Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi 27 (1):166-173, 2022
(*Mustafa Kemal University Journal of Agricultural Sciences* 27 (1):166-173, 2022)
e-ISSN: 2667-7733
http://dergipark.org.tr/mkutbd

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Detection and characterization of phytoplasmas in some cucurbits (Cucurbitaceae) and bindweed (Convolvulaceae) in Hatay Province of Turkey

Hakan ÇARPAR[1], Gülşen SERTKAYA[2]

[1]*Hatay Mustafa Kemal University, Department of Plant Protection, Faculty of Agriculture Antakya, Hatay, Turkey.*

MAKALE BİLGİSİ / ARTICLE INFO

Makale tarihçesi / Article history:
DOI: 10.37908/mkutbd.1041286
Geliş tarihi /Received:24.12.2021
Kabul tarihi/Accepted:14.03.2022

Keywords:
Ca. phytoplasma solani, *Ca*. phytoplasma trifolii, phytoplasma, summer squash (*Cucurbita pepo*),Turkey.

Corresponding author: Hakan ÇARPAR
✉: hcarpar@mku.edu.tr

Ö Z E T / A B S T R A C T

Aims: During a disease survey conducted in 2019, typical symptoms including yellowing of the leaves, stunting, shortening of internodes, proliferation of auxiliary shoots (witches'-broom), the bunchy appearance of growth at the ends of stems (rosetting), virescence of flowers or sterility, phyllody, small and deformed leaves and fasciation were observed on suspected cucurbit plants growing in Hatay province. Therefore, this study was carried out to determine the phytoplasma infection(s) on cucurbit crops and bindweed in Hatay province of Turkey.
Methods and Results: In total; 4 pumpkins (winter squash: *Cucurbita moschata* Duchesne), 12 summer squash (*Cucurbita pepo* L.) and 2 bindweed (*Convolvulus arvensis* L.) samples were collected from suspected plants in cucurbit fields in Hatay province. Some of the plants (2 pumpkins, 10 summer squash and 2 bindweeds) exhibited symptoms related to phytoplasmas. Total nucleic acid was extracted by CTAB method. PCR amplification of 16S rDNA with phytoplasma specific primer pairs; F1/R0 followed by R16F2n/R2 confirmed the phytoplasma presence. Expected size amplicons of ~1.2 kb were obtained from infected samples from symptomatic cucurbits (12/12) and bindweed (1/2), but not from asymptomatic cucurbit plants (0/4). The sequences of the 4 symptomatic samples were deposited in NCBI GenBank (MT163353, MT163393, MT163396, and MT163469).
Conclusions: After sequencing and phylogenetic analyses it was revealed that the squash isolates had 99% sequence identity with "*Candidatus* phytoplasma trifolii" (16SrVI) and the bindweed isolate had 99% sequence identity with "*Candidatus* phytoplasma solani" (16SrXII).
Significance and Impact of the Study: There is a lack of knowledge on the status of phytoplasma infections and natural hosts in cucurbits in Turkey. According to our knowledge, this is the first report of a member of 16SrVI group, *Candidatus* phytoplasma trifolii associated with phytoplasma infection in winter squash-pumpkin (*C. moschata*) and summer squash (*C. pepo*) in Turkey.

Atıf / Citation: Çarpar H, Sertkaya G (2022) Detection and characterization of phytoplasmas in some cucurbits (Cucurbitaceae) and bindweed (Convolvuluceae) in Hatay province of Turkey. *MKU. J. Agric. Sci.* 27(1) : 166-173. DOI: 10.37908/mkutbd.1041286

INTRODUCTION

Turkey is an important agricultural producer of a variety of crops due to its geographical location. Cucurbitaceae has been one of the most important crops in Turkey (Sarı et al., 2008).

Pumpkin and squash cultivation has a long history among the cucurbits, and they are related with the beginnings of agriculture (Whitaker and Robinson, 1986). Furthermore, these plants can be cultivated in almost all arable regions of the world. *Cucurbita pepo*, *C. maxima* and *C. moschata* are considered

economically important species which are widely grown in agricultural regions worldwide (Robinson and Decker-Walters, 1997; Paris and Brown 2005; Wu et al., 2007). Phytoplasmas are plant pathogens that are classified in Mollicutes, can be transmitted by vectors and localized in plant phloem. They are associated with numerous plant diseases and have been reported to cause destructive losses in both crops and natural ecosystems (Seemüller et al., 1998; Khan et al., 2003; Bertaccini 2007; Hogenhout and Musić 2010). Economic losses may reach up to 100% in infected plants (Salehi et al., 2015; Martini et al., 2018).

Pumpkins are widely cultivated crops in Turkey (Sarı et al., 2008; Balkaya et al., 2010). Summer squash (*Cucurbita pepo* L.) and pumpkin (winter squash or butternut squash) (*Cucurbita moschata* Duchesne) are the most commonly grown cucurbits in Hatay province of Turkey. According to FAO, China ranked first with an estimated annual production of cucurbits of approximately 8 million tons in 2017, while Turkey ranked seventh in the world with a production of 616.777 tons (FAO, 2018). According to the reports of the Turkish Statistical Institute, 590.414 tons of cucurbits were produced in 2019 in Turkey (TUIK, 2019).

Phytoplasmas affecting *C. moschata* and *C. pepo* have been reported in Italy, Brasil, Egypt and the diseases were reported as "pumpkin yellows" (Seemüller et al., 1998; Montano et al., 2006), pumpkin yellow leaf curl (PYLC), squash phyllody (Salehi et al., 2015) and squash virescence (Omar and Foissac, 2012).

'*Ca*. Phytoplasma australiense' and pumpkin yellow leaf curl (PYLC) disease were reported as mixed infection in pumpkin plants (*C. maxima* and *C. moschata*) for the first time in Queensland, Western Australia and the Northern Territory (Streten et al. 2005). '*Ca*. Phytoplasma trifolii' was first reported in sesame plants in Turkey in 2007 (Sertkaya et al., 2007). Lately, '*Ca*. Phytoplasma trifolii' and '*Ca*. Phytoplasma solani' were reported in cucumbers in Van province of Turkey (Usta et al., 2017).

This study was conducted to detect phytoplasma diseases of cucurbits and the other host/s in cucurbit growing areas in Hatay province of Turkey.

MATERIALS and METHODS

Plant samples

Leaves of pumpkin-winter squash (*Cucurbita moschata*), summer squash (*C. pepo*) and bindweed (*Convolvulus arvensis*) were collected from plants exhibiting phytoplasma symptoms and symptomless plants from cucurbit fields in Hatay province of Turkey in 2019.

DNA extraction and PCR amplifications

CTAB method was used to extract total DNA from plant material according to Doyle and Doyle (1990). The universal primer sets were used to detect phytoplasma DNA isolated from fresh squash and bindweed leaf midribs. F1/R0 (Lee et al., 1994) and R16F2n/R2 (Gundersen and Lee, 1996) universal primers were employed in Direct and Nested PCRs respectively for amplification of the 16SrRNA gene (Table 1).

PCR products were dyed with ethidium bromide then run on 1.2% agarose gel using Tris-Borate EDTA (TBE) buffer, and visualized with a UV trans-illuminator.

Table 1. Primer pairs used in PCRs, their size and references

	Primer sets		Base pairs length	References
Direct	**F1**	5'-AAGACGAGGATAACAGTTGG-3'	1800	Lee et al. 1994
PCR	**R0**	5'- GGATACCTTGTTACGACTTAACCCC-3'		Lee et al. 1994
Nested	**F2n**	5'-CGACTGCTAAGACTGG-3'	1200	Gundersen and Lee 1996
PCR	**R2**	5'-TGACGGGCGGTGTGTACAAACCCCG-3'		Gundersen and Lee 1996

Sequencing and BLAST

Nested PCR products were sequenced in both directions with forward and reverse and aligned using the MEGA-X software. Based on 16SrDNA gene sequences of the phytoplasmas in this study and those of selected reference strains accessible from NCBI were compared.

Characters considered to be uninformative were omitted from the sequences. The phylogenetic relationships were calculated using 1000 bootstrap replicates by using the Neighbor-Joining method. The phylogenetic tree was created for the 4 phytoplasmas studied in this work and 12 phytoplasmas representing distinct phytoplasma groups. As the root of the phylogenetic tree *Acholeplasma laidlawii* (FJ226570) was selected to be an outgroup (Volokhov 2008).

Virtual RFLP (Restriction Fragment Length Polymorphism) and phylogenetic analysis

*i*PhyClassifier program was used for virtual (RFLP) analysis. *Alu*I, *Hae*III, *Hha*I, *Mse*I, *Rsa*I, *Taq*I (Lee et al. 1998) restriction enzymes were used for 16S rDNA digestions in virtual RFLP analysis, sequences of phytoplasmas isolated from cucurbits and bindweed were compared with each reference strains for group and subgroup recognized by *i*PhyClassifier.

RESULTS and DISCUSSION

Phytoplasma detection

Phytoplasma symptoms typical on cucurbit plants include yellowing of the leaves, stunting, shortening of internodes, proliferation of auxiliary shoots (witches' broom), the bunchy appearance of growth at the ends of stems (rosetting), virescence of flowers or sterility, phyllody, small and deformed leaves and fasciation (Figure 1). Out of a total of 4 pumpkins, 12 summer squash and 2 bindweed samples collected, 2 pumpkins, 10 summer squash and 1 bindweed plants exhibited symptoms considered to be related to phytoplasma diseases were found to be infected with phytoplasmas. Samples exhibiting phytoplasma symptoms gave positive bands (about 1.2 kb) in agarose gel but there was not any band observed from the symptomless plants (Figure 2).

Figure 1. Winter and summer squash plants showing dwarfing, phyllody and virescence (A,B), fasciation (C), small and yellow leaves naturally infected by phytoplasma in cucurbit fields in Antakya district of Hatay province

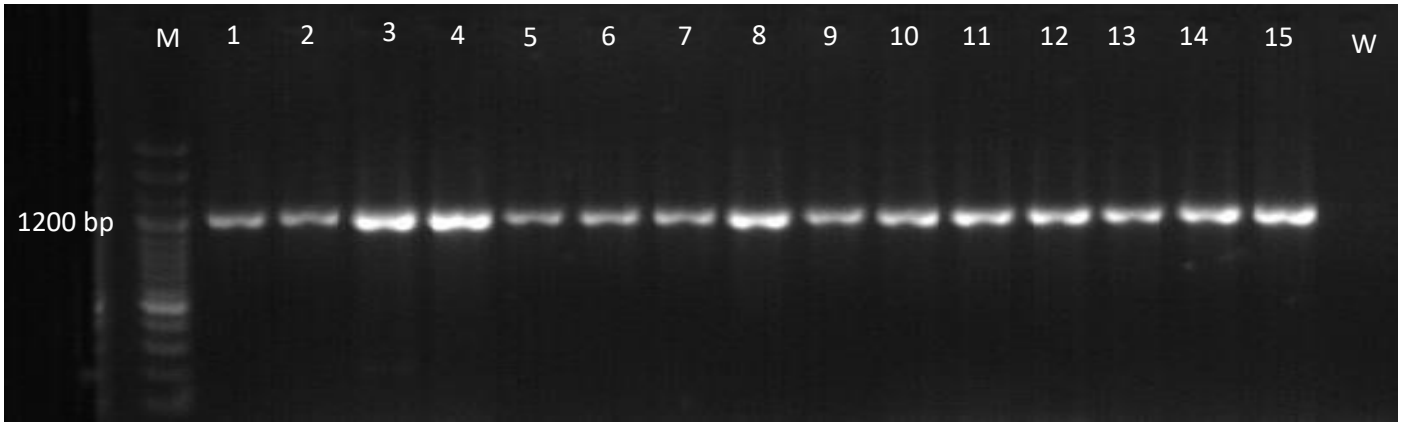

Figure 2. Gel electrophoresis photo of Nested PCR products, 1-12 cucurbit samples, 13 bindweed sample and 14-15 positive controls, W: negative control. M: Marker (1 kb DNA marker)

Sequencing and phylogenetic tree

Selected from the positive samples, one winter squash (pumpkin), two summer squash and one bindweed (*C. arvensis*) isolates were designated as TUR Squ 804 HC, TUR Squ 805 HC, TUR Squ 822 HC and TUR Con 806 HC, respectively. The obtained nucleotide sequences of 16S rDNA from three squash (*C. pepo* and *C. moschata*) and one bindweed (*C. arvensis*) isolates were deposited in GenBank (Accession numbers: MT163353, MT163393, MT163396 and MT163469 respectively).

Comparing the sequences of 16SrVI phytoplasma isolates and other phytoplasma groups' 16S rDNA with that of the cucurbit phytoplasma by MEGA (version X) software affirmed its closest phylogenetic relationship with the members of 16SrVI group (Figure 3). Therefore, *Ca*. Phytoplasma trifolii (16SrVI) was identified from *Cucurbita pepo* and *C. moschata*. The phytoplasma isolate from *C. arvensis* sample taken from a *C. pepo* field was identified as *Ca*. Phytoplasma solani (16SrXIV-A subgroup). *Ca*. Phytoplasma trifolii and *Ca*. Phytoplasma solani were found to cause natural infections in squash and bindweed in cucurbit fields in Hatay.

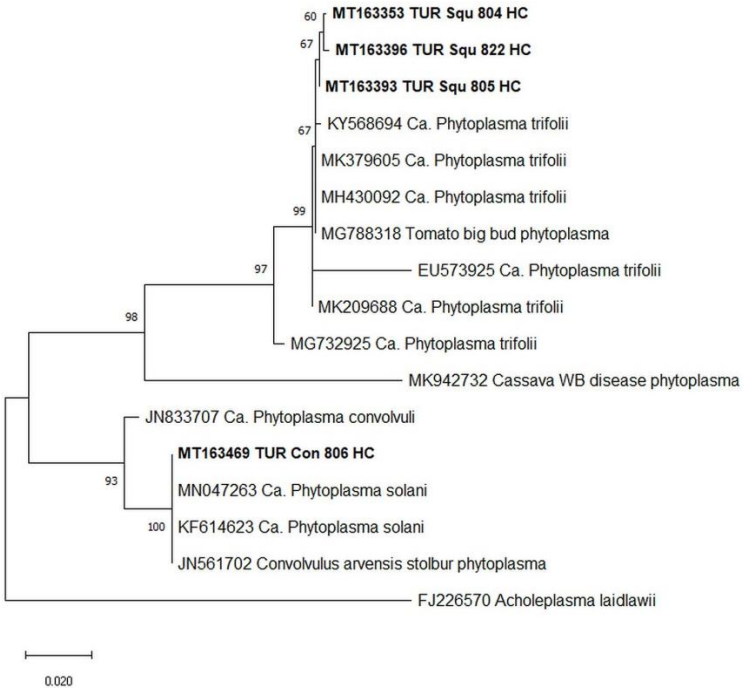

Figure 3. Phylogenetic tree constructed with neighbor joining algorithm of 16S rDNA sequences of '*Ca*. P. solani' and '*Ca*. P. trifolii'. Sequences for '*Ca*. Phytoplasma' species were retrieved from NCBI Genbank. '*Ca*. P. trifolii'; MT163353, MT163393, MT163396 (this publication), '*Ca*. P. trifolii values for 1000 replicates are shown on branches. GenBank accession numbers shown in brackets. Acholeplasma laidlawii was used as an outgroup to root the tree. The tree was constructed by the neighbor-joining method using MEGA X software

Virtual RFLP

Virtual RFLP analyses were conducted according to Zhao et al. (2009). Nested PCR products with the primer pairs of R16F2n/R2 were digested with restriction endonucleases *Alu*I, *Hha*I, *Hae*III, *Mse*I, *Rsa*I and *Taq*I. Results showed that the pumpkins and squash plants were infected by '*Ca*. Phytoplasma trifolii' and *Convolvulus arvensis* were infected by '*Ca*. Phytoplasma solani' (Figure 4).

The disease of squash phyllody (SqP) caused by phytoplasma was reported to be a very economically important disease reducing the yield and quality of the crop. It is reported that crop loss may reach up to 100% in case of early infections in Iran (Salehi et al., 2015).

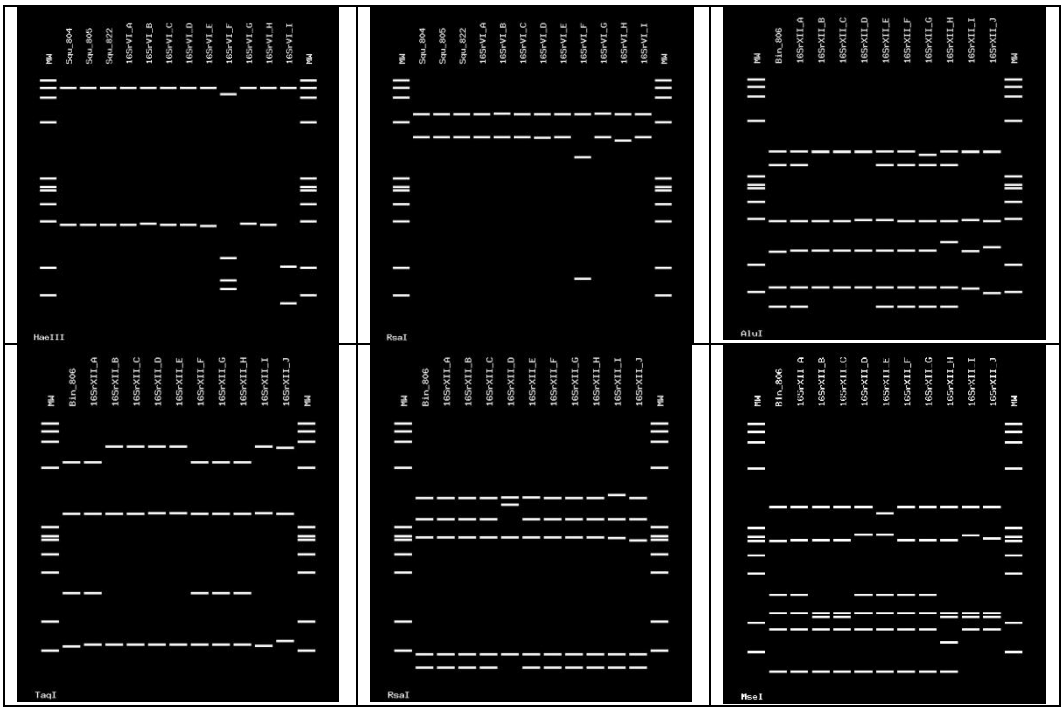

Figure 4. Virtually generated RFLP patterns (Zhao et al. 2009) of cucurbits (Squ 804, Squ 805 and Squ 822) and bindweed (Con 806). Computer-simulated virtual RFLP patterns illustrating in silico digestions with six key enzymes: *Alu*I, *Hae*III, *Hha*I, *Mse*I, *Rsa*I, *Taq*I showing fragments of 16S rDNA sequence of Nested PCR products from detected phytoplasmas and representative strains of groups 16SrVI. MW, Molecular weight marker (derived from øX174 RFI DNA *Hae*III digest, Size range: 72 bp to 1,353 bp) (Zhao et al. 2009)

In conclusion, phytoplasmas belonging to different groups associated with cucurbit diseases have been reported worldwide, such as Loofah witches' broom (16SrVIII-A)(Davis et al., 2017), *Cucurbita moschata* (16SrIII-J) and *Cucurbita pepo* (16SrIII) (Montano et al., 2006 and 2007), Little leaf of luffa (Kumar et al., 2010), Squash phyllody (16SrI-B) (Rao et al., 2017), Cucumber phyllody and Squash phyllody 16SrII-D, (Salehi et al., 2015; Al-Subhi et al., 2018), Little leaf disease of bitter gourd (*Momordica charantia* L.) (NangKyuKyu et al., 2014), phyllody of squash (*Cucurbita* spp.) (16SrVI-A) (Zibadoost et al., 2016), *Cucurbita pepo* (16SrVI-A) (Kastalyeva et al., 2016), bottle gourd virescence and phyllody (16SrIX)(Tripathi et al., 2017), *Ca*. P. trifolii' and '*Ca*. P. solani' in cucumber (16SrVI-A and 16SrXII-A) (Usta et al. 2017), Edible gourd phyllody (*Lagenaria siceraria* L.) (Sertkaya and Yüksel, 2018) and 16SrVI-A (Kumari et al., 2019), and a little leaf of bitter gourd and Loofah and cucumber phyllody (Borines et al., 2020).

The phytoplasma group 16SrVI CP (clover proliferation) (subgroup VI-A) has been observed to infect a wide variety of plants all around the world (Khasa et al., 2016; Zibadoost et al., 2016; Choueiri et al., 2007). It has been reported that many crops belong to different families such as cucumber, tomato, pepper, cabbage, maize etc. were also affected by phytoplasma group 16SrVI from Turkey (Sertkaya et al., 2007; Usta et al., 2017; Yılmaz et al., 2019; Güller and Usta, 2020; Ulubaş-Serçe and Yılmaz 2020; Şimşek et al., 2021) and group 16SrXII from Turkey (Çağlar et al., 2021; Usta et al., 2021). In the current study the phytoplasmas identified in squash belong to group 16SrVI, and in bindweed belong to 16SrXII.

Phytoplasmas affecting cucurbit crops have been appeared to prevalent among the main producing areas such as Antakya district of Hatay province. The role of winter and summer squash as a source of phytoplasmas was important not only for cucurbits but also for the other economically important crops grown in Hatay,Turkey. As vectors insects are important to distribute phytoplasma diseases (Weintraub and Beanland, 2006). Therefore, insect vectors and alternative hosts of phytoplasma diseases in cucurbit crops need to be investigated and unknown transmission paths of the diseases should be revealed by sensitive and reliable diagnostic approaches. This will be of help for developing future management strategies of the disease in other potential vegetable hosts as well as cucurbits in the region.

According to our knowledge, this is the first report of a member of 16SrVI phytoplasma group (*Ca*. Phytoplasma trifolii) associated with winter squash-

pumpkin (*C. moschata*) and summer squash (*C. pepo*) in Turkey.

ÖZET

Amaç: 2019'da bir hastalık surveyinde, şüpheli kabak bitkilerinde yaprakların sararması, bodurluk, boğum aralarının kısalması, çoklu sürgün oluşumu, tepe sürgünlerinde çalı görünümü, çiçeklerin yeşillenmesi ve kısırlık, fillodi, küçük ve şekli bozulmuş yapraklar ve gövde yassılaşması gibi fitoplazmalara özgü simptomlar gözlenmiştir. Böylelikle, bu çalışma Hatay ili kabak bitkilerinde ve tarla sarmaşığında fitoplazma hastalıklarının araştırılması amacıyla yürütülmüştür.

Yöntem ve Bulgular: Hatay ili kabak tarlalarından şüpheli toplam 4 kış kabağı (*Cucurbita moschata*), 12 yaz kabağı (*Cucurbita pepo*) ve 2 tarla sarmaşığı (*Convolvulus arvensis*) örnekleri toplanmıştır. Bitkilerden bazıları (2 kış kabağı, 10 yaz kabağı ve 2 tarla sarmaşığı) fitoplazmalara özgü simptom sergilemiştir. Toplam nükleik asit izolasyonu, CTAB metoduna göre yapılmıştır. Direct ve Nested PCR çalışmalarında sırasıyla F1/R0 ve R16F2n/R2 evrensel primer çiftleri kullanılmıştır. Simptom sergileyen kabak bitkilerinde (12/12) ve tarla sarmaşığında (1/2) beklenen ~1.2 kb büyüklüğünde bant elde edilirken simptomsuz kabaklarda elde edilememiştir. Simptom gösteren örneklerden 4 tanesi NCBI Genbankası'na (MT163353, MT163393, MT163396 ve MT163469) yüklenmiştir.

Genel Yorum: Sekans ve filogenetik analizlerin ardından, kabak izolatlarının "*Candidatus* phytoplasma trifolii" (16SrVI), tarla sarmaşığı izolatının ise "*Candidatus* phytoplasma solani" (16SrXII) olduğunu göstermiştir.

Çalışmanın Önemi ve Etkisi: Türkiye'de kabakgillerde fitoplazma enfeksiyonlarının ve doğal konakçıların durumu hakkında bilgi eksikliği bulunmaktadır. Mevcut bilgilerimize göre, Türkiye'de 16SrVI grubunun bir üyesi olan *Candidatus* phytoplasma trifolii'nin kışlık kabaklardan bal kabağı (*C. moschata*) ve yazlık kabaklardan sakız kabağı (*C. pepo*) bitkilerinde enfeksiyon oluşturduğu ilk kez belirlenmiştir.

Anahtar Kelimeler: *Ca*. phytoplasma solani, *Ca*. phytoplasma trifolii, fitoplazma, kabak, Türkiye.

ACKNOWLEDGEMENTS

We would like to thank Dr. İlhan ÜREMİŞ for identification of weed samples and Res. Assist. Ahmet Emin YILDIRIM for his helpful efforts on improving the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

REFERENCES

Al-Subhi AM, Hogenhout SA, Al-Yahyai RA, Al-Sadi AM (2018) Detection, identification, and molecular characterization of the 16SrII-D phytoplasmas infecting vegetable and field crops in Oman. Plant Dis. 102: 576-588.

Balkaya A, Özbakır M, Karaağaç O (2010) Pattern of variation for seed characteristics in Turkish populations of *Cucurbita moschata* Duch. Afr. J. Agric. Res. 5(10): 1068-1076.

Bertaccini A (2007) Phytoplasmas: diversity, taxonomy and epidemiology. Front. Biosci. 12: 673-689.

Borines LM, Nuñez JAC, Duero ND, Sagarino-Borines R, Gerona RG (2020) Detection and molecular characterization of phytoplasma affecting vegetables in Eastern Visayas, Philippines. Ann. Trop. Res. 42(2): 1-20.

Choueiri E, Salar P, Jreijiri Fouad, Zammar SE, Masaad R, Abdul-Nour H, Bove´ JM, Danet JL, Foissac X (2007) Occurrence and distribution of 'Candidatus Phytoplasma trifolii' associated with diseases of solanaceous crops in Lebanon. Eur. J. Plant Pathol. 118: 411-416.

Çağlar BK, Şimşek E, Dikilitas M, Bertaccini A (2021) Characterization of 'Candidatus Phytoplasma solani' associated with a maize leaf reddening disease in Turkey. J Phytopathol. 169(11-12): 658-666.

Davis, RE, Zhao Y, Wei W, Dally EL, Lee IM (2017) 'Candidatus Phytoplasma luffae', a novel taxon associated with witches' broom disease of loofah, *Luffa aegyptica* Mill. 2017. Int. J. Syst. Evol. Microbiol. 67(8): 3127-3133.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (San Francisco, Calif.)12, 13–15.

FAO (2018) http://www.fao.org/faostat/en/#data/QC (verified October 10, 2020).

Gundersen DE, Lee IM (1996) Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol. Mediterr. 35: 144-151.

Güller A, Usta M (2020) Stolbur and clover proliferation phytoplasma infections in tomato from Bingöl province, Turkey. Türk Tarım Doğa Bilim. Derg. 7(4): 855-866.

Hogenhout SA, Musić MS (2010) Phytoplasma Genomics, from Sequencing to Comparative and Functional Genomics- What Have We Learnt? Page: 19-36. Ed. Phyllis G. Weintraub and Phil Jones, Phytoplasmas Genomes, Plant Hosts and Vectors. CABI, London, UK.

Kastalyeva TB, Bogoutdinov DZ, Bottner-Parker KD, Girsova NV, Lee I (2016) Diverse phytoplasmas associated with diseases in various crops in Russia - pathogens and vectors. Sel'skokhozyaistvennaya Biologiya (Agricultural Biology) 51: 367-375.

Khan AJ, Botti S, Subhi AMA, Zaidi MA, Altosaar I, Alma A, Bertaccini A (2003) Molecular characterization of the 16S RNA gene of phytoplasma detected in two leafhopper species associated with alfalfa plants infected with witches' broom in Oman. Phytopathol. Mediterr. 42(3): 257-267.

Khasa E, Gopala Taloh A, Prabha T, Madhupriyal, Rao GP (2016) Molecular characterization of phytoplasmas of 'Clover proliferation' group associated with three ornamental plant species in India. Biotech. 6(2): 237.

Kumar S, Singh V, Lakhanpaul S (2010) First report of 'Candidatus Phytoplasma asteris' (16SrI) associated with little leaf of cotton and luffa in India. Australas. Plant Dis. 5(1): 117-119.

Kumari S, Nagendran K, Rai AB, Singh B, Rao GP, Bertaccini A (2019) Global status of phytoplasma diseases in vegetable crops. Front. Microbiol. 10: 1349.

Lee IM, Gundersen DE, Hammond RW, Davis RE (1994) Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed- MLO infections in a single host plant. Phytopathology 84(6): 559-566.

Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk, IM (1998) Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int. J. Syst. Bacteriol. 48: 1153-1169.

Martini M, Delić D, Liefting L, Montano H (2018) Phytopsma infecting vegetable, Pulse and Oil crops. In: Phytoplasmas: Plant Pathogenic Bacteria – I.

Montano HG, Brioso PST, Pimentel JP, Figueiredo DV, Cunha Junior JO (2006) *Cucurbita moschata*, new phyto-plasma host in Brazil. J. Plant Pathol. 88: 226.

Montano HG, Paulo STB, Roberta CP, Joao PP (2007) *Sicana odorifera* (*Cucurbitaceae*) a new phytoplasma host. Bull. Insectology 60: 287-288.

NangKyuKyu W, YoungHwan K, HeeYoung, J (2014) Bitter gourd little leaf disease associated to 'Candidatus Phytoplasma asteris'. Trop. Plant Pathol. 39(1): 82-88.

Omar AF, Foissac X (2012) Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. Eur. J. Plant Pathol. 133: 353-360.

Paris HS, Brown RN (2005) The genes of pumpkin and squash. HortScience 40(6): 1620-1630.

Rao GP, Gopala GS, Rao A (2017) First report of a 'Candidatus Phytoplasma asteris'-related strain (16SrI-B subgroup) associated with witches' broom disease in *Cucurbita pepo* in India. New Dis. Rep. 35: 33.

Robinson RW, Decker-Walters DS (1997) Cucurbits. New York Cab. International. p. 226 (Crop Production Science in Horticulture).

Salehi MT, Siampour M, Esmaeilzadeh-Hosseini SA, Bertaccini A (2015) Characterization and vector identification of phytoplasmas associated with cucumber and squash phyllody in Iran. Bull. Insectology 68(2): 311-319.

Sarı N, Tan A, Yanmaz R, Yetişir H, Balkaya A, Solmaz I, Aykas L (2008) General Status of Cucurbit Genetic Resources in Turkey. Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M.ed.) INRA. Avignon, France, 21-32s.

Seemüller E, Marcone C, Lauer U, Ragozzino A, Goschl M (1998) Current status of molecular classification of the phytoplasmas. J. Plant Pathol. 80: 3-26.

Sertkaya G, Martini M, Musetti R, Osler R (2007) Detection and molecular characterization of phytoplasmas infecting sesame and solanaceous crops in Turkey. Bull. Insec. 60(2): 141-142.

Sertkaya G, Yüksel SD (2018) Virus and phytoplasma diseases of edible gourd (*Lagenaria siceraria* L.) in Hatay province of Turkey. I. International Agricultural Science Congress, Van Congress 2018 (09-12 May 2018 Van, Turkey): 633.

Streten C, Conde B, Herrington M, Moulden J, Gibb K (2005) Candidatus Phytoplasma australiense is associated with pumpkin yellow leaf curl disease in Queensland, Western Australia and the Northern Territory. Australasian Plant Pathol. 34: 103-105.

Şimşek E, Ayvacı H, Akkurak H, Dikilitas M, Güldür, ME (2021) First report of a 'Candidatus Phytoplasma trifolii'-related strain (16SrVI-A) associated with white leaf disease in Spirea japonica. Australasian Plant Dis. Notes 16: 28.

Tripathi S, Thorat V, Verma R, Shouche Y, Yadav A (2017) First Report of 'Candidatus Phytoplasma asteris' (subgroup 16SrIX) associated with bottle gourd virescence and phyllody disease in India. Plant

Disease (Disease Notes), 101(11): 1949.

TUIK (2019) https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2019-30685 (verified June 16, 2021).

Ulubaş-Serçe Ç, Yılmaz S (2020) First report of 'Candidatus Phytoplasma trifolii' (16SrVI group) infecting cabbage (Brassica oleracea) in Turkey. J. Plant Pathol. 102: 553.

Usta M, Güller A, Sipahioğlu HM (2017) Detection and Characterization of two Phytoplasma lineages on Cucumber (*Cucumis sativus* L.) with Same Symptomatology based on Virtual RFLP and Nucleotide Sequence Analysis of 16S rDNA. YYÜ Tar. Bil. Derg. (YYU J AGR SCI) 27(3): 299-308.

Usta M , Güller A, Demirel S (2021) Molecular identification of 'Candidatus Phytoplasma solani' using SecY and Vmp1 Genes in Tomato Plants from Van province . YYÜ Tar. Bil. Derg., 31(4): 951-960.

Volokhov DV (2008) Phylogenetic relationships among members of the class Mollicutes (Unpublished data).

Weintraub PG, Beanland L (2006) Insect vectors of phytoplasmas. Annu. Rev. Entomol. 51(1): 91-111.

Whitaker TW, Robinson RW (1986) Squash breeding. In: Bassett M.J. (Ed.). Breeding Vegetable. Crops. Westport, Connecticut: Avi, (209-242), 584.

Wu T, Zhou J, Zhang Y, Cao J (2007) Characterization and inheritance of a bush-type in tropical pumpkin (*Cucurbita moschata* Duchesne). Sci. Hortic. 114(1): 1-4.

Yılmaz S, Çağlar BK, Djelouah K (2019) Molecular characterization of phytoplasma diseases of pepper in Turkey. J Phytopathol. 167(9): 479-483.

Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE (2009) Construction of an interactive online phytoplasma classification tool, iPhyClassifier and its application in analysis of the peach X-disease phytoplasma group (16SrIII). Int. J. Syst. Evol. Microbiol 59: 2582-2593.

Zibadoost S, Rastgou M, Asghari S, Tazehkand SA (2016) Detection and molecular identification of 'Candidatus phytoplasma trifoli' infecting some cultivated crops and vegetables in West Azarbaijan province. Australas. Plant Dis. Notes. 11: 3.