	16SrIV-A	USA
Ca. Phytoplasma ziziphi'	KC478660	16SrV	China
Ca. Phytoplasma cocostanzaniae	EU168739	16SrIV-B	Tanzania
Ca. Phytoplasma ulmi	EU168741	16SrV-A	France
Ca. Phytoplasma trifolii'	AY390261	16SrVI-A	Canada
Ca. Phytoplasma trifolii	EU168742	16SrVI-A	USA
Ca. Phytoplasma trifolii	EU168743	16SrVI-A	India
Ca. Phytoplasma fraxini'	AF092209	16SrVII-A	USA
Ca. Phytoplasma vitis	AF176319	16SrVIII	France
Ca. Phytoplasma phoenicium'	AF515636	16SrIX-D	Lebanon"
Ca. Phytoplasma mali'	AJ542541	16SrX-A	Italy
Ca. Phytoplasma mali	EU168747	16Sr X-A	Italy
Ca. Phytoplasma prunorum	EU168749	16Sr X-B	Germany
Ca. Phytoplasma oryzae'	D12581	16SrXI-A	Japan
Ca. Phytoplasma australiense'	L76865	16SrXII-B	Australia
Ca. Phytoplasma fragariae	EU168751	16SrXII	UK
No '*Candidatus*' name proposed	AF248960	16SrXIII	Mexico
Ca. Phytoplasma cynodontis'	AJ550984	16SrXIV-A	Italy
Ca. Phytoplasma brasiliense	AF147708	16SrXV	Brazil
Ca. Phytoplasma graminis	AY725228	16SrXVI	Cuba
Ca. Phytoplasma caricae	AY725234	16SrXVII	Cuba
Ca. Phytoplasma americanum	DQ174122	16SrXVIII	USA
Ca. Phytoplasma castaneae	AB054986	16SrXIX	South Korea
Ca. Phytoplasma rhamni	X76431	16SrXX	NA
Ca. Phytoplasma pini	AJ632155	16SrXXI	Spain
Ca. Phytoplasma cocosnigeriae	Y14175	16SrXXII	UK
No '*Candidatus*' name proposed	AY083605	16SrXXIII	Australia
No '*Candidatus*' name proposed	AF509322	16SrXXIV	Australia
No '*Candidatus*' name proposed	AF521672	16SrXXV	Australia
No '*Candidatus*' name proposed	AJ539179	16SrXXVI	Mauritius
No '*Candidatus*' name proposed	AJ539180	16SrXXVII	Mauritius
Ca. Phytoplasma omanense	EF666051	16SrXXIX	Oman
Ca. Phytoplasma tamaricis	FJ432664	16SrXXX	USA

Phylogenetic trees were constructed using 16SrDNA (R16F2n/ R16R2) and *SecA* (SecAFor2/ SecARev3) gene sequences (Table 1). The phylogenetic relationship of bitter gourd little leaf phytoplasma was assembled with other phytoplasma strains available in GenBank for both genes. Bio Edit software was used for multiple alignments of sequences obtained from Gen Bank. Phylogenetic trees were constructed using MEGA7 software using neighbour joining method done in 1,000 replicates. *Acholeplasma laidlawii* (Accession No. M23932) and *Bacillus subtillis* (Accession No. D10279) were included as outgroups.

3. Results and Discussion

The nested PCR using primers R16F2n and R16R2 yielded an approximately 1.2 kb amplicon from all the symptomatic bitter gourd vine samples (Figure 2). In addition, an amplicon of approximately 480 bp was also observed in all symptomatic samples using the semi nested PCR assay for the amplification of *SecA* gene using SecAFor2/ SecARev3 primers. The same size PCR products were also observed in the positive control (periwinkle phyllody), while no amplicons of the expected size were observed in all the non-symptomatic samples (Figure 2). These results associate the little leaf disease and malformations in the bitter gourd vines with a phytoplasma.

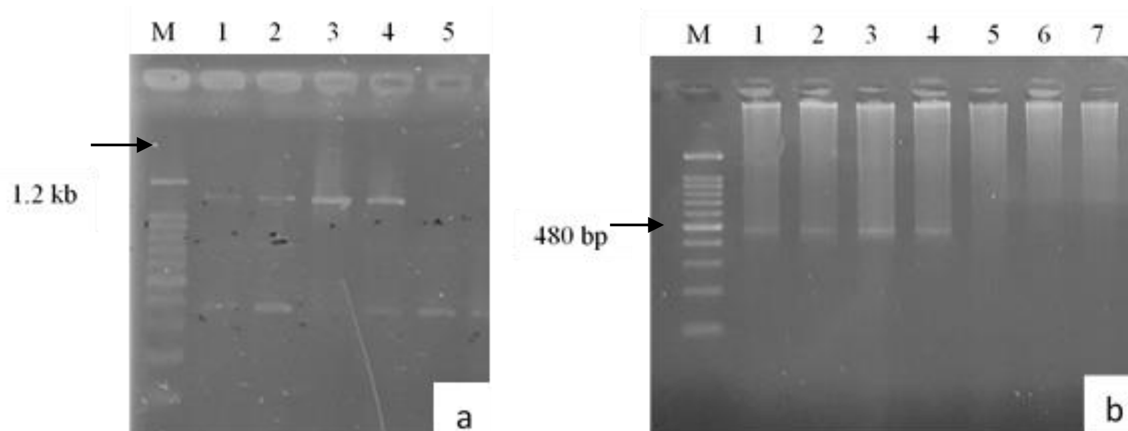

Figure 2: Nested PCR amplification of phytoplasma 16rDNA and *SecA* gene sequences from infected bitter gourd samples. (a) 1.2 kb fragment amplified using the primer pair P1/P7 followed by R16F2n/R16R2. (1,2,3 – symptomatic bitter gourd samples, 4 – positive control, 5- negative control, M- 100bp marker), (b) 480 bp fragment amplified using the primer pair SecFor1/SecARev3 followed by SecAFor2/SecARev3. (1,2,3 – symptomatic bitter gourd samples, 4 – positive control, 5,6,7- negative control, M – 100bp marker)

Near full length of 16SrDNA gene sequence of 1185 bp was obtained from the nested PCR using R16F2n and R16R2 primes. The blast analysis of the 16SrDNA sequence of bitter gourd little leaf phytoplasma showed the highest identity (99%) with 'Prunus sp.' witches'-broom phytoplasma (16SrI, Accession no. MN877917). The sequence obtained in this study was submitted to GenBank with the accession number of MT422719. In addition, *SecA* gene sequence analysis of bitter gourd little leaf phytoplasma showed 100% sequence identity to *SecA* gene sequence of Sesame phyllody phytoplasma (JN977034), belongs to 16SrI-B group of phytoplasma. The *SecA* gene sequence was submitted to GenBank with the accession number of MT478168. Both results

confirmed that little leaf bitter gourd phytoplasma in Malaysia belongs to the *Candidatus* phytoplasma asteris (16SrI) group. This is the most common phytoplasma group which infects a wide range of host plants and vectors worldwide [15]. Phylogenetic analysis of 16SrDNA gene sequences showed that the bitter gourd little leaf phytoplasma, (MT422917) was clustered with 16SrI phytoplasma strains (Figure 3), confirmed by the branching pattern of the phylogenetic tree clearly. The phylogenetic results obtained from 16SrDNA was further supported by the observation of the *SecA* gene pattern of the tree (Figure 4). Both gene sequences of bitter gourd phytoplasma showed close phylogenetic relationship with members of *Candidatus* phytoplasma asteris (16SrI). Phylogenetic analysis of organisms using a single highly conserved genes such as 16SrRNA has its limitations when defining groups, thus the phylogenetic trees should be analysed using a range of different genes [5]. One such gene is the *SecA* gene, which has been used for identification and classification of phytoplasma from various crops [5,8,9,10]. Phytoplasma have been previously reported in bitter gourds in India [16] and Myanmar [3] causing little leaf disease. Symptoms associated with the little leaf disease include reduced leaf and flower size, yellowish green leaves and shortened internodes [3,16]. Symptoms observed in this study were similar to those described with the bitter gourd little leaf disease in Myanmar [3] and India [16]. In both cases, the bitter gourd phytoplasma belonged to the *Candidatus* phytoplasma asteris (16SrI) group which occur on a broad range of vegetable crops such as onion [17], carrots [18], tomatoes [19], potatoes [20], lettuce [21] and brinjal [22]. While in Brazil, bitter gourd was infected with phytoplasma causing the symptoms of witches'- broom, yellowing, small leaves and stunting. It was identified as phytoplasma of 16SrIII [23]. Sometimes a disease of phytopasma is given a common name in different parts of the world since having same symptoms [5]. At the same time the same plant species can be infected with different 16Sr groups even in the same country of origin. [21,24]. This study provides the first evidence that bitter gourd in Malaysia is infected with the *Candidatus* .phytoplasma asteris (16SrI) group but further analysis is needed to identify the sub group under the 16SrI group. Additional studies on symptomatology, detection, diversity analysis, in vitro and virtual restriction analysis will provide invaluable basic knowledge and improved understanding of the disease. This would enable a better understanding of the epidemiology and host-vector interactions that can be used to formulate novel management strategies for phytoplasma diseases.

4. Conclusion

Leaf and floral malformation and stunting of the bitter gourd vines in this study was found to be associated with a phytoplasma infection. The presence of the phytoplasma was confirmed by nested and semi nested PCR using two different genes. The phytoplasma in bitter gourd was identified as belonging to the *Candidatus* phytoplasma asteris (16SrI) group and associated with the bitter gourd little leaf disease. This is the first record of phytoplasma in bitter gourd in Malaysia.

5. Recommendations

The current study emphasizes the importance of identification of the correct pathogen of diseases in bitter gourd, which will help in managing the pathogen. Nevertheless, further analysis is needed to identify the sub group under the 16SrI group. *In vitro* and virtual restriction enzyme analysis should be able to provide invaluable basic knowledge and improved understanding of the pathogen.

Acknowledgements

The authors thank the Sri Lanka Council for Agriculture Research policy (SLCARP), the Department of Agriculture, Sri Lanka and Department of Plant Protection, Universiti Putra Malaysia for supporting this study.

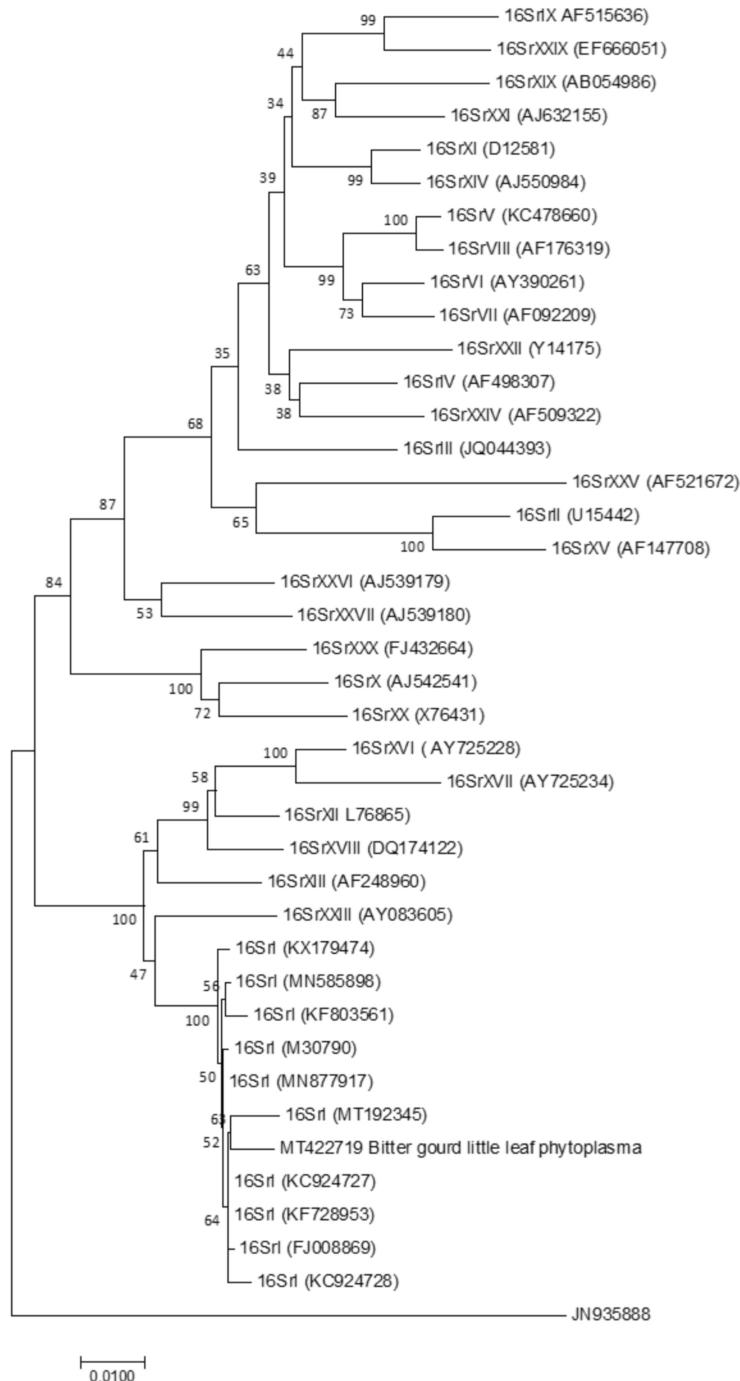

Figure 3: Phylogenetic tree constructed by the neighbour joining method showing the phylogenetic relationship for 16SrDNA sequences of different groups of phytoplasmas. Bootstrap values obtained from 1,000 replicates are shown. *Acholeplasma laidlawii* (JN935888) was employed as the out group of the root.

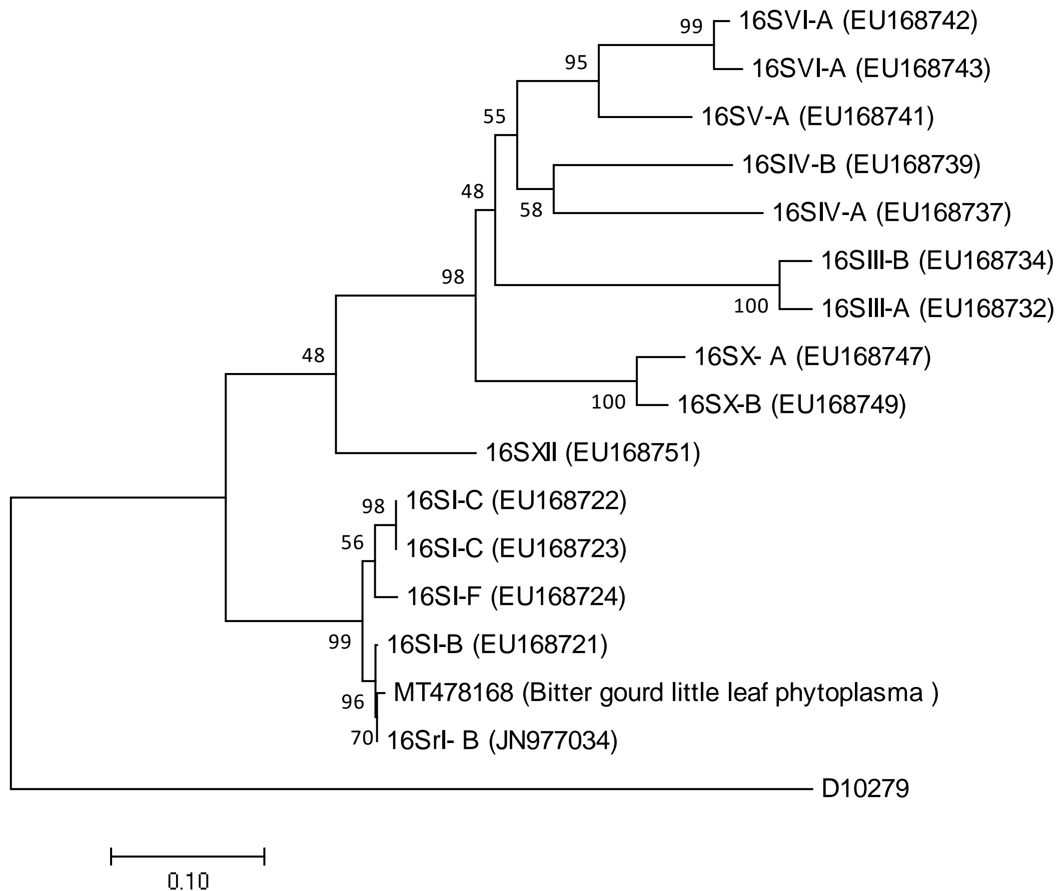

Figure 4: Phylogenetic tree constructed by the neighbour joining method showing the phylogenetic relationship for *SecA* sequences of different groups of phytoplasmas. Bootstrap values obtained from 1,000 replicates are shown. *Bacillus subtilis* (D10279) was employed as the out group of the root.

References

[1]. J. K. Grover and S. P. Yadav, "Pharmacological actions and potential uses of Momordica charantia: A review," J. Ethnopharmacol., vol. 93, no. 1, pp. 123–132, 2004, doi: 10.1016/j.jep.2004.03.035.

[2]. S. Zhang, M. Lamberts, G. Mcavoy, and F. W. Fusarium, "Diseases of Bitter Melon in South Florida 1 Target Leaf Spot (Corynespora," pp. 1–3, 2015.

[3]. N. K. K. Win, Y. H. Kim, and H. Y. Jung, "Bitter gourd little leaf disease associated to 'Candidatus Phytoplasma asteris,'" Trop. Plant Pathol., vol. 39, no. 1, pp. 82–88, 2014, doi: 10.1590/S1982-56762014000100010.

[4]. I. Lee, R. E. Davis, and D. E, "Phytopathogenic Mollicutes," Annu. Rev. Microbiol., vol. 54, pp. 221–255, 2000.

[5]. M. D. &Jennifer Hodgetts, Ed., phytoplasma Methods and Protocols. London, 2013.

[6]. C. D. Smart et al., "Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region," Appl. Environ. Microbiol., vol. 62, no. 8, pp. 2988–2993, 1996, doi: 10.1128/aem.62.8.2988-2993.1996.

[7]. N. A. Harrison, M. Womack, and M. L. Carpio, “Detection and characterization of a lethal yellowing (16SrIV) group phytoplasma in Canary Island date palms affected by lethal decline in Texas,” Plant Dis., vol. 86, no. 6, pp. 676–681, 2002, doi: 10.1094/PDIS.2002.86.6.676.

[8]. N. Nejat, K. Sijam, S. Nor, A. Abdullah, G. Vadamalai, and M. Dickinson, “Molecular characterization of an aster yellows phytoplasma associated with proliferation of periwinkle in Malaysia,” vol. 9, no. 15, pp. 2305–2315, 2010.

[9]. N. Nejat, K. Sijam, S. N. A. Abdullah, G. Vadamalai, and M. Dickinson, “First report of a 16SrXIV , ‘ Candidatus Phytoplasma cynodontis ’ group phytoplasma associated with coconut yellow decline in Malaysia First report of ‘ Candidatus Liberibacter asiaticus ’ associated with Huanglongbing in Cuba,” no. August 2008, p. 3059, 2009, doi: 10.1111/j.1365-3059.2008.01923.x.

[10]. N. Nejat, G. Vadamalai, R. E. Davis, N. A. Harrison, K. Sijam, and M. Dickinson, “‘ Candidatus Phytoplasma malaysianum ’, a novel taxon associated with virescence and phyllody of Madagascar periwinkle (Catharanthus roseus),” pp. 540–548, 2013, doi: 10.1099/ijs.0.041467-0.

[11]. Y. P. Zhang, J. K. Uyemoto, and B. C. Kirkpatrick, “A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay,” J. Virol. Methods, vol. 71, no. 1, pp. 45–50, 1998, doi: 10.1016/S0166-0934(97)00190-0.

[12]. S. Deng and C. Hiruki, “Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes,” J. Microbiol. Methods, vol. 14, no. 1, pp. 53–61, 1991, doi: 10.1016/0167-7012(91)90007-D.

[13]. D. E. Gundersen and I.-M. Lee, “Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs,” Phytopathol. Mediterr., vol. 35, no. 3, pp. 144–151, 1996.

[14]. J. Hodgetts, N. Boonham, R. Mumford, N. Harrison, and M. Dickinson, “Phytoplasma phylogenetics based on analysis of secA and 23S rRNA gene sequences for improved resolution of candidate species of ‘Candidatus Phytoplasma,’” Int. J. Syst. Evol. Microbiol., vol. 58, no. 8, pp. 1826–1837, 2008, doi: 10.1099/ijs.0.65668-0.

[15]. P. G. Weintraub and L. Beanland, “Insect Vectors of Phytoplasmas,” Annu. Rev. Entomol., vol. 51, no. 1, pp. 91–111, 2006, doi: 10.1146/annurev.ento.51.110104.151039.

[16]. V. Venkataravanappa, L.R.C. Narasimha Reddy, S. Polam, S.K Subbanna, R. Manem Krishna “Detection and characterization of ‘Candidatus Phytoplasma asteris’ associated with littleleaf disease of bitter gourd from India by 16S rRNA phylogenetic and RFLP (in vitro and virtual) analysis” vol. 69, no. 4, pp. 707–714, 2017.

[17]. R. Jomantiene, R.E. Davis, I.-M. Lee, Y. Zhao, K. Bottner-parker, D. Valiunas, and R. Petkauskaite. "Onion is host for two phytoplasma lineages, subgroups 16SrI-a and 16SrI-(b/l)l, in Lithuania: a HinfI site revealed a SNP marking divergent branches of evolution." journal of plant pathology 92, no. 2 (2010): 461-70. Accessed may 30, 2020.

[18]. B. Duduk et al., “Phytoplasmas in carrots: Disease and potential vectors in Serbia,” Bull. Insectology, vol. 61, no. 2, pp. 327–331, 2008.

[19]. E. Vellios and F. Lioliopoulou, “Detection and characterization of phytoplasmas infecting tomato plants in Greece,” Bull. Insectology, vol. 60, no. 2, pp. 157–158, 2007.

[20]. P. Jones, Y. Arocha, O. Antesana, E. Montellano, and P. Franco, “‘Brotes grandes’ (big bud) of potato:

A new disease associated with a 16SrI-B subgroup phytoplasma in Bolivia," Plant Pathol., vol. 54, no. 2, p. 234, 2005, doi: 10.1111/j.1365-3059.2005.01137.x.

[21]. I. M. Lee, K. D. Bottner-Parker, Y. Zhao, R. E. Davis, and M. C. Black, "Aster yellows group (16SrI), subgroups 16SrI-A and 16SrI-B, phytoplasmas associated with lettuce yellows in Texas," New Dis. Reports, vol. 29, p. 5, 2014, doi: 10.5197/j.2044-0588.2014.029.005.

[22]. J. Kumar, S. Gunapati, S. P. Singh, A. Lalit, N. C. Sharma, and R. Tuli, " First report of a ' Candidatus Phytoplasma asteris' (16SrI group) associated with little leaf disease of Solanum melongena (brinjal) in India ," New Dis. Reports, vol. 26, no. December, p. 21, 2012, doi: 10.5197/j.2044-0588.2012.026.021.

[23]. M.S. Avis, G.M.Ribeiro, A.N.S. Souza, and C. M. Carvalho, "First Report of a 16SrIII-B Phytoplasma Associated with Momordica charantia Witches'-Broom in Brazil," Plant Dis., vol. 1314, no. 101, p. 7, 2017.

[24]. M. E. Santos-Cervantes, J. A. Chávez-Medina, J. Acosta-Pardini, G. L. Flores-Zamora, J. Méndez-Lozano, and N. E. Leyva-López, "Genetic diversity and geographical distribution of phytoplasmas associated with potato purple top disease in Mexico," Plant Dis., vol. 94, no. 4, pp. 388–395, 2010, doi: 10.1094/PDIS-94-4-0388.