

ORIGINAL ARTICLE

Molecular identification of *Candidatus Phytoplasma* spp. associated with *Sophora* yellow stunt in Iran

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Abstract

In the spring of 2012, *sophora* (*Sophora alopecuroides* L.) plants showing symptoms of leaf yellowing, little leaves and stunting were observed in Firooz-kuh (Tehran province), Sari (Mazandaran province) and Urmia (West Azerbaijan province) in Iran. Symptomatic plants from the three locations were subjected to nested polymerase chain reaction (PCR) to amplify 16SrRNA using primer pair P1/P7 followed by primer pair R16F2n/R16R2. The amplicons were purified, sequenced and the nucleotide sequences were analyzed by virtual restriction fragment length polymorphism (RFLP). The phytoplasmas associated with the yellows disease were identified as members of the 16SrIX group (*Candidatus Phytoplasma phoenicium*) and the 16SrXII group (*Candidatus Phytoplasma solani*). The two phytoplasmas were placed in 16SrIX-C and 16SrXII-A subgroups, respectively, in constructed phylogenetic trees. This is the first report on *sophora* yellows associated with *Candidatus Phytoplasma phoenicium*.

Key words: *Candidatus Phytoplasma phoenicium*, nested-PCR, *Sophora* yellows

Introduction

Phytoplasmas are important phloem-limited, insect-transmitted agents associated with approximately a thousand plant diseases, many of which are lethal in hundreds of plant species including weeds throughout the world (Marcone *et al.* 1997). Plants infected with phytoplasma exhibit an array of symptoms that suggests profound disturbances in the balance of growth regulators (Duduk and Bertaccini 2011). Typical symptoms include virescence, phyllody, sterility of flowers, proliferation of axillary buds resulting in witches broom growth, elongation of internodes and generalized stunting (Bertaccini *et al.* 1996, 2014). Phytoplasmas cause diseases in several weeds which may act as alternative natural hosts facilitating the spread of phytoplasmas to economically important plants and thereby increasing economic losses. So far more than 100 weed species have been reported to have phytoplasma infections in many regions of the world. Phytoplasmas are able to survive in many economical crops and the

insect vectors are capable of transmitting phytoplasmas from weeds to crops which are known as phytoplasma hosts. The chance of transmission in the future seems high, given the large phytoplasma reservoirs already existing and the propensity of new phytoplasma strains to evolve (Mall *et al.* 2010). Phytoplasma identity is essential for phytoplasma disease management, especially in providing information about the mode of disease spread (insect vectors, propagation material, etc.). *Sophora alopecuroides* L. (*sophora*) is one of several herbaceous species of Leguminosae (Fabaceae), native to southeast Europe, southern Asia, Australasia, the Pacific Islands, western South America and Puerto Rico and possesses medicinal properties. It grows along the edges of fields, banks and less frequently on sand dunes, from sea level to 1750 m (Chamberlin 1970). However, in many areas of Iran it is regarded as a rather noxious weed species necessitating control. This plant grows around and in orchards, especially in

plum, peach and nectarine orchards, showing phytoplasma-like symptoms. Its infectivity by *Candidatus Phytoplasma solani* was verified previously (Allahverdipour *et al.* 2013a, b) and it exhibits phytoplasma-like symptoms including leaf yellowing, little leaf and phyllody. Since weeds can serve as reservoirs for phytoplasmas the aim of the present study was to verify the presence of phytoplasma diseases in symptomatic sophora plants in three different geographical regions of Iran using polymerase chain reaction (PCR) assay. The phytoplasmas detected were characterized and classified using sequence analysis of PCR-amplified 16S rDNA and virtual restriction fragments length polymorphism (RFLP).



Fig. 1. Sophora with yellowing symptoms, left – healthy plant, right – infected plant

Materials and Methods

During the spring season of 2012, leaves and shoots from 24 symptomatic *S. alopecuroides* plants showing typical symptoms of leaf yellowing, little leaf and phyllody were collected in Firooz-kuh (Tehran province), Sari (Mazandaran province) and Urmia (West Azerbaijan province) regions of Iran (Figs. 1, 2) around and in plum, peach and nectarine orchards showing phytoplasma-like symptoms. At least eight symptomatic sophora plants were collected from each region. Asymptomatic sophora plants were also collected and used in molecular analysis as negative controls. Total DNA was extracted from 0.25 g of leaves and midribs using the CTAB extraction method (Maixner *et al.*

1995) and DNeasy Plant Mini kit (Qiagen, UK) and maintained at -80°C until use. Total DNA of healthy plants was extracted and used as negative controls. The universal primer pair P1/P7 (Schneider *et al.* 1995) was employed in direct PCR to amplify a 1.8 kbp fragment of 16S rDNA. A 30-fold dilution of the direct PCR product was used as templates for nested PCR using primer pair R16f2n/R16R2 which amplifies an internal fragment of 1.2 kbp from the 16S rDNA species in the genus *Candidatus Phytoplasma* (Lee *et al.* 1993). A total volume of 20 μl PCR reaction mixtures contained 20 ng DNA template, 0.2 mM of each dNTPs (Cinnagen, Iran), 1.6 mM MgCl_2 , 1U of *Taq* DNA polymerase (Cinnagen, Iran), 0.5 μl of each primer pair (20 pmol $\cdot \mu\text{l}^{-1}$) and 1X PCR buffer was used. The reaction mixtures were subjected to 35 cycles under



Fig. 2. Sophora with phyllody symptoms, healthy plant is on the top

Table 1. Strains of *Candidatus* Phytoplasma isolated from *Sophora alopecuroides* with yellow stunt and phyllody symptoms from various regions of Iran with GenBank accession numbers of their DNA spacer sequence

Strain	Location	GenBank accession No.
<i>Candidatus</i> Phytoplasma solani strain S141	Sari-Mazandaran province	KX172137
<i>Candidatus</i> Phytoplasma solani strain S135	Firoozkuh-Tehran province	KF923878
<i>Candidatus</i> Phytoplasma solani strain S139	Firoozkuh-Tehran province	KX172136
<i>Sophora alopecuroides</i> phyllody Phytoplasma isolate S191	Sari-Mazandaran province	KJ001834
<i>Sophora alopecuroides</i> phyllody Phytoplasma isolate S194	Urmia-West Azarbaijan province	KX172134
<i>Sophora alopecuroides</i> phyllody Phytoplasma isolate S196	Sari-Mazandaran province	KX172135

the following conditions: 1 min (2 min for the first cycle) denaturation step at 94°C, 2 min for annealing at 50°C and 3 min (10 min for the last cycle) for primer extension at 72°C. PCRs were performed in a thermal cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany). The PCR products were analyzed by electrophoresis in 1% agarose gel and stained with ethidium bromide. An ultraviolet (UV) trans-illuminator was used to visualize DNA bands. PCR products of nested PCR were purified and directly sequenced. Sequencing was performed by Macrogen (South Korea) on both strands with three replicates. Sequences were compared with the phytoplasmas sequences deposited in GenBank database using BLASTn. Nucleotide sequence similarity and multiple alignment and phylogenetic tree construction of phytoplasma 16S rDNA gene sequences were carried out using the neighbor-joining (NJ) method and bootstrap analysis replicated 1,000 times employing MEGA6 software (Tamura *et al.* 2011). *Acholeplasma laidlawii* was used as an out-group. Assignment of phytoplasmas to 16Sr group/subgroup was done by *in silico* RFLP analyses of F2n/R2

amplicons using the software iPhyClassifier (Zhao *et al.* 2009). RFLP profiles of the test phytoplasmas were compared to those of 16SrIX-subgroups A to G using: *AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI* and *TaqI* recognition sites.

Results

Sophora plants with phytoplasma symptoms were collected from different regions of Iran including Firoozkuh, Sari and Urmia during 2012 and DNA fragments of approximately 1.8 kbp and 1.25 kbp were amplified using phytoplasma universal primer pairs, P1/P7 and R16f2n/R16R2 in direct and nested PCR, respectively from all 24 symptomatic plants. No DNA was amplified from asymptomatic plants with either of the PCR methods. Comparison of 1,200 bp of 16S rDNA sequences of six of these phytoplasmas with those present in GenBank revealed that the three phytoplasma

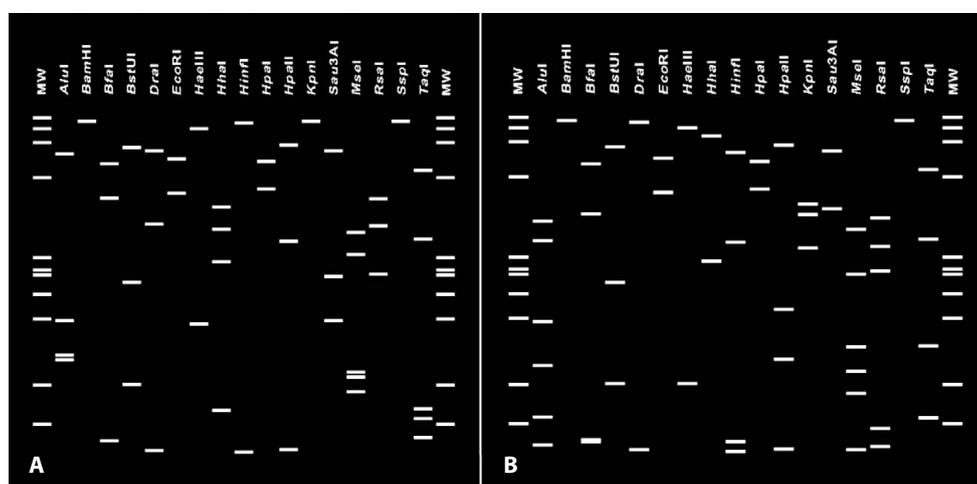


Fig. 3. Virtual restriction fragment length polymorphism (RFLP) pattern of R₁₆F_{2n}/R₁₆R₂ PCR product sequence from *Sophora*. A – *Candidatus* Phytoplasma solani strain S135, B – *Sophora alopecuroides* phyllody phytoplasma isolate S191. Restriction sites for the 17 restriction enzymes used in the simulated digestions: *AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI* and *TaqI*

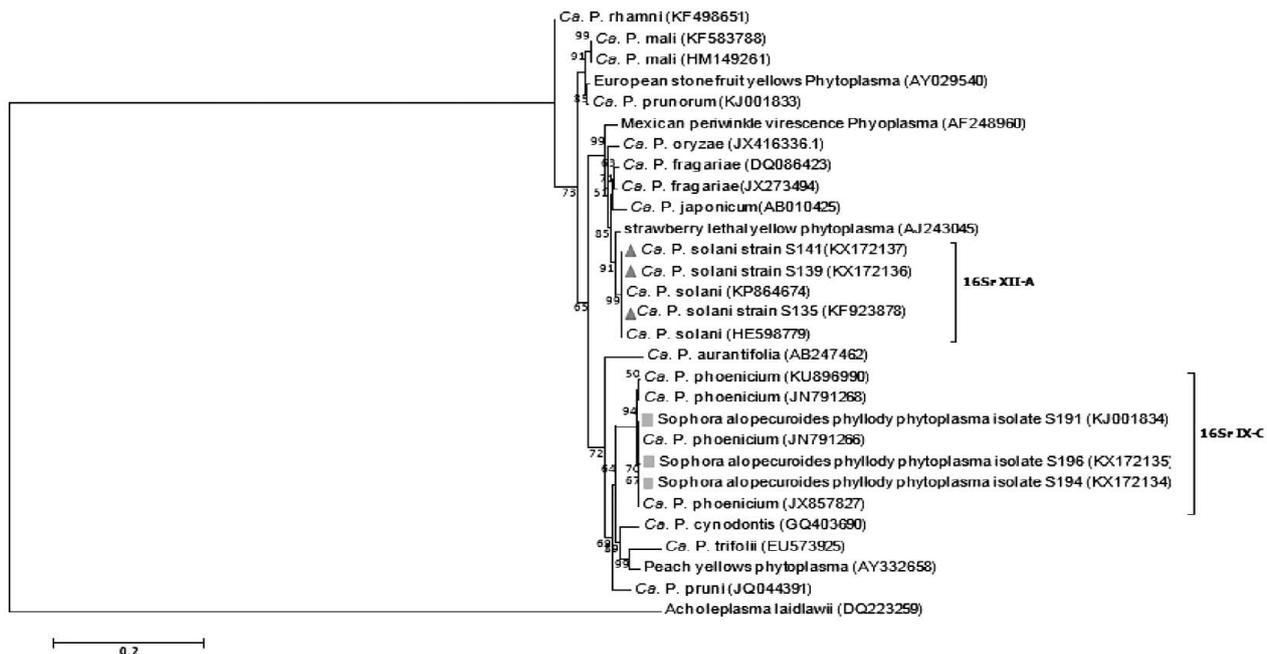


Fig. 4. Phylogenetic tree constructed by the neighbor joining method of 16S rRNA gene sequences from 22 phytoplasma and the strains isolated from *Sophora* plants. *Acholeplasma laidlawii* was used as outgroup. Numbers at the nodes are bootstrap values based on 1,000 repetitions. GenBank accession numbers for sequences are given in parentheses. Ca. P. – *Candidatus Phytoplasma*

strains under study shared 99-100% sequence identity with *Candidatus Phytoplasma phoenicium* (accession number AF515636), ribosomal RNA group IX and the other three strains had 100% identity to *Candidatus Phytoplasma solani* (accession number AF248959), ribosomal RNA group XII. Nucleotide sequences determined in the present study were deposited in the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers: KJ001834, KX172134, KX172135, KX172136, KX172137 and KF923878. Computer-simulated restriction analyses were carried out on R16f2n/R16R2 amplified sequences from *sophora* together with 22 reference phytoplasmas and representative strains belonging to 16SrIX and 16SrXII subgroups. Comparison of virtual gel images revealed that RFLP patterns of *sophora* shared a virtual RFLP similarity coefficient >98% with *Candidatus Phytoplasma phoenicium* strains of subgroup IX-C and *Candidatus Phytoplasma solani* strains of subgroup XII-A (Fig. 3). Phylogenetic analyses of sequences presented in this survey with those of phytoplasmas present in GenBank clearly demonstrated that the three phytoplasmas (with accession numbers: KJ001834, KX172134 and KX172135) clustered with the strains belonging to group 16SrIX, and the other three strains (with accession numbers: KX172136, KX172137 and KF923878) belonged to group 16SrXII and were well separated from the members of other phytoplasma groups (Fig. 4). Furthermore, these phytoplasmas clustered together with the strains of *Candidatus Phytoplasma phoenicium* (subgroup 16SrIX-C) and

Candidatus Phytoplasma solani (subgroup 16SrXII-A), verifying their close similarity to the above-mentioned subgroups.

Discussion

Sophora plants with little leaf, yellowing and phyllody symptoms were collected around and in phytoplasma-infected plum, peach and nectarine orchards. Approximately 50% of the plants from the three sampled regions were symptomatic at the time of collection. Sequence analyses and virtual RFLP of *Sophora* phytoplasma strains clearly showed that three of the studied phytoplasma strains belonged to phytoplasma group 16SrIX-C (*Candidatus Phytoplasma phoenicium*) and three other strains belonged to the 16SrXII-A group (*Candidatus Phytoplasma solani*). Phytoplasmas of ribosomal group 16SrIX (pigeon pea witches' broom group) are associated with diseases affecting crops and wild plants in different geographic areas worldwide (Kenyon *et al.* 1998; Verdin *et al.* 2003; Khan *et al.* 2007; Davis *et al.* 2010). Several strains of *Candidatus Phytoplasma phoenicium* have previously been reported from a number of plants including *Lactuca serriola*, *L. sativa*, *Solanum lycopersicon*, *Sonchus* sp. [16SrIX-E], *Carthamus tinctorius*, *Chrysanthemum morifolium* cv. Paniz and *Prunus amygdalus* [16SrIX-B] from Iran (Salehi *et al.* 2006, 2011; Bayat *et al.* 2013). It has also been shown that *Candidatus Phytoplasma*

phoenicium is associated with a lethal disease of almond, peach and nectarine, called almond witches' broom disease (AlmWB) (Abou-Jawdah *et al.* 2009). Molino Lova *et al.* (2011) showed that Iranian phytoplasma strains associated with AlmWB and almond brooming shared >99% similarity with phytoplasmas of subgroup IX-C.

Phytoplasmas classified in the 16S rRNA gene RFLP group 16SrXII infect a wide range of wild and cultivated plants including grapevine (where it induces Bois noir, BN), potato, and strawberry worldwide (Davis *et al.* 1997; Valiunas *et al.* 2006; Quaglino *et al.* 2013). Our study in Iran also showed that the 16SrXII group of phytoplasmas, especially *Candidatus* Phytoplasma solani, is widespread in Iran and has been reported on different crops such as potato (Hosseini *et al.* 2011) plum, peach, nectarine (Zirak *et al.* 2009a, b, 2010; Allahverdipoor *et al.* 2013a, b) and grapevine (Mirchenari *et al.* 2015).

Previous reports of phytoplasmas affecting sophora species include a 16SrXII phytoplasma associated with *S. japonicum* yellows (Duduk *et al.* 2010) in China. Other phytoplasmas have also been identified on *S. japonicum* including *Candidatus* Phytoplasma ziziphi in China associated with witches' broom disease and a 16SrI *Candidatus* Phytoplasma asteris associated with sophora yellows (Yu *et al.* 2012; Chen *et al.* 2013). Allahverdi *et al.* (2014) reported a 16SrXII phytoplasma association with *S. alopecuroides* from Firooz-kuh (Iran) with leaf yellowing, little leaf and stunting symptoms. The association of a phytoplasma belonging to 16SrVI (*Candidatus* Phytoplasma trifolii) was previously established in sophora exhibiting yellowing and witches' broom symptoms in China (Li *et al.* 2013) and West Azarbaijan province of Iran (Zibadoost and Rastgou 2016).

Phytoplasma infections have been documented in more than 100 weed plant species thus far (Singh and Upadhyaya 2013). Sophora is widely distributed as a volunteer plant and grows frequently in or around farms and orchards in the studied regions. Our results suggest the potential of sophora acting as a significant alternative host plant for *Candidatus* Phytoplasma phoenicium and *Candidatus* Phytoplasma solani, posing a threat to crops such as almond, nectarine, plum, peach and grapevine in geographical regions where these plants are main crops. The wide host ranges of these phytoplasmas implicate them as alternative hosts in which the phytoplasmas are maintained, perpetuated and transmitted by insect vectors to commercial crops and they can be a severe threat to them. This is, to our knowledge, the first report of a phytoplasma of the 16SrIX group member infecting sophora. The emergence of this phytoplasma in the sophora represents ongoing evolution in the adaptation of *Candidatus* Phytoplasma phoenicium to a new ecological niche (Arocha-Rosete

et al. 2011). Additional studies are needed to determine the distribution, insect vectors, and economic impact of *Candidatus* Phytoplasma solani and *Candidatus* Phytoplasma phoenicium on plum, peach and nectarine orchards and other crops in the studied regions.

References

- Abou-Jawdah Y., Sobh H., Akkary M. 2009. First report of Almond witches' broom phytoplasma (*Candidatus* Phytoplasma phoenicium) causing a severe disease on nectarine and peach trees in Lebanon. Bulletin OEPP/EPPO Bulletin 39 (1): 94–98. DOI: <https://doi.org/10.1111/j.1365-2338.2009.02223.x>
- Allahverdipoor T., Rahimian H., Rastgou M., Babaiezed V. 2013a. Molecular identification of the phytoplasma associated with peach and nectarine trees in West Azarbaijan province. 2th National Conference on Climate Change and its Impact on Agriculture and Nature, 14 August: 65–70.
- Allahverdipoor T., Rahimian H., Babaiezed V., Rastgou M. 2013b. *Candidatus* Phytoplasma solani associated with European and Japanese plums leaf roll in north of Iran. National Student Congress of Biosciences, 25–26 September: 71–72.
- Allahverdi T., Rahimian H., Babaiezed V. 2014. First report of a *Candidatus* Phytoplasma solani isolate affecting *Sophora alopecuroides* in Iran. New Diseases Report 30: 22. DOI: <https://doi.org/10.5197/j.2044-0588.2014.030.022>
- Arocha-Rosete Y., Zunnoon-Khan S., Krukovets I., Crosby W., Scott J., Bertaccini A., Michelutti R. 2011. Identification and molecular characterization of the phytoplasma associated with peach rosette-like disease at the Canadian Clonal Genebank based on the 16S rRNA gene analysis. Canadian Journal of Plant Pathology 33 (2): 127–134. DOI: <https://doi.org/10.1080/07060661.2011.558854>
- Bayat H., Verhoeven T.Th.J., Botermans M., Peters D., Hassani-Mehraban A. 2013. First report of a 16SrIX group (*Candidatus* Phytoplasma phoenicium'-Related) phytoplasma associated with a chrysanthemum disease. Plant Disease 97 (8): 1110. DOI: <https://doi.org/10.1094/pdis-01-13-0050-pdn>
- Bertaccini A., Bellardi M.G., Vibio M. 1996. Virus diseases of ornamental shrubs. X. *Euphorbia pulcherrima* Willd. Infected by viruses and phytoplasmas. Phytopathologia Mediterranea 35 (2): 129–132.
- Bertaccini A., Duduk B., Paltrinieri S., Contaldo N. 2014. Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. American Journal of Plant Sciences 5 (12): 1763–1788. DOI: <https://doi.org/10.4236/ajps.2014.512191>
- Chamberlain D.F. 1970. Sophora L. p. 11–12. In: "Flora of Turkey and the East Aegean Islands". Vol. 3 (P.H. Davis, ed.). Edinburgh University Press, Edinburgh, 628 pp.
- Chen X.F., Liang Y.C., Chen N., Su W.M., Xiao H., Wang X., Zhu X.P. 2013. Molecular identification of a phytoplasma associated with sophora root yellows. Forest Pathology 43: 415–421. DOI: <https://doi.org/10.1111/efp.12048>
- Davis R.E., Dally E.L., Gunderson D.E., Lee I.-M. Habili N. 1997. '*Candidatus* Phytoplasma australiense', a new phytoplasma taxon associated with Australian grapevine yellows. International Journal of Systematic Bacteriology 47 (2): 262–269. DOI: <https://doi.org/10.1099/00207713-47-2-262>
- Davis R.E., Dally E.L., Zhao Y., Lee I.-M., Jomantiene R., Detweiler A.J., Putnam M.L. 2010. First report of a new subgroup 16SrIX-E (*Candidatus* phytoplasma phoenicium'-related) phytoplasma associated with juniper witches' broom disease in Oregon, USA. Plant Pathology 59 (6): 1161. DOI: <https://doi.org/10.1111/j.1365-3059.2010.02294.x>

- Duduk B., Bertaccini A. 2011. Phytoplasma classification: Taxonomy based on 16S ribosomal gene, is it enough? *Phytopathogenic Mollicutes* 1 (1): 3–13. DOI: <https://doi.org/10.5958/j.2249-4669.1.1.001>
- Duduk B., Tian J., Contaldo N., Fan X., Paltrinieri S., Chen Q., Zhao Q., 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. DOI: <https://doi.org/10.1111/j.1439-0434.2009.01586.x>
- Hosseini P., Bahar M., Madani G., Zirak L. 2011. Molecular characterization of phytoplasmas associated with potato purple top disease in Iran. *Journal of Phytopathology* 159 (4): 241–246. DOI: <https://doi.org/10.1111/j.1439-0434.2010.01757.x>
- Khan A.J., Al-Subhi A.M., Calari A., Al-Saady N.A., Bertaccini A. 2007. A new phytoplasma associated with witches' broom of *Cassia italica* in Oman. *Bulletin of Insectology* 60 (2): 269–270.
- Kenyon L., Harrison N.A., Ashburner G.R., Boa E.R., Richardson P.A. 1998. Detection of a pigeon pea witches'-broom-related phytoplasma in trees of *Gliricidia sepium* affected by little-leaf disease in Central America. *Plant Pathology* 47 (5): 671–680. DOI: <https://doi.org/10.1046/j.1365-3059.1998.00281.x>
- Lee I.-M., Hammond R.W., Davis R.E., Gundersen D.E. 1993. Universal amplification and analysis of pathogen 16SrDNA for classification and identification of mycoplasma-like organisms. *Phytopathology* 83 (8): 834–842. DOI: <https://doi.org/10.1094/phyto-83-834>
- Li C.L., Du Y.J., Xiang B.C. 2013. Molecular detection and identification of *Sophora alopecuroides* witches' broom phytoplasma. *Xinjiang Agricultural Sciences* 50: 92–99.
- Mall S., Rao G., Marcone C. 2010. Phytoplasma diseases of weeds, taxonomy and diversity. p. 87–108. In: "Recent Trends in Biotechnology and Microbiology" (R.K. Gaur, P. Sharma, R. Pratap, K.P. Sharma, M. Sharma, R. Dwivedi, eds.). Nova Science Publishers, Inc. 231 pp.
- Maixner M., Ahrens U., Seemüller E. 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and vector by a specific PCR procedure. *European Journal of Plant Pathology* 101 (3): 241–250. DOI: <https://doi.org/10.1007/bf01874780>
- Marcone C., Ragozzino A., Seemüller E. 1997. Detection and identification of phytoplasmas infecting vegetable, ornamental and foliage crops in southern Italy. *Journal of Plant Pathology* 79: 211–217.
- Mirchenari S.A., Massah A., Zirak L. 2015. 'Bois noir': new phytoplasma disease of grapevine in Iran. *Journal of Plant Protection Research* 55 (1): 88–93. DOI: <https://doi.org/10.1515/jppr-2015-0012>
- Molino Lova M., Quaglino F., Abou-Jawdah Y., Choueiri E., Sobh H., Casati P., Tedeschi R., Alma A., Bianco P.A. 2011. Identification of new 16SrIX subgroups, -F and -G, among 'Candidatus Phytoplasma phoenicium' strains infecting almond, peach and nectarine in Lebanon. *Phytopathologia Mediterranea* 50: 273–282.
- 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 (8): 2879–2894. DOI: <https://doi.org/10.1099/ijs.0.044750-0>
- Salehi M., Izadpanah K., Nejat N. 2006. A new phytoplasma infecting lettuce in Iran. *Plant Disease* 90 (2): 247. DOI: <https://doi.org/10.1094/pd-90-0247c>
- Salehi M., Haghshenas F., Khanchezar A., Esmailzadeh-Hosseini S.A. 2011. Association of 'Candidatus Phytoplasma phoenicium' with GF-677 witches' broom in Iran. *Bulletin of Insectology* 64: 113–114.
- Schneider B., Seemüller E., Smart C.D., Kirkpatrick B.E. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. p. 369–380. In: "Molecular and Diagnostic Procedures in Mycoplasmaology" (S. Razin, J.G. Tully, eds.). Academic Press, 483 pp.
- Singh N., Upadhyaya P.P. 2013. Analysis of medicine weeds associated with phytoplasma by morphological characters. *International Journal of Medicinal Plants and Alternative Medicine* 2 (1): 001–004.
- Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, Evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28 (10): 2731–2739. DOI: <https://doi.org/10.1093/molbev/msr121>
- Valiunas D., Staniulis J., Davis R. E. 2006. 'Candidatus Phytoplasma fragariae', a novel phytoplasma taxon discovered in yellow-diseased strawberry, *Fragaria x ananassa*. *International Journal of Systematic and Evolutionary Microbiology* 56 (1): 277–281. DOI: <https://doi.org/10.1099/ijs.0.63935-0>
- Verdin E., Salar P., Danet J., Choueiri E., Jreijiri F., El Zamar S., Gelie B., Bove J.M., Garnier M. 2003. 'Candidatus Phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. *International Journal of Systematic and Evolutionary Microbiology* 53 (3): 833–838. DOI: <https://doi.org/10.1099/ijs.0.02453-0>
- Yu Z.C., Cao Y., Zhang Q., Deng D.F., Liu Z.Y. 2012. 'Candidatus Phytoplasma ziziphi' associated with *Sophora japonica* witches' broom disease in China. *Journal of General Plant Pathology* 78 (4): 298–300. DOI: <https://doi.org/10.1007/s10327-012-0385-7>
- Zhao Y., Wei W., Lee I.-M., Shao J., Suo X., Davis R.E. 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). *International Journal of Systematic and Evolutionary Microbiology* 59 (10): 2582–2593. DOI: <https://doi.org/10.1099/ijs.0.010249-0>
- Zirak L., Bahar M., Ahoonmanesh A. 2009a. Characterization of phytoplasmas associated with almond diseases in Iran. *Journal of Phytopathology* 157 (11–12): 736–741. DOI: <https://doi.org/10.1111/j.1439-0434.2009.01567.x>
- Zirak L., Bahar M., Ahoonmanesh A. 2009b. Molecular characterization of phytoplasmas related to peanut witches-broom and stolbur groups infecting plum in Iran. *Journal of Plant Pathology* 91 (3): 713–716. DOI: <http://dx.doi.org/10.4454/jpp.v91i3.567>
- Zirak L., Bahar M., Ahoonmanesh A. 2010. Molecular characterization of phytoplasmas associated with peach diseases in Iran. *Journal of Phytopathology* 158 (2): 105–110. DOI: <https://doi.org/10.1111/j.1439-0434.2009.01585.x>
- Zibadoost S., Rastgou M. 2016. Molecular identification of phytoplasmas associated with some weeds in West Azarbaijan province, Iran. *Acta Agriculturae Slovenica* 107 (1): 129. DOI: <https://doi.org/10.14720/aas.2016.107.1.13>