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Identification of '*Candidatus* Phytoplasma solani' phytoplasma-associated diseases in eggplants exhibiting abnormal flower structure (phyllody and virescence) and witches' broom symptoms in Şanlıurfa province

Şanlıurfa ilinde anormal çiçek yapısı (phyllody ve virescence) ve cadı süpürgesi simptomları gösteren patlıcan bitkilerinde '*Candidatus* phytoplasma solani fitoplazma-ilişkili hastalığın tanımlanması

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ABSTRACT

Phytoplasmas cause infections in numerous plants in agricultural ecosystems, causing significant yield and quality losses in products. In recent years, it has been known that diseases caused by phytoplasmas cause economic losses in eggplant (*Solanum melongena* L.) cultivation. In Turkey, research on infections caused by phytoplasmas in eggplant growing areas is quite limited. This study was carried out to detect phytoplasma infections symptomatologically and molecularly in eggplant production areas in Şanlıurfa province. Fourteen samples were collected from eggplants exhibiting symptoms such as witches' broom, flower abnormalities (virescence, phyllody), elongation of the pedicle, arising of new shoots from flower parts, yellowing and proliferation. Phytoplasma infection was detected in 8 symptomatic samples using 16S rRNA-specific primers, P1/P7 and R16F2n/R16R2, by direct and nested PCR. Sequence information of fragments obtained as a result of molecular studies was extracted and BLAST analyses were performed. According to nucleotide sequence similarity in the 16S rRNA gene region, it was determined that the genetic group of phytoplasma causing infection in eggplant was related to '*Candidatus* Phytoplasma solani' (CaPsol) belonging to 16SrXII-A subgroup with 98% sequence identity. To our best knowledge, this study suggests comprehensive symptomatic diagnosis of CaPsol infecting eggplants in Türkiye.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an important crop that can be easily cultivated in tropical and subtropical regions, and it provides nutrition and economic value with a high yield and a short maturation period. Eggplant, which is commonly grown in China, India, Egypt, France, Italy and Spain (Rao and Kumar 2017), has been largely produced in

Türkiye with 817.591 tons in an area of 166.619 acres (TÜİK 2023). Şanlıurfa province has a significant potential in terms of eggplant production in the Southeastern Region.

Among these biotic stress factors, phytoplasmas, which have no direct control method, are of particular importance. Phytoplasmas are bacterial plant pathogens, a group of microorganisms that lack a cell wall and are genetically related to a Gram-positive ancestor (Weisburg et al. 1989). They are restricted to the phloem tissue in plants and are transmitted by sap-sucking insect vectors such as Cicadellidae, Fulgoromorpha, and Psyllidae (Weintraub and Beanland 2006). Phytoplasmas, whose molecular differences were defined using the 16S ribosomal gene as the basic standard (Duduk et al. 2010), have been classified into 48 tentative species and more than 150 subgroups so far, and 27 complete genomes have been sequenced (Wang et al. 2024). Among these genetic groups, '*Candidatus* Phytoplasma solani' (CaPsol) (subgroup 16SrXII-A), which is particularly widespread in the vineyards of the Euro-Mediterranean basin and poses a potential threat to viticulture worldwide and causes losses in members of the Solanaceae, has become remarkable in agroecosystems (Navrátil et al. 2009, Quaglino et al. 2019). Infections caused by CaPSol have been reported from different geographical regions of Türkiye and various agricultural ecosystems where perennial or annual plants such as tobacco, pepper, tomato and grapevine are grown (Erilmez et al. 2022, Usta et al. 2022, Zelyüt 2023, Zelyüt et al. 2022). Studies on phytoplasma-associated infections in eggplant cultivation areas of our country are quite limited and have only been reported from the Eastern Mediterranean and Eastern Anatolia regions of Turkey (Sertkaya et al. 2007, Usta et al. 2022). Moreover, it has been reported from different countries of the world that phytoplasma-induced infections in eggplant plants are associated with six different ribosomal groups (16SrI, 16SrII, 16SrIII, 16SrVI, 16SrIX, 16SrXII) (Rao and Kumar 2017). More specifically, phytoplasma-associated diseases have been reported to cause losses of up to 40% in eggplant plants (Mitra et al. 1993, Rao et al. 2011).

Phytoplasmas were identified in more than 1000 plant species with diseases and different symptoms exhibited in field crops, horticultural and ornamental plants and weeds (Bertaccini 2022, Bertaccini et al. 2022) and exhibit quite diverse symptoms from the behaviour and physiology of normal plants. However, eggplants infected with phytoplasma-associated diseases show widespread symptoms including dwarfism, witches' broom, little leaf formation, phyllody (Li et al. 2019, Gawande et al. 2022), hypertrophy of the calyces (Usta et al. 2022) and abnormal development of floral parts into leafy structures (Arocha et al. 2007, Asudi et al. 2021, Bertaccini et al. 2014, Karthikeyan et al. 2024, Šafárˇová et al. 2016).

Phytoplasmas have a wide host range, infecting plants and replicating within the bodies of insect vectors. Beyond their transmission by phloem-feeding insect vectors such as leafhoppers, planthoppers, and psyllids (Asudi et al. 2021, Gonella et al. 2008, Huang et al. 2021), they can also spread through alternative mechanisms. These include parasitic plants like dodder that connect to the vascular tissues of host plants (Akhtar et al. 2009, Montano et al. 2001), vegetative propagation materials such as grafts, cuttings, storage roots, rhizomes, bulbs (Omar and Foissac 2012), and stem segments (Bertaccini 2007, Tedeschi et al. 2006, Wang et al. 2024), as well as, in some cases, seeds (Kirdat et al. 2023, Randa-Zelyüt et al. 2022, Wang et al. 2024).

In phytoplasma-infected plants, specific symptoms can sometimes help diagnose the disease. However, in certain cases, infected plants may remain asymptomatic or display symptoms that are hard to differentiate from those caused by viral infections or physiological disorders (Wang et al. 2024). Thus, identification relies on techniques like electron microscopy, histochemical staining, serological assays, and molecular diagnostic methods. In additions, with the technological advances in recent years, the use of the *rp* (ribosomal protein) operon, *tuf*, *secY*, *secA*, *groEL* (*cpn60*) and *rpoB* marker genes (Botti and Bertaccini 2003, Lee et al. 2006, Lee et al. 2010, Marcone et al. 2000, Martini et al. 2002, Martini et al. 2007, Mitrovic et al. 2011, 2015, Hodgetts et al. 2008, Valiunas et al. 2013) has been developed to distinguish phytoplasmas and various genes encoding surface proteins such as *vmp1* (Cimerman et al. 2009, Fialová et al. 2009), *imp* (Danet et al. 2011), *amp* (Kakizawa et al. 2006), *stamp* (Fabre et al. 2011), and *hflB* (Schneider and Seemüller 2009) which appear to be more determinant at the strain level, has become widespread.

Almost half of the phytoplasma diseases observed in vegetables belong to *Solanaceae* family diseases and in eggplants. Distinct phytoplasma groups and subgroups have been reported in different countries, including the 16SrI group in Bangladesh (Kelly et al. 2009) and India (Kumar et al. 2012); 16SrI-B subgroup in Japan (Lee et al. 1998, Okuda et al. 1997); 16SrII-D subgroup in India (Kumar 2015, Yadav et al. 2016), Iran (Siampour et al. 2013), Oman (Al-Subhi et al. 2011) and Egypt (Omar and Foissac 2012); 16SrIII-J and -U subgroup in Brazil (Amaral Mello et al. 2007, Barros et al. 1998); 16SrVI-A subgroup in Türkiye (Sertkaya et al. 2007); 16SrVI-D subgroup in India (Azadvar and Branwal 2012, Kumar 2015); Bangladesh (Siddique et al. 2001, Wei

et al. 2008); 16SrIX-C subgroup in Iran (Tohidi et al. 2015) 16SrXII-A subgroup in Russia (Ember et al. 2011) and Türkiye (Usta et al. 2018).

This study was conducted to identify and characterize the presence of phytoplasma in eggplant plants, which exhibit suspicious phytoplasma symptoms in fields where eggplant is cultivated in Şanlıurfa province. PCR-based techniques were employed for this purpose.

MATERIALS AND METHODS

Sample collection

A total of 14 samples exhibiting phytoplasma symptoms such as phyllody, virescence, little leaf formation, witches' broom and yellowing were collected from eggplant cultivation fields in July-August 2023 in Eyyübiye, Haliliye, Karaköprü and Siverek districts of Şanlıurfa province. All plant samples were transported to Niğde Ömer Halisdemir University-Plant Production and Technologies Laboratory and stored at -20 °C prior to molecular analysis.

DNA extraction and PCR analysis

Total DNA was extracted from 0.5 g of plant tissue from young shoots, midribs, and flowers belonging to symptomatic eggplants using the cetyl trimethyl ammonium bromide (CTAB) technique (Doyle and Doyle 1990). To amplify the 16S rRNA gene region, the extracted DNA was amplified with the P1 (5'-AAGAGTTTGATCCTGGCTCAGGATT-3') / P7 (5'-CGTCCTTCATCGGCTCTT-3') (Deng and Hiruki 1991, Schneider et al. 1994) primer pair for direct PCR and with the R16F2n (5'-GAAACGACTGCTAAGACTGG-3') / R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG-3') (Gundersen and Lee 1996) primer pair for nested PCR in two-stage (direct and nested) PCR studies. Amplicons obtained from direct PCR products were diluted at a 1/50 ratio and used as template DNA in nested PCR. Thermocycling conditions were regulated to direct and nested PCR. For direct PCR: 3 min at 94 °C for the first denaturation, followed by 35 cycles of 1 min at 94 °C, 2 min at 50 °C and 3 min at 72 °C, finally 10 min at 72 °C. For nested 5 min at 9 °C for the first denaturation, followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 2 min at 72 °C, finally 10 min at 72 °C. The 25 µl of PCR reaction mixes consisted of 1 µl of DNA as a template, 2.5 µl of 10x PCR buffer, 1.5 µl of MgCl2 (25 mM), 1 µl of dNTP (10 mM), 1 µl of reverse and forward primer (10 µM), 0.2 µl of Taq DNA polymerase (2 unite) (Thermo-Fisher Scientific). A 'Ca. P. mali' (AP) isolate, used as a positive control, was kindly provided by Dr. B. Schneider (Germany). To visualize the products amplified by nested PCR, they were subjected to electrophoresis on a 1% agarose gel in 1xTAE (Tris Acetic EDTA) buffer at 120 V for 40 minutes. The gel was then stained with Ethidium Bromide and visualized with a UV transilluminator (Biorad).

DNA analysis

Amplified nested PCR products were sequenced using the amplification primers from both sides with Applied Biosystems®3500 by MedSanTek (İstanbul/Türkiye). Geneious Prime software was used to check the quality of the sequences and to expand the entire sequence of the fragments by merging the overlaps. The obtained sequence was blasted in the NCBI database (www.ncbi.nlm.nih.gov) and for further analysis similar sequences were retrieved. The aligned sequences were deposited in the GenBank database and an accession numbers were obtained.

The Neighbor-Joining (NJ) method inferred the phylogenetic tree (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021).

RESULTS

Symptomatology

The most dramatic symptoms related to phytoplasma agents in diseased eggplants were observed as flower organ abnormalities (phyllody, virescence). Additionally, yellowing of the entire plant, little leaf formation, new shoots from the flower parts, elongation of the flower stalk, and witches' broom were commonly observed symptoms (Figure 1).

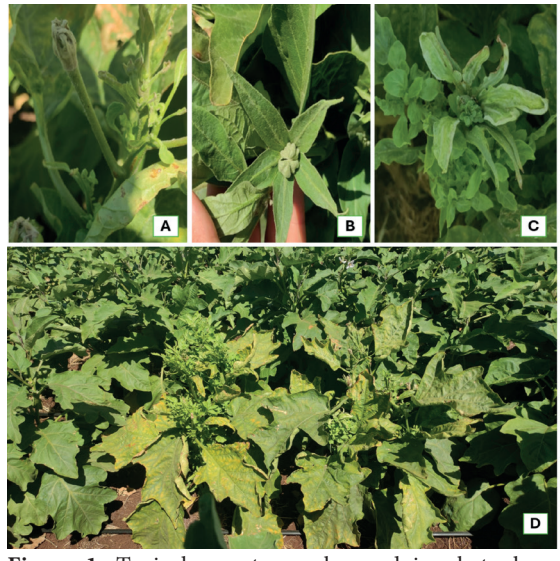

Figure 1. Typical symptoms observed in phytoplasma-infected plants: A. elongation of the flower stalk, B. Phyllody, C. Virescence and formation of new plants from flower parts, D. yellowing, witches' broom and proliferation

Detection and identification of phytoplasma agents

Fourteen symptomatic plants were tested against phytoplasma using 16S rRNA-specific primers. Amplifications of approximately 1200 bp in length expected for primer pairs R16F2n / R16R2 were obtained in 8 of all samples. The trimmed 991 nt nucleotide sequence of the PH50 isolate showed 98.85% similarity to the member of the 16SrXII-A 'Ca P. solani' sequence, an eggplant isolate from Turkey deposited in NCBI with the accession number KT595210. The phylogenetic tree constructed with CaPsol strains detected in Türkiye from different hosts was shown in Figure 2. The eggplant isolate showed 98.85% identity with all CaPSol strains from Türkiye.

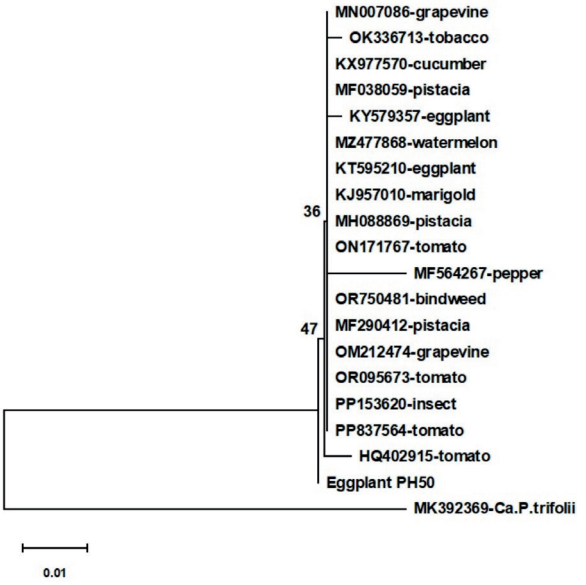

Figure 2. The phylogenetic tree constructed using the CaPsol strains detected in Türkiye from different hosts. 'Ca. Phytoplasma trifolii' used as outgroup (Bootstrap is 1000)

DISCUSSION

Phylogenetic analyses of 16S rRNA sequences of phytoplasmas worldwide revealed that phytoplasma-associated diseases in eggplants belong to different groups and subgroups of the pathogen. In Brazil, Amaral-Mello et al. (2011) identified that 16SrIII-B subgroups were associated with eggplant giant calix disease exhibiting symptoms such as leaf chlorosis, proliferation, shortened internodes, dwarfism, enlarging of calyces, small flowers and reduction of fruit size. In a study, carried out on eggplant in India, Venkataravanappa et al. (2018) detected mixed infections with both phytoplasma and begomovirus in showing little leaf formation and mosaic symptoms, and identified the phytoplasma-associated disease as Clover proliferation belonging to the 16SrVI group. Li et al. (2019) detected that the phyllody phytoplasma (eggplant phyllody phytoplasma – EPP) strain was associated to the 16SrII-D group in phylogenetic analyses based on 16S rRNA and *secA* gene sequences of eggplant plants showing phyllody, little leaf and witches' broom symptoms. Omar et al. (2020) identified peanut witches' broom phytoplasma belonging to 16SrII-X subgroup in infected eggplants displaying symptoms including phyllody, little leaf formation and witches' broom. Usta et al. (2022) detected CaPsol belonging to 16SrXII-A in eggplants exhibiting symptoms such as fruit deformation, hypertrophy of the calyces and yellowing. Gawande et al. (2022) found that '*Candidatus* Phytoplasma trifolii' was associated with Brinjal little leaf (BLL) disease in eggplants showing symptoms including little leaf formation, phyllody and witches' broom. Darabakula et al. (2024) detected phytoplasma associated diseases in infected eggplants showing brinjal little leaf symptoms; 16SrI, -II, -V, -VI, and -XII phytoplasma groups identified in the first-generation seedling produced from these infected eggplants whereas only 16SrI and 16SrXII groups identified in the second-generation seedlings. Karthikeyan et al. (2024) identified 'Ca. P. trifolii' belonging to 16SrVI Clover proliferation group in eggplants exhibiting symptoms such as little leaf formation, excessive growth of axillary shoots, virescence, phyllody, stunted growth, leaf chlorosis and witches' broom symptoms, and insect vectors.

In this study, CaPsol belonging to the 16SrXII-A phytoplasma group was identified based on the 16S rRNA conserved region in eggplant plants. CaPSol, the causative agent of stolbur disease, has a wide host range that includes both cultivated and wild plants (CABI 2024). In the 4 samples where phytoplasma could not be detected, it is likely that the phytoplasma density was at an undetectable density. CaPsol affects various members of the same species such as Solanaceae plants, grapes, lavender, strawberry, sugarcane, bindweed and common morning glory (Danet et al. 2003, Fos et al. 1992, Garnier 2000, Langer and Maixner 2004, Quaglino et al. 2013). In particular, the period when eggplant is grown at the same time as other crops raises concerns about the natural spread of phytoplasmas among different plant species. Therefore, effective control methods should be implemented in an integrated manner by choosing resistant plant species and taking into account regional conditions.

In this study, both symptomatic detection and molecular detection based on the 16S rRNA conserved region were performed in eggplant which is a significant host

of phytoplasma-associated diseases. Symptoms such as witches' broom, flower abnormalities (virescence, phyllody), elongation of the peduncle, production of new shoots from flower parts, yellowing and proliferation were observed in infected eggplants. Pathogen was identified as 16SrXII-A subgroup belonging to CaPSol with bioinformatics' analysis. To detect other possible hosts belonging to this phytoplasma species and insect vectors, advanced studies are necessary. Further studies are needed to identify other possible hosts and vector insects of this phytoplasma species.

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Author's Contributions
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ÖZET

Fitoplazmalar tarımsal ekosistemlerdeki çok sayıda bitkide enfeksiyonlara neden olarak ürünlerde önemli verim ve kalite kayıplarına yol açmaktadır. Son yıllarda patlıcan (*Solanum melongena* L.) yetiştiriciliğinde fitoplazmalardan kaynaklanan hastalıkların ekonomik kayıplara neden olduğu bilinmektedir. Türkiye'de patlıcan yetiştirilen alanlarda fitoplazmaların neden olduğu enfeksiyonlara ilişkin araştırmalar oldukça sınırlıdır. Bu çalışma, Şanlıurfa ilinde patlıcan üretim alanlarında görülen fitoplazma enfeksiyonlarının simptomatolojik ve moleküler olarak tespiti amacıyla yürütülmüştür. Cadı süpürgesi hastalığı, çiçek anormallikleri (viresens, fillodi), çiçek sapının uzaması, çiçek kısımlarından yeni sürgünlerin çıkması, sararma ve çoğalma gibi belirtiler gösteren patlıcanlardan 14 örnek toplanmıştır. 8 simptomatik örnekte 16S rRNA-spesifik primerler, P1/P7 ve R16F2n/R16R2 kullanılarak direkt ve nested PCR ile fitoplazma enfeksiyonu tespit edilmiştir. Moleküler çalışmalar sonucunda elde edilen fragmentlerin sekans bilgileri çıkarılmış ve BLAST analizleri yapılmıştır. 16S rRNA gen bölgesindeki nükleotid dizi benzerliğine göre, patlıcanda enfeksiyona neden olan fitoplazmanın genetik grubunun %98 dizi benzerliği ile 16SrXII-A alt grubuna ait '*Candidatus* Phytoplasma solani' (CaPsol) ile ilişkili olduğu belirlenmiştir. Bilgilerimize göre, bu çalışma Türkiye'de patlıcanları enfekte eden CaPsol'ün kapsamlı semptomatik teşhisini önermektedir.

Anahtar kelimeler: patlıcan, Şanlıurfa, CaPsol, nested-PCR, moleküler karakterizasyon

REFERENCES

Akhtar K.P., Sarwar G., Dickinson M., Ahmad M., Haq M.A., Hameed S., Iqbal M.J., 2009. Sesame phyllody disease: symptomatology, etiology and transmission in Pakistan. Turkish Journal of Agriculture and Forestry, 33 85), 477–486. doi:10.3906/tar-0901-23

Al-Subhi A.M., Al-Saady N.A., Khan A.J., Deadman M.L., 2011. First report of a group 16srII phytoplasma associated with witches'-broom of eggplant in Oman. Plant Disease, 95 (3), 360. doi: 10.1094/PDIS-10-10-0761

Amaral Mello A.P.O., Bedendo I.P., Camargo L.E.A., 2007. Identidade molecular dos fitoplasmas associados aos enfezamentos do tomateiro e da berinjela com base na ana´lise do gene 16S rDNA. Summa Phytopathologica, 33, 258–263 (in Portuguese).

Amaral Mello P., Eckstein B., Flores D., Kreyci P.F., Bedendo I.P., 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 sub- group 16SrIII-U. International Journal of Systematic and Evolutionary Microbiology, 61 (Pt 6), 1454–1461. doi: 10.1099/ijs.0.019141-0

Arocha Y., Antesana O., Montellano E., Franco P., Plata G., Jones P., 2007. '*Candidatus* Phytoplasma lycopersici', a phytoplasma associated with 'hoja de perejil' disease in Bolivia. International Journal of Systematic and Evolutionary Microbiology, 57 (Pt 8), 1704–1710. doi:10.1099/ijs.0.64851-0

Asudi G.O., Omenge K.M., Paulmann M.K., Reichelt M., Grabe V., Mithöfer A., Oelmüller R., Furch A.C.U., 2021. The physiological and biochemical effects on Napier grass plants following napier grass stunt phytoplasma infection. Phytopathology, 111 (4), 703–712. doi:10.1094/PHYTO-08-20-0357-R

Azadvar M., Baranwal V.K., 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. doi: 10.5958/j.2249-4669.2.1.001

Barros T.S.L., Kitajima E.W., Resende R.O., 1998. Diversidade de isolados brasileiros de fitoplasmas atrave´s da ana´lise do 16S rDNA. Phytopathology of Brasil, 23, 459–465 (in Portuguese).

Bertaccini A., 2007. Phytoplasmas: diversity, taxonomy, and epidemiology. Frontiers in Bioscience-Landmark, 12, 673–689. doi: 10.2741/2092

Bertaccini A., 2022. Plants and phytoplasmas: when bacteria modify plants. Plants, 11 (11), 1425. https://doi.org/10.3390/plants11111425.

Bertaccini A., Duduk B., Paltrinieri S., Contaldo N., 2014. Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. American Journal of Plant Sciences, 5, 1763–1788. doi: 10.4236/ajps.2014.512191

Bertaccini A., Arocha-Rosete Y., Contaldo N., Duduk B., Fior N., Montano H.G., Kube M., Kuo C.H., Martini M., Oshima K., 2022. Revision of the '*Candidatus* Phytoplasma' species description guidelines. International Journal of Systematic and Evolutionary Microbiology, 74, 005353.

Botti S., Bertaccini A., 2003. Variability and functional role of chromosomal sequences in phytoplasmas of 16SrI-B subgroup (aster yellows and related strains). Journal of Applied Microbiology, 94 (1), 103–110. doi: 10.1046/j.1365-2672.2003.01809.x

CABI, 2024. Invasive Species Compendium. *Candidatus* Phytoplasma solani (Stolbur Phytoplasma). https://www.cabi.org/isc/datasheet/108243/htm (accessed date: 18.09.2024).

Cimerman A., Pacifico D., Salar P., Marzachì C., Foissac X., 2009. Striking diversity of vmp1, a variable gene encoding a putative membrane protein of the "stolbur" phytoplasma. Applied and Environmental Microbiology, 75 (9), 2951–2957. doi: 10.1128/AEM.02613-08

Danet J.L., Foissac X., Zreik L., Salar P., Verdin E., Nourrisseau J.G., Garnier M., 2003. "Candidatus Phlomobacter fragariae" is the prevalent agent of marginal chlorosis of strawberry in French production fields and is transmitted by the planthopper *Cixius wagneri* (China). Phytopathology, 93 (6), 644–649. doi: 10.1094/PHYTO.2003.93.6.644

Danet J.L., Balakishiyeva G., Cimerman A., Sauvion N., Marie-Jeanne V., Labonne G., Lavina A., Batlle A., Krizanac I., Skoric D., Ermacora P., Ulubas Serce C., Caglayan K., Jarausch W., Foissac X., 2011. Multilocus sequence analysis reveals the genetic diversity of European fruit tree phytoplasmas and supports the existence of inter-species recombination. Microbiology, 157 (2), 438–450. https://doi.org/10.1099/mic.0.043547-0

Darabakula M., Mateeti S.T., Pacini F., Bertaccini A., Contaldo N., 2024. Eggplant little leaf-associated phytoplasma detection in seedlings under insect-proof conditions. International Journal of Plant Biology, 15 (2), 217–229. doi.org/10.3390/ijpb15020018

Deng S., Hiruki C., 1991. Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. Journal of Microbiological Methods, 14, 53–61.

Doyle J.J., Doyle J.L., 1990. Isolation of plant DNA from fresh tissue. Focus, 12, 13–15.

Duduk B., Tian J.B., Contald N., Fan X.P., Paltrinieri S., Chen Q.F., Zhao Q.F., Bertaccini A., 2010. Occurrence of phytoplasmas related to "stolbur" and to '*Candidatus* Phytoplasma japonicum' in woody host plants in China. Journal of Phytopathology, 158 (2), 100–104. https://doi.org/10.1111/j.1439-0434.2009.01586.x

Ember I., Acs Z., Munyaneza J.E., Crosslin J.M., Kolber M., 2011. Survey and molecular detection of phytoplasmas associated with potato in Romania and southern Russia. European Journal of Plant Pathology, 130 (3), 367-377. doi: 10.1007/s10658-011-9759-5

Erilmez S., Kaya A., Üstün N., Güven N., Altındişli F.Ö., Özsemerci F., 2022. Ege Bölgesi bağ alanlarında fitoplazma hastalıklarının ve olası vektör böcek türlerinin belirlenmesi. ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 32 (2), 264-276. https://doi.org/10.18615/anadolu.1225552

Fabre A., Danet J.L., Foissac X., 2011. The "stolbur" phytoplasma antigenic membrane protein gene stamp is submitted to diversifying positive selection. Gene, 472 (1-2), 37–41. doi: 10.1016/j.gene.2010.10.012

Felsenstein J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39 (4), 783-791. doi: 10.1111/j.1558-5646.1985.tb00420.x

Fialová R., Válová P., Balakishiyeva G., Danet J.L., Šafárová D., Foissac X., Navrátil M., 2009. Genetic variability of "stolbur" phytoplasma in annual crop and wild plant species in south Moravia. Journal of Plant Pathology, 91, 411–416. doi:10.4454/jpp.v91i2.971

Fos A., Danet J.L., Zreik L., Garnier M., Bove J.M., 1992. Use of a monoclonal-antibody to detect the stolbur mycoplasma-like organism in plants and insects and to identify a vector in France. Plant Disease, 76 (11), 1092–1096.

Garnier M., 2000. The stolbur phytoplasma: an ubiquitous agent. Comptes Rendus de l'Academie d'Agriculture de France, 86, 27–33.

Gawande Priya Y., Karthikeyan M., Johnson I., Swarnapriya R., Manikanda Boopathi N., 2022. Overview of little leaf disease in eggplant in Tamil Nadu. The Pharma Innovation Journal, 11 (9S), 2178-2182.

Gonella E., Negri I., Marzorati M., Brusetti L., Pajoro M. Mandrioli M., 2008. Study of the bacterial community affiliated to *Hyalesthes obsoletus*, the insect vector of "bois noir" phytoplasma of grape. Bulletin of Insectology, 61, 221–222.

Gundersen D., Lee I., 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathologia Mediterranea, 35, 144–151.

Hodgetts J., Boonham N., Mumford R., Harrison N., Dickinson M., 2008. Phytoplasma phylogenetics based on analysis of secA and 23S rRNA gene sequences for improved resolution of candidate species of 'Candidatus Phytoplasma'. International Journal of Systematic and Evolutionary Microbiology, 58 (Pt 8), 1826–1837. doi: 10.1099/ijs.0.65668-0

Huang W., MacLean A.M., Sugio A., Maqbool A., Busscher M., Cho S.T., Kamoun S., Kuo C.H., Immink R.G.H., Hogenhout S.A., 2021. Parasitic modulation of host development by ubiquitin-independent protein degradation. Cell, 184 (20), 5201-5214.e12. doi: 10.1016/j.cell.2021.08.029

Kakizawa S., Oshima K., Namba S., 2006. Diversity and functional importance of phytoplasma membrane proteins. Trends in Microbiology, 14 (6), 254–256. doi: 10.1016/j.tim.2006.04.008

Karthikeyan M., Yogiraj G.P., Elaiyabharathi T., Jesu B.A.J., Johnson I., Jaffer S.B., Dhanabalan S.P., Boopathi N.M., Marimuthu S., Nejad H.S., Adorada D.L., 2024. Comprehensive analysis of little leaf disease incidence and resistance in eggplant. BMC Plant Biology, 24 (1), 576. https://doi.org/10.1186/s12870-024-05257-4

Kelly P.L., Arocha Y., Dider S.Z., 2009. First report of a 16SrI, '*Candidatus* Phytoplasma asteris' isolate affecting eggplant and *Mikania* sp. in Bangladesh. Plant Pathology, 58 (4), 789. https://doi.org/10.1111/j.1365-3059.2009.02070.x

Kimura M.A., 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16 (2), 111-120. doi: 10.1007/BF01731581

Kirdat K., Tiwarekar B., Sathe S., Yadav A., 2023. From sequences to species: charting the phytoplasma classification and taxonomy in the era of taxogenomics. Frontiers in Microbiology, 14, 1123783. doi: 10.3389/fmicb.2023.1123783

Kumar J., Gunapati S., Singh S.P., Lalit A., Sharma N.C., Tuli R., 2012. First report of a '*Candidatus* Phytoplasma asteris' (16SrI group) associated with little leaf disease of *Solanum melongena* (brinjal) in India. New Disease Reports, 26 (1), 21. https://doi.org/10.5197/j.2044-0588.2012.026.021

Kumar M., 2015. Genetic diversity and natural spread sources of brinjal little leaf phytoplasma. M.Sc. Thesis. Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India, p. 103.

Langer M., Maixner M., 2004. Molecular characterisation of grapevine yellows associated phytoplasmas of the stolbur-group based on RFLP-analysis of non-ribosomal DNA. Vitis, 43 (4), 191–200.

Lee I.M., Gundersen-Rindal D.E., Davis R.E. Bartoszyk I.M., 1998. Revised classification scheme of phytoplasma based on RFLP analyses of 16S rDNA and ribosomal protein gene sequences. International Journal of Systematic and Evolutionary Bacteriology, 8 (4), 1153–1169. https://doi.org/10.1099/00207713-48-4-1153

Lee I-M, Zhao Y., Bottner K.D., 2006. SecY gene sequence analysis for finer differentiation of diverse strains in the aster yellows phytoplasma group. Molecular and Cellular Probes, 20 (2), 87–91. doi: 10.1016/j.mcp.2005.10.001

Lee I.M., Bottner-Parker K.D., Zhao Y., Davis R.E., Harrison N.A., 2010. Phylogenetic analysis and delineation of phytoplasmas based on secY gene sequences. International Journal of Systematic and Evolutionary Microbiology, 60, 2887–2897. doi: 10.1099/ijs.0.019695-0

Li Z., Tang Y., She X.M., Yu L., Lan G., He Z., 2019. First report of 16SrII-D phytoplasma associated with eggplant phyllody in China. Canadian Journal of Plant Pathology, 41, 339-344. doi:10.1080/07060661.2019.1596162

Marcone C., Lee I-M., Davis R.E., Ragozzino A., Seemüller E., 2000. Classification of aster yellows-group phytoplasmas based on combined analyses of rRNA and tuf gene sequences. International Journal of Systematic and Evolutionary Microbiology, 50, 1703–1713. doi: 10.1099/00207713-50-5-1703

Martini M., Botti S., Marcone C., Marzachì C., Casati P., Bianco P.A., Benedetti R., Bertaccini A., 2002. Genetic variability among "flavescence dorée" phytoplasmas from different origins in Italy and France. Molecular and Cellular Probes, 16, 197–208. doi: 10.1006/mcpr.2002.0410

Martini M., Lee I-M., Bottner K.D., Zhao Y., Botti S., Bertaccini A., Harrison N.A., Carraro L., Marcone C., Khan J., Osler R., 2007. Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. International Journal of Systematic and Evolutionary Microbiology 57, 2037–2051. doi: 10.1099/ijs.0.65013-0

Navrátil M., Válová P., Fialová R., Lauterer P., Šafářová D., Starý M., 2009. The incidence of stolbur disease and associated yield losses in vegetable crops in South Moravia (Czech Republic). Crop Protection, 28 (10), 898-904. https://doi.org/10.1016/j.cropro.2009.05.008

Mitra D.K., 1993. Little leaf, a serious disease of eggplant (*Solanum melongena*). In: Raychaudhuri, S.P., Teakle, D.S. (Eds.), Management of Plant Diseases Caused by Fastidious Prokaryotes. Associated Publishing Co, New Delhi, India, pp. 73-78.

Mitrovic J., Kakizawa S., Duduk B., Oshima K., Namba S., Bertaccini A., 2011. The cpn60 gene as an additional marker for finer differentiation of '*Candidatus* Phytoplasma asteris'-related strains. Annals of Applied Biology, 159, 41–48. http://dx.doi.org/10.1111/j.1744-7348.2011.00472.x

Mitrovic J., Smiljković M., Seemüller E., Reinhardt R., Hüttel B., Büttner C., Bertaccini A., Kube M., Duduk B., 2015. Differentiation of 'Candidatus Phytoplasma cynodontis' based on 16S rRNA and groEL genes and identification of a new subgroup, 16SrXIV-C. Plant Disease, 99, 1578–1583.

Montano H.G., Dally E.L., Davis R.E., Pimentel J.P., Brioso P.S.T., 2001. First report of natural infection by "Candidatus Phytoplasma brasiliense" in *Catharanthus roseus*. Plant Disease, 85 (11), 1209. doi: 10.1094/PDIS.2001.85.11.1209C

Okuda S., Prince J.P., Davis R.E., Dally E.L., Lee I.M., Mogen B., Kato S., 1997. Two groups of phytoplasma from Japan distinguished on the basis of amplification and restriction analysis of 16S rDNA. Plant Disease, 81 (3), 301–305. doi: 10.1094/PDIS.1997.81.3.301

Omar A.F., Alsohim A.S., Dumonceaux T.J., Pérez-López E., 2020. Molecular characterization of '*Candidatus* Phytoplasma australasia' 16SrII subgroups associated with eggplant, cabbage, beetroot, and celery in Saudi Arabia, Crop Protection, 127, 104970. https://doi.org/10.1016/j.cropro.2019.104970

Omar A.F., Foissac X., 2012. Occurrence and incidence of phytoplasmas of the 16SrII-D subgroup on solanaceous and cucurbit crops in Egypt. European Journal of Plant Pathology, 133, 353–360. doi: 10.1007/s10658-011-9908-x

Quaglino F., Sanna F., Moussa A., Faccincani M., Passera A., Casati P., Bianco P.A., Mori N., 2019. Identification and ecology of alternative insect vectors of '*Candidatus* Phytoplasma solani' to grapevine. Scientific Reports, 9 (1), 19522. https://doi.org/10.1038/s41598-019-56076-9

Quaglino F., Zhao Y., Casati P., Bulgari D., Bianco P.A., Wei W., Davis R.E. 2013. "*Candidatus* Phytoplasma solani", a novel taxon associated with stolbur- and bois noir-related diseases of plants. International Journal of Systematic and Evolutionary Microbiology, 63 (Pt 8), 2879–2894. doi:10.1099/ijs.0.044750-0

Randa Zelyüt F., Ertunç F., Şenal D., 2022. The association of 16SrVI and 16SrI phytoplasma groups with carrot seeds and weeds in Ankara and Konya provinces in Turkey. Plant Protection Bulletin, 62 (1), 24-33. https://doi.org/10.16955/bitkorb.1014427

Rao G.P., Kumar M., 2017. World status of phytoplasma diseases associated with eggplant. Crop Protection, 96, 22–29. https://doi.org/10.1016/j.cropro.2017.01.005

Rao G.P., Mall S., Raj S.K., Snehi S.K., 2011. Phytoplasma diseases affecting various plant species in India. Acta Phytopathologica et Entomologica Hungarica, 46 (1), 59-99. https://doi.org/10.1556/aphyt.46.2011.1.7

Šafárˇová D., Zemánek T., Válová P., Navrátil M., 2016. '*Candidatus* Phytoplasma cirsii', a novel taxon from creeping thistle [*Cirsium arvense* (L.) Scop]. International Journal of Systematic and Evolutionary Bacteriology, 66 (4), 1745–1753. doi: 10.1099/ijsem.0.000937

Saitou N., Nei M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4 (4), 406-425. doi: 10.1093/oxfordjournals.molbev.a040454

Schneider B., Seemüller E., 2009. Strain differentiation of '*Candidatus* Phytoplasma mali' by SSCP- and sequence analyses of the *hflB* gene. Journal of Plant Pathology, 91 (1), 103–112. doi: 10.4454/jpp.v91i1.630

Schneider B., Seemüller E., 1994. Presence of two sets of ribosomal genes in phytopathogenic mollicutes. Applied and Environmental Microbiology, 60 (9), 3409-3412. doi: 10.1128/aem.60.9.3409-3412.1994

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

Siampour M., Izadpanah K., Galettob L., Salehic M., Marzachi, C., 2013. Molecular characterization, phylogenetic comparison and serological relationship of the *Imp* protein of several 'Candidatus Phytoplasma aurantifolia' strains. Plant Pathology, 62, 452-459. doi: 10.1111/J.1365-3059.2012.02662.X

Siddique A.B.M., Agrawal G.K., Alam N., Krishina Reddy M., 2001. Electron microscopy and molecular characterization

of phytoplasmas associated with little leaf disease of brinjal (*Solanum melongena* L.) and periwinkle (*Catharanthus roseus*) in Bangladesh. Journal of Phytopathology, 149 (5), 237–244. https://doi.org/10.1046/j.1439-0434.2001.00590.x

Tamura K., Stecher G., Kumar S., 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38 (7), 3022-3027 https://doi.org/10.1093/molbev/msab120

Tedeschi R., Alma A., 2006. *Fieberiella florii* (Homoptera: Auchenorrhyncha) as a vector of "*Candidatus* Phytoplasma mali". Plant Disease, 90 (3), 284–290. https://doi.org/10.1094/PD-90-0284

Tohidi Z., Salehi M., Ghasemi S., Khanchezar A., Shahamiri S.M., 2015. Association of a 16SrIX-C phytoplasma with eggplant phyllody in Iran. Journal of Crop Protection, 4 (2), 247–256.

TUİK, 2023. Bitkisel Üretim İstatistikleri. http://www.tuik.gov.tr. (accessed date: 15.03.2025).

Usta M., Guller A., Sipahioğlu H.M., 2018. Molecular analysis of '*Canditatus* Phytoplasma trifolii' and '*Canditatus* Phytoplasma solani' associated with phytoplasma diseases of tomato (PDT) in Turkey. International Journal of Agriculture and Biology, 20, 1991-1996. https://doi.org/10.17957/IJAB/15.0721

Usta M., Güller A., Sipahioğlu H.M., 2022. Detection, *in silico* analysis and molecular diversity of phytoplasmas from solanaceous crops in Turkey. Plant Protection Science, 58 (1), 31–39. https://doi.org/10.17221/115/2021-PPS

Valiunas D., Jomantiene R., Davis R.E., 2013. Evaluation of the DNA-dependent RNA polymerase β-subunit gene (rpoB) for phytoplasma classification and phylogeny. International Journal of Systematic and Evolutionary Microbiology, 63 (Pt 10), 3904–3914. doi: 10.1099/ijs.0.051912-0

Venkataravanappa V., Prasanna H.C., Lakshminarayana C.N., Reddy M.K., 2018. Molecular detection and characterization of phytoplasma in association with begomovirus in eggplant. Acta Virologica, 62 (3), 246-258. doi: 10.4149/av_2018_218

Wang R., Bai B., Li D., Wang J., Huang W., Wu Y., Zhao L., 2024. Phytoplasma: a plant pathogen that cannot be ignored in agricultural production-research progress and outlook. Molecular Plant Pathology, 25 (2). e13437. doi: 10.1111/mpp.13437

Wei W., Lee M., Davis R.E., Suo X., Zhao Y., 2008. Automated RFLP pattern comparison and similarity coefficient calculation for rapid delineation of new and distinct phytoplasma 16Sr subgroup lineages. International Journal of Systematic and Evolutionary Microbiology, 58 (10), 2368-2377. https://doi.org/10.1099/ijs.0.65868-0

Weintraub P.G., Beanland L., 2006. Insect vectors of phytoplasmas. Annual Review of Entomology, 51 (1), 91-111. doi: 10.1146/annurev.ento.51.110104.151039

Weisburg W.G., Tully J.G., Rose D.L., Petzel J.P., Oyaizu H., Yang D., Van Etten J., 1989. A phylogenetic analysis of the mycoplasmas: basis for their classification. Journal of Bacteriology, 171 (12), 6455-6467. 10.1128/jb.171.12.6455-6467.1989

Yadav V., Mahadevakumar S., Tejaswini G.S., Shilpa N., Amruthavalli C., Janardhana G.R., 2016. First report of 16SrII-D phytoplasma associated with eggplant big bud (*Solanum melongena* L.) in India. Plant Disease, 100, 517. doi: 10.1094/PDIS-07-15-0761-PDN

Zelyüt F.R., Santosa A.I., Karanfil A., 2022. '*Candidatus* Phytoplasma solani' (subgroup 16SrXII-A) associated with Nicotiana tabacum leaf abnormality in Turkey. Tekirdağ Ziraat Fakültesi Dergisi, 19 (3), 571-581. https://doi.org/10.33462/jotaf.1028263

Zelyüt F.R., 2023. Genetic diversity and molecular variability of *Candidatus* Phytoplasma solani based on multilocus sequence typing analysis in tomato plantations of western Turkey. Physiological and Molecular Plant Pathology, 127, 102120. https://doi.org/10.1016/j.pmpp.2023.102120