Phytoparasitica (2015) 43:303–310
DOI 10.1007/s12600-014-0449-7

Occurrence and identification of grapevine phytoplasmas in main viticultural regions of Turkey

Filiz Ertunc · Didem Canik Orel · Serife Bayram · Samanta Paltrinieri · Assunta Bertaccini · Serife Topkaya · Gokhan Soylemezoglu

Received: 24 December 2013 / Accepted: 18 December 2014 / Published online: 8 January 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Intensive surveys were conducted in the main viticultural areas of Turkey in 2009-2010. Two hundred eighty nine symptomatic and 20 non-symptomatic plant samples were collected and subjected to nucleic acid extraction followed by PCR, nested PCR and RFLP analyses to detect phytoplasma presence and for their identification. The incidence rate of phytoplasma infection was 18.33% and the majority of the symptomatic grapevines were infected with grapevine yellows phytoplasmas belonging to 16SrXII-A subgroup ("bois noir"). Phytoplasmas of 16SrV group, aster yellows (16SrI-B) and pigeon pea witches' broom (16SrIX) groups were also detected in the surveyed vineyards. Phytoplasma-associated infections were present more on wine grapevine cultivars (73.6%), such as Alphonse Lavallée, Alicante Bouschet, Chardonnay, Shiraz, Cabernet Sauvignon, Merlot, Sauvignon Blanc and Pinot Noir, compared to table grapes (26.4%), such as Bogazkere, Sirfani, Tahannebi and Emir.

Keywords Phytoplasma · grapevine · "bois noir" · molecular identification

F. Ertunc (✉) · D. C. Orel · S. Bayram · S. Topkaya
Faculty of Agriculture, Department of Plant Protection, Ankara University, 06110 Ankara, Turkey
e-mail: ertunc@agri.ankara.edu.tr

F. Ertunc
e-mail: fertunc@gmail.com

S. Paltrinieri · A. Bertaccini
Department of Agricultural Sciences, DipSA, Plant Pathology, Alma Mater Studiorum, University of Bologna, 40100 Bologna, Italy

G. Soylemezoglu
Faculty of Agriculture, Department of Horticulture, Ankara University, 06110 Ankara, Turkey

Introduction

Turkey is located in the subtropic climatic region of the world and its Northeastern part, the Anatolian peninsula, located between Black sea and Caspian sea regions, includes the location from which many important grapevine varieties originate. In Turkey, grapevine cultivation is present since more than 6000 years, and there is very rich potential of both wild (*Vitis vinifera* ssp. *sylvestris*) and cultivated grapevine (*Vitis vinifera* ssp. *sativa*) germplasms. According to FAO data, Turkey has 540,000 ha of grapevine cultivated area, ranking fourth after Spain, France and Italy, and a grapevine production of 3,923 million tons, ranking sixth after Italy, France, China, USA and Spain (Celik *et al*. 2010). Viticulture provides 24.8% of the total yearly fruit production and comes after field crops, fruit and vegetable production (Tuik 2008). The main viticultural region is the Aegean Region with 151,401 ha of vineyard area, that is the 31% of the grapevine cultivated area of the country, followed by Mediterranean, Central Anatolia, Eastern Anatolia, and Marmara regions. Manisa province is the leader in viticulture with 68,751 ha of vineyards and 1,075,013 tons of grapevine production and it is followed by Denizli and İzmir (Tuik 2008). Wide spectrum of

wine and local table grapevine varieties are grown in Turkey such as Cabernet Sauvignon, Merlot, Syrah, Malbec, Cabernet Franc, Pinot noir, Chardonnay, Kalecik Karası, Öküzgözü, Boğazkere, Çal Karası, Bornova Misketi, Çavuş, Sultani Seedless, and Besni.

Grapevine is worldwide infected by several pathogens including phytoplasmas that are insect transmitted, wall-less, phloem-limited bacteria of the class *Mollicutes*. They can cause devastating losses in crops and natural ecosystems worldwide (Weintraub and Jones 2010). They have extreme genome reductions, compared to their Gram-positive bacteria relatives, and are identified according to the 16S ribosomal DNA sequence (Lee *et al*. 1998; Seemüller *et al*. 1998). Phytoplasmas are transmitted by phloem feeding hemipteran insects mainly leafhoppers (Weintraub and Beandland 2006; Maixner 2011).

Worldwide several phytoplasmas, were associated with diseases of grapevine such as, aster yellows (group 16SrI), elm yellows (group 16SrV) and "stolbur" (subgroup 16SrXII-A), together with phytoplasmas belonging to 16SrII, 16SrIII, 16SrVII, and 16SrX groups (Angelini 2010; Constable 2010; Duduk *et al*. 2010). "Bois noir" (BN) and "flavescence dorée" (FD) together with Australian grapevine yellows and North American grapevine yellows phytoplasmas are known as grapevine yellows phytoplasmas (GY diseases). Similar symptoms are associated with these diseases including severe redness of the leaves of red varieties and irregular yellowing of the leaves of white varieties, with inward curling of leaves, lack of lignification, and shriveling of berries followed by early drying of whole cluster on infected plants (Kuzmanovic *et al*. 2008; Martini *et al*. 1999; 2002; Weintraub and Jones 2010). These symptoms cause significant reduction in yields in many European countries (Angelini *et al*. 2001) and in Turkey neighboring countries such as Iran, Bulgaria and Syria (Avramov *et al*. 2008; Karimi *et al*. 2009; Angelini 2010). "Bois noir" phytoplasma, '*Candidatus* Phytoplasma solani' (Quaglino *et al*. 2013), belongs to 16SrXII-A subgroup and is transmitted by *Hyalesthes obsoletus* Signoret (Cixiidae), a polyphagous planthopper which seldomly and erratically feeds on grapevine and also by *Reptalus panzeri* (Low) (Maixner 1994; Sforza *et al*. 1998; Alma *et al*. 2002; Sabaté *et al*. 2007; Cvrković *et al*. 2014). "Flavescence dorée" phytoplasma which belongs to 16SrV-C and -D subgroups, is transmitted by the leafhopper *Scaphoideus titanus* Ball, a monophagous insect, feeding on grapevine (Schvester *et al*. 1961; Lessio and Alma 2004). This phytoplasma is a quarantine pathogen in Europe while the "stolbur" phytoplasma is a quarantine pathogen only for potato in both Europe and Turkey (Gthb 2012; Weintraub and Jones 2010).

Recently, severe redness and inward curling of the leaves were observed in wine vineyards in Turkey, therefore intensive surveys were conducted in the main viticultural production areas to verify phytoplasma presence and identity. This is the first report about the presence and distribution of grapevine phytoplasma infections in Turkey.

Materials and Methods

Phytoplasma survey

Periodical surveys were conducted in main viticultural areas located in Thrace (Edirne, Kirklareli, Tekirdag), Aegean region (Izmir, Manisa and Denizli), Central Anatolia (Ankara, Nevsehir) and Eastern Anatolia region (Elazig, Malatya, Diyarbakir and Mardin) of Anatolian Peninsula from July-September in 2009-2010. Leaf and young shoot samples were collected from 289 symptomatic and 20 non-symptomatic plants. Surveyed area is shown in Fig. 1.

Nucleic acid extraction

Nucleic acids were extracted from 1 g of grapevine leaf ribs, using a chloroform/phenol protocol described in Prince *et al*. (1993), suspended in 50-100 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and stored at -20°C until they were processed. DNA's of non-symptomatic plants were used as negative control.

PCR and nested PCR assays

Direct PCR assays with ribosomal P1/P7 universal primer pair (Deng and Huruki 1991; Schneider *et al*. 1995), followed by nested-PCR with R16F2n/R2 (Gundersen and Lee 1996 or M1/B6 (Duduk *et al*. 2004) were carried out. R16F2n/R2 amplicons were used as templates in a second nested PCR with R16(I)F1/R1, R16(V)F1/R1 (Lee *et al*. 1994) and M1/M2 (16R758f/16S1232r) primer pairs (Gibbs *et al*. 1995).



Fig. 1 The surveyed grapevine growing areas in Turkey

PCR mixture for direct PCR assays in a final volume of 25 μl was as follows: 2.5 μl 10 X PCR buffer, 250 μM dNTP mix, 0.3 μM each primers, 20 ng target DNA, 2 U Taq DNA polymerase (Promega, Madison, USA). Nucleic acid concentration was determined by Nanodrop 2000 (Thermo Scientific) and 20-60 ng of DNA were used per reaction. PCR products were subjected to 1.5% agarose gel electrophoresis; the gels were stained with ethidium bromide (1 mg ml^{-1}) and visualised by bioimage analysing system of Syngene (Cambridge, UK).

PCR mixture for nested PCR assays was the same described above and 1 μl of 1:30 dilutions of direct or nested PCR amplicons was used as template. PCR cycling for direct and nested PCR assays were the same and were applied for 35 cycles as: 1 min (2 min for the first cycle) denaturation step at 94°C, 2 min for annealing at 55°C, 3 min at 72°C for primer extension (10 min for the last cycle). Positive controls were DNAs extracted from periwinkle infected with elm yellows (16SrV-A subgroup), "stolbur" (16SrXII-A) and pear decline (PD) (16SrX-C) phytoplasmas. DNAs of non-symptomatic plants and sterile distilled water were used as negative controls.

RFLP analyses

Preliminary identification of the detected phytoplasmas was achieved by RFLP analysis of R16(I)F1/R1, M1/B6 and M1/M2 amplicons with restriction endonucleases *Tru1*I and *Taq*I, (Fermentas, Vilnius, Lithuania). Results of the reaction were visualised by agarose 5% or polyacrylamide 6.7% gel electrophoresis, then the gels were stained and visualized as described above.

Sequencing of 16S rDNA

Selected amplicons obtained with R16(I)F1/R1, M1/B6 and M1/M2 primers were subjected to direct sequencing. The sequences were assembled, using Sequencher 4.1 software, compared with selected nucleotide sequences in the GenBank database, using BLAST (version BLASTN 2.2.18) (National Center for Biotechnology Information, Bethesda, MD). Sequence alignments were performed, using ClustalX and BioEdit (Hall 1999; Thompson *et al*. 1997).

Results

Survey results

During the surveys, 159 and 130 symptomatic grapevine samples were collected in the main viticultural areas of Turkey in 2009 and 2010, respectively. Main symptoms observed were severe redness and inward curling of the mature leaves (Fig. 2). Other typical yellows symptoms such as leaf yellowing and triangle shaped, were not detected. Symptoms were usually present on the old mature leaves, occasionally, in some grapevine varieties, the whole canopy was completely red. The redness of the leaves was more severe on wine



Fig. 2 Typical symptoms of grapevine yellows: reddening of leaves and shriveling of berries in cv. Merlot

grapevine varieties comparing to table grapevines. Phytoplasma-associated symptomatology was present in most of the surveyed provinces, especially in almost all vineyards located in Marmara and Aegean Regions. There were no phytoplasma symptoms in the vineyards located in Diyarbakır and Mardin except in the germplasm collection vineyard of Dicle University (Diyarbakir). Phytoplasma-associated symptoms were detected in the local table-grapevine varieties Trakya İlkeren (Ankara), Bogazkere (Manisa), Şirfani

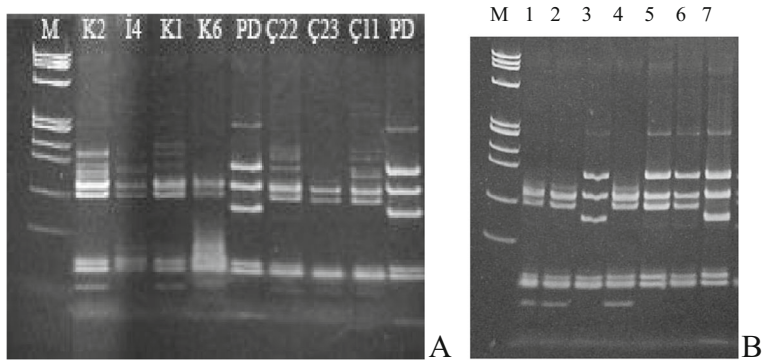


Fig. 3 In A: restriction profiles of BN infected samples after *Tru1*I enzyme restriction analyses after second nested-PCR amplification with M1/M2 primer pair on the nested amplicons obtained with R16F2n/R2 primer pair. K1, K2 and K6: Kırklareli samples; I4: Izmir sample; Ç11, Ç22 and Ç23: Çanakkale samples, PD: pear decline; in B reference phytoplasmas: 1 and 4 "stolbur" from pepper (STOL, 16SrXII-A); 2, grapevine BN (16SrXII-A), 3 and 7, pear decline (PD, 16SrX-C); 5 and 6, aster yellows (16SrI-B); M: ØX174 DNA marker

Table 1 Distribution of the phytoplasmas detected in theTurkey viticultural areas

Province	Grapevine cultivar	Positive/analyzed	Phytoplasma group	GeneBank Accession No.
Ankara	Unknow	1/1	16SrXII-A	KJ810648
	Trakya İlk Eren	1/1	16SrXII-A	KJ810649
	Others	0/9		
İzmir	Alphonse Lavallée	5/7	16SrXII-A	KJ810633
	Alicante	1/1	16SrXII-A	
	Others	0/13		
Manisa	Chardonnay	1/3	16SrXII-A	
	Alicante	2/6	16SrXII-A	
		1/6	16SrIX	HQ714331
	Boğazkere	1/1	16SrXII-A	
	Syrah	1/9	16SrXII-A	
	Others	0/19		
Kırklareli	Cabernet Sauvignon	3/5	16SrXII-A	KJ810634
		1/5	16SrI-B	
	Merlot	6/7	16SrXII-A	KJ810635 and KP015027
	Malbec	1/5	16SrV	
Elazığ	Unknown	5/10	16SrXII-A	KJ810636; KJ810637; KJ810638; KJ810639; KJ810640
	Şırfani	1/1	16SrXII-A	KJ810641
	Others	0/8		
Diyarbakır	Unknown	3/10	16SrXII-A	
	Others	0/18		
Malatya	Tahannebi	2/4	16SrXII-A	
Nevşehir	Chardonnay	1/7	16SrXII-A	
	Emir	1/1	16SrXII-A	KJ810642
	Sauvignon blanc	1/1	16SrXII-A	KJ810643
	Others	0/14		
Çanakkale	Syrah	1/1	16SrXII-A	KP015028
	Syrah	1/1	16SrV	
	Others	0/14		
Tekirdağ	Pinot Noir	3/4	16SrXII-A	KJ810644; KJ810645; KJ810647
	Papaz karası	1/1	16SrXII-A	
	Gamay	1/1	16SrXII-A	KJ810646
	Alicante	2/2	16SrXII-A	
	Çıtlık	1/1	16SrXII-A	
	Others	0/17		
Edirne	Cabernet Sauvignon	2/2	16SrXII-A	
	Merlot	1/1	16SrXII-A	
	Syrah	1/1	16SrXII-A	
	Others	0/5		

(Elazığ), Tahannebi (Malatya), Emir (Nevşehir), and in grapevine cultivars for wine production Alphonse Lavallée (İzmir), Alicante Bouschet (İzmir, Manisa, Tekirdağ), Chardonnay (Manisa), Shiraz (Manisa,

Çanakkale), Cabernet Sauvignon (Kırklareli, Çanakkale), Merlot (Kırklareli), Sauvignon Blanc (Nevşehir), and Pinot Noir (Tekirdağ).

Phytoplasma identification

After double nested-PCR assays with primers M1/M2, 53 out of the 289 grapevine sample tested were detected as phytoplasma-infected. The detection rate of phytoplasma-infected plant samples was 18.33% (53/289); in 49 samples '*Ca*. P. solani', was identified according to the restriction profiles indicating the presence of phytoplasmas belonging to 16SrXII-A, (Fig. 3). Positive samples were also amplified in second nested PCR assays with primers R16(V)F1/R1 and R16(I)F1/R1. In two samples, phytoplasmas belonging to 16SrV group were detected (data not shown), while all other samples were amplified with R16(I)F1/R1 primers and after RFLP analyses, 16SrI-B and 16SrXII-A phytoplasmas were detected respectively in 1 and 49 samples (data not shown). One sample showing a profile not referable to any one reported in literature was subjected to sequencing and the 1,063 bp sequence (accession number HQ714331), showed 99% homology with phytoplasmas belonging to group 16SrIX. M1/M2 (about 440 nt) amplicons of the BN strains showed 100% homology with sequences of phytoplasmas belonging to the same group present in the NCBI Genebank (accession numbers KJ810633-KJ810649, Table 1). Sequence analyses of two samples (K11 KP015027 and C24 KP015028, Table 1) amplified with M1/B6 primers (843 and 845 nt respectively) showed 99% homology with '*Ca*. P. solani'-related strains, while the RFLP analyses with *Taq*I showed the presence of an extra band of 700 bp in strain K11 that was not referable to other reported phytoplasmas (Fig. 4) nor retrivable by *in silico* RFLP. Considering the consistent presence of this band in all the repetitions of PCR/RFLP analyses on this sample its presence could indicate a mixed phytoplasma infection with not identified strains.

The incidence of grapevine phytoplasmas in Turkish vineyards was low and the predominant phytoplasma, infection was associated to the presence of "bois noir" phytoplasma as shown in Table 1. The phytoplasma was present mostly in grapevine varieties for wine production of imported origin such as Alphonse Lavallée (Izmir), Chardonnay (Manisa, Nevsehir), Alicante Bouchet (Manisa, Tekirdag), Shiraz (Manisa), Cabernet Sauvignon (Kirklareli, Canakkale),

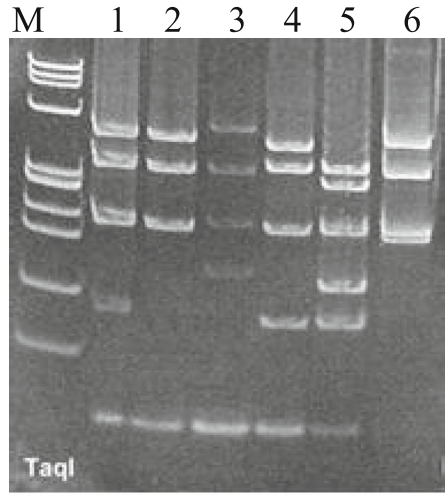


Fig. 4 Profiles of M1/B6 amplicons obtained after *Taq*I restriction analyses. 1 and 2: grapevine samples K11 and G24; 3: elm yellows, 4: "flavescence dorée" strain 16SrV-C; 5: "flavescence dorée" strain 16SrV-D; 6: "bois noir"; M: ØX174 DNA marker

Sauvignon Blanc (Nevsehir), Merlot and Malbec (Kirklareli) and Pinot Noir (Tekirdag). The infection was also detected in a small number of samples in local varieties: Bogazkere (Manisa), Tahannebi (Malatya), Emir (Nevsehir), Papaz karasi and Gamay (Tekirdag).

Phytoplasmas belonging to group 16SrIX were detected in one of the samples from Manisa and aster yellows phytoplasmas were detected in one of the samples, from Kırklareli, from symptomatic Cabernet Sauvignon grapevine. There was no difference in symptoms associated with the detected phytoplasmas; no phytoplasma infection was detected in Denizli and Mardin regions.

Discussion

In recent years, epidemics of phytoplasma-associated diseases of grapevine became one of the major problem in Turkish viticulture. "Bois noir" phytoplasma was detected in most of the symptomatic plants of this survey. Morevoer '*Ca*. P. asteris' (aster yellows) and '*Ca*. P. phoenicium' were found as unique infections and grapevine was detected as a new host species of the latter phytoplasma within this research (Canik *et al*. 2011). None of the identified phytoplasmas was epidemic in the surveyed viticultural areas.

Aster yellows phytoplasma was detected in one of the Cabernet Sauvignon cultivar samples. This phytoplasma was associated with an epidemic grapevine

yellow outbreak in South African vineyards, causing serious damages ranging from lower yields to death of grapevines, and Chardonnay was reported as the most sensitive grapevine cultivar (Carstens *et al*. 2012). Transmission of grapevine phytoplasmas occurs through the use of phytoplasma infected propagation material and by vector insects. The main phytoplasma infections detected in the vineyards of Turkey were associated with BN present in all surveyed regions except in the provinces Denizli and Mardin. It was detected from both wine grapevine and some local table grapevine varieties. BN is considered an endemic disease vectored by *H. obsoletus* which is a polyphagous insect and feeds on grapevine occasionally. BN phytoplasma is transmitted to grapevine from several wild plant species which can host both insect and phytoplasma, especially *Urtica dioica* (nettle) and *Convolvulus arvensis* (bindweed) (Maixner 2011). The cixiid vector is present in Turkey and it has been reported in the potato fields in Erzurum plato (Güçlü and Özbek 1988), however it was very rarely present in the inspected vineyards. Insect vectors were found as less effective in dissemination of grapevine yellows phytoplasmas in Turkey (Bayram *et al*. 2014) therefore it has been thought that phytoplasma infection in grapevines occurred by infected plant material. The incidence of symptomatic grapevines was higher in the vineyards located in Thrace and Aegean region compared to Central and Eastern Anatolia regions where local table grapevine varieties are usually grown and there was no introduction of new imported varieties. Although the incidence rate of phytoplasma associated diseases was low, it does not mean that they will not become epidemic in the near future so, regulation and certification procedures of grapevine material need to be improved.

Acknowledgments The authors are grateful to Turkish Scientific and Technological Research Council for supporting this research by Project coded COST-109O005 and also to COST Action FA0807 for founding COST-STSM-090110. Part of the work was carried out in the frame of the COST Action FA0807 "Integrated managemant of Phytoplasma Epidemics in Different Crop Systems" at Bologna University.

References

Alma, A., Soldi, G., Tedeschi, R., & Marzachì, C. (2002). Role of *Hyalesthes obsoletus* Signoret in transmission of grapevine Bois Noir in Italy. *Petria, 12*, 411–412.

Angelini, E., Clair, D., Borgo, M., Bertaccini, A., & Boudon-Padieu, E. (2001). Flavescence dorée in France and Italy-occurence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and alder phytoplasma. *Vitis, 40*, 79–86.

Angelini, E. (2010). Field assestment and diagnostic methods for detection of grapevine phytoplasmas. In S. Delrot, H. O. E. Medrano, L. Bavaresco, & S. Grando (Eds.), *Methodologies and Results in Grapevine Research* (pp. 247–255). New York: Springer.

Avramov, Z., Gillet, J., & Laginova, M. (2008). First detection of stolbur phytoplasma grapevine (*Vitis vinifera* cv Merlot) affected in Bulgaria. *Journal of Phytopathology, 56*(2), 112–114.

Bayram, S., Zeybekoglu, U., Soylemezoglu, G., Canik, D., Karavin, M., Cakır, A., & Ertunc, F. (2014). Putative vectors of grapevine yellows phytoplasmas in Turkey. *Phytopathogenic Mollicutes, 4*(1), 22–26.

Canik, D., Ertunc, F., Paltrinieri, S., Condalto, N., & Bertaccini, A. (2011). Identification of different phytoplasmas infecting grapevine in Turkey. *Bulletin of Insectology, 64*, 226–227.

Carstens, R., Petersen, Y., Stephan, D., & Burger, J. T. (2012). *Incidence of aster yellows disesase in South African vineyards*. (pp. 242–243). UC-Davis, CA, USA: Proceedings of the 17th Congress of ICVG.

Constable, F. E. (2010). Phytoplasma Epidemiology: Grapevine as a model. In P. G. Weintraub & P. Jones (Eds.), *Phytoplasmas Genomes, Plant Hosts And Vectors* (pp. 188–213). London, UK: CABI.

Cvrković, T., Jović, J., Mitrović, M., Krstic, O., & Toševski, I. (2014). Experimental and molecular evidence of *Reptalus panzeri* as a natural vector of bois noir. *Plant Pathology, 63*(1), 42–53.

Celik, H., Kunter, B., Soylemezoglu, G., Ergul, A., Celik, H., Karatas, H., Ozdemir, G., & Atak, A. (2010). Bağcılığın Geliştirilmesi Yöntem Ve Üretim Hedefleri, Ziraat Mühendisliği 7. Teknik Kongresi, 23 p, Ankara.

Deng, S., & Huruki, C. (1991). Amplifications of 16S rRNA genes from culturable and non-culturable mollicutes. *Microbiological Methods, 14*, 53–61.

Duduk, B., Botti, S., Ivanović, M., Krstić, B., Dukić, N., & Bertaccini, A. (2004). Identification of phytoplasmas associated with grapevine yellows in Serbia. *Journal of Phytopathology, 152*, 575–579.

Duduk, B., Tian, J. B., Contaldo, N., Fan, X. P., Paltrinieri, S., Chen, Q. F., Zhao, Q. F., & Bertaccini, A. (2010). Occurence of phytoplasmas related to stolbur and to '*Candidatus* Phytoplasma japonicum' in woody host plants in China. *Journal of Phytopathology, 158*, 100–110.

Gthb, T. C. (2012). Gıda, Tarım ve Hayvancılık Bakanlığı, Bitki Karantinası Yönetmeliği, 55 p.

Gibbs, K. S., Padovan, A. C., & Mogen, B. A. (1995). Studies on sweet potato little-leaf phytoplasmas detected in sweet potato and other species growing in Northern Australia. *Phytopathology, 85*, 169–174.

Güçlü, S., & Özbek, H. (1988). Some studies on the biology of *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) in the conditions of Erzurum. *Türkiye Entomology Dergisi, 12*, 103–111.

Gundersen, D. E., & Lee, I.-M. (1996). Ultrasensitive detection of phytoplasmas by nested PCR assays using two

universal primer pairs. *Phytopathologia Mediterranea, 35*, 144–151.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Karimi, M., Contaldo, N., Mahmodi, B., Duduk, B., & Bertaccini, A. (2009). Identification of stolbur related phytoplasma in grapevine showing decline symptoms in Iran. Le Progrès agricole et viticole, HS, 208-209.

Kuzmanovic, S., Martini, M., Ermacora, P., Ferrini, F., Starovic, M., Tosic, M., Carraro, L., & Osler, R. (2008). Incidence and molecular characterization of Flavescence dorée in grapevine cultivars in different viticultural areas in Serbia. *Vitis, 47*, 105–111.

Lee, I.-M., Gundersen, D. E., Hammond, R. W., & Davis, R. E. (1994). Use of mycoplasma like organism (MLO) group-specific oligonucleotide primers for nested PCR assays to detect mixed–MLO infections in a single host plants. *Phytopathology, 84*, 559–566.

Lee, I.-M., Davis, R. E., Gundersen-Rindal, D. E., Davis, R. E., & Bartoszyk, I. (1998). Revised classification scheme of phytoplasmas based on RFLP analyses of 16 Sr RNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology, 48*, 1153–1169.

Lessio, F., & Alma, A. (2004). Dispersal patterns and chromatic response of *Scaphoideus titanus* Ball (Homoptera, Cicadellidae), vector of the phytoplasma agent of grapevine flavescence dorée. *Agricultural and Forest Entomology, 6*, 121–127.

Maixner, M. (1994). Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis, 33*, 103–104.

Maixner, M. (2011). Recent advances in Bois Noir research. *Petria, 21*(2/3), 17–32.

Martini, M., Murari, E., Mori, N., & Bertaccini, A. (1999). Identification and epidemic distribution of two flavescence dorée related phytoplasmas in Veneto (Italy). *Plant Disease, 83*, 925–930.

Martini, M., Botti, S., Marcone, C., Marzachi, 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 Cellular Probes, 16*, 197–208.

Prince, J. P., Davis, R. E., Wolf, T. K., Lee, I.-M., Mogen, B. D., Dally, E. L., Bertaccini, A., Credi, R., & Barba, M. (1993). Molecular detection of diverse mycoplasmalike organisms associated with grapevine yellows and their classification with aster yellows, x-disease and elm yellows MLOs. *Phytopathology, 83*, 1130–1137.

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 Evolutionary Microbiology, 63*(8), 2879–2894.

Sabaté, J., Lavina, A., Legorburu, J., Fortanete, J., Perez De Obanos, J. J., Perez Marin, J. L., Reyes, J., & Batlle, A. (2007). Incidence of "Bois Noir" phytoplasma in different wine-growing regions of Spain and its relation to *Hyalesthes obsoletus. Bulletin of Insectology, 60*(2), 367–368.

Schneider, B., Seemüller, E., Smart, C. D., & Kirkpatrick, B. C. (1995). Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas. In S. Razin & J. G. Tully (Eds.), *Molecular and Diagnostic Procedures in Mycoplasmology* (pp. 369–380). New York: Academic Press.

Schvester, D., Carle, P., & Moutous, G. (1961). Sur la transmission dela flavescence dorée des vignes par une cicadelle. *Comptes Rendus des Séances de l'Académie d'Agriculture de France, 47*, 1021–1024.

Seemüller, E., Marcone, C., Lauer, U., Ragozzino, A., & Goschl, M. (1998). Current status of molecular classification of the phytoplasmas. *Journal of Plant Pathology, 80*, 3–26.

Sforza, R., Clair, D., Daire, X., Larrue, J., & Boudon-Padieu, E. (1998). The role of *Hyalesthes obsoletus* (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevines in France. *Journal of Phytopathology, 146*, 549–556.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*, 4876–4882.

Tuik. (2008). *Tarımsal Yapı Ve Üretim*. Ankara: Başbakanlık Devlet İstatistik Enstitüsü yayınları.

Weintraub, P. G., & Beandland, L. (2006). Insect vectors of phytoplasmas. *Annual Review of Entomology, 51*, 91–111.

Weintraub, P. G., & Jones, P. (2010). *Phytoplasmas, Genomes, Plant Hosts And Vectors*. United Kingdom: Cab International.