Ankara Üniv Vet Fak Derg, **60**, 221-224, 2013

Review / *Derleme*

A new threat for Turkish horticulture: phytoplasma diseases and their vectors

Filiz ERTUNC

Ankara University, Faculty of Agriculture, Department of Plant Protection, Diskapi, Ankara, Turkey.

Summary: Our country is one of the nations settled in temperate region which enables it high agricultural potential. Agriculture is done as viticulture, field's crops, variable fruits, vegetables and ornamental crops production and constitutes great impact on Turkish economy. Plant diseases are one of the important factors which limit the production of all those crops. Recently phytoplasma diseases are the most important agents that limit the agricultural production in Turkey and in the world. Transmission by vegetative plant material and also by vectors causes epidemics in very short period. Stolbur on potato and tomato, Apple proliferation and Pear decline on pomes fruits, European stone fruit yellows on stone fruits, Flavescense doree, Bois Noir on vineyards are the most important phytoplasma infections in our country. AP, PD and ESFY are transmitted by the vector species belonging to Hemiptera order, Psyllidae family whereas FD and BN are transmitted by the species of Cicadellidae, Cixiidae families.

Key words: Phytoplasma, vector, Hemiptera.

Türk bahçeciliğinde yeni bir tehdit: fitoplazma hastalıkları ve vektörleri

Özet: Türkiye ılıman iklim kuşağında yer alan ve bu nedenle tarım potansiyeli yüksek ender ülkelerden biridir. Ülkemizde tarım, tarla bitkileri, bağ, çeşitli meyve, sebze ve süs bitkilerinin tarımı şeklinde yapılmakta olup, ülke ekonomisinde önemli yere sahiptir. Tüm bu ürünlerde üretimi kısıtlayan en önemli unsurlardan biri de bitkilerde görülen hastalıklardır. Fitoplazma hastalıkları da son yıllarda ülkemiz ve dünya tarımında üretimi kısıtlayan en önemli etmenleri oluşturmaktadır. Vegetatif olarak üretim materyali ile ayrıca vektör böceklerle kolaylıkla yayılmaları, kısa sürede epidemi oluşturmalarına neden olmaktadır. Patates ve domateste stolbur, yumuşak çekirdekli meyvelerde gözlenen Apple proliferation (AP), Pear Decline (PD), sert çekirdekli meyve türlerinde görülen European stone fruit yellows (ESFY), bağlarda görülen Flavescense doree (FD) and Bois Noir (BN) hastalıkları ülkemizde üretimi kısıtlayan en etkin enfeksiyonlardır. AP, PD ve ESFY enfeksiyonları Hemiptera takımında yer alan ve Psyllidae, FD ve BN hastalıkları ise Cicadellidae, Cixiidae familyasında yer alan türlerle taşınmaktadır.

Anahtar sözcükler: Fitoplazma, vektör, Hemiptera.

Turkey is located in the subtropic climatic region in the world so enables it to produce many different horticultural products such as different pome and stone fruits and grapevines varieties. Plant pathogenic phytoplasmas are insect transmitted, wall-less, unculturable phloem-limited bacteria of the class *Mollicutes* with a small genome size, which ranges from 530-1350 kilobases. They can cause devastating losses in crops and natural ecosystem worldwide. They are small bacteria (almost 500 nm in diameter) which appear to have suffered extreme genome reductions compared to their Gram-positive relatives and are identified according to the 16S ribosomal DNA (5, 10, 39). Phytoplasmas continuously cycle between plants and insects and insects and in nature require both organisms for survival and dispersal. They are present in gut lumen, haemolymph, saliva and endocellular niches of various organs of their insect hosts (40). In diseased plants, they reside almost exclusively in the phloem sieve tube elements, to which they are introduced by phloem feeding hemipteran insects mainly leafhoppers (28). Phytoplasmas are transmitted by the insects in the order Hemiptera. However the vector species are restricted in only a few families of the suborder Auchenorrhyncha namely Cercopidae, Cixiidae, Derbidae, Delphacidae, Cicadellidae, Psyllidae (40). Within a family, some species are known to be phytoplasma vectors while others are not. Transmission of phytoplasmas by insects involves at several levels elements of host-pathogen specificity. Insect vectors can acquire more than one phytoplasma species either by feeding on multiple- infected source plants or by feeding on different plants infected by different phytoplasmas (8, 9, 16). Phytoplasma infections can only be identified by PCR and nested-PCR, followed

by PCR-RFLP according to the ribosomal 16Sr RNA gene by using general and group specific primers (24, 25, 34).

Grapevine yellows phytoplasmas: Turkey is one of the nations native to grapevine in the Middle East and table and wine grape varieties have been grown in Central Anatolia, Meditarrenean, Aegean and Eastern Anatolia regions (15). According to FAO, is the 4th row in grapevine cultivation area and the 6th row in grapevine production in the world (1). Grapevine is affected by several plant diseases, including phytoplasma-associated diseases worldwide. Aster yellows (16SrI group), elm yellows (16SrV group) and stolbur (16SrXII group) phytoplasmas together with 16SrII, 16SrIII, 16SrVII and 16SrX groups were present in different countries in the world (14, 17). *Bois noir* (BN) and *Flavescense doree* (FD) which have similar symptoms as severe redness of the leaves on infected plants are known as Grapevine yellows phytoplasmas (22, 29, 30) and both cause significant reduction in yields of many European countries and Iran (3, 4, 21). BN is a member of stolbur (16SrXII group) (*Candidatus* phytoplasma solani) (33) and FD is a member of elm yellows (16SrV group) (*Candidatus* phytoplasma vitis) (36). FD is a quarantine pathogen but stolbur is a quarantine pathogen only on potato in Turkey and in Europe. Recently, severe redness and inward curling of foliage were observed in the wine vineyards in Turkey, severe redness of pear leaves resembling to phytoplasma infections therefore intensive surveys were conducted to the main viticultural and horticultural areas in Turkey in 2006-2010.

Detection of Grapevine yellows phytoplasmas: In order to detect grapevine yellows phytoplasma infections, reddish and inwardly curved foliage of grapevines were collected from main viticultural areas of Turkey in between 2009-2010. One year old stems with foliage were collected from the symptomatic plants.

Nucleic acids were extracted from midribs of the infected plants leaves according to chloroform/phenol protocol (34). Direct PCR with ribosomal P1/P7 universal primer pair, followed by nested PCR with R16F2n/R2, R16 (I)F1/R1 and then with R16(V) F1/VR1 primer pairs were carried out. Final amplified RCR products were digested with *Tru*I enzyme for the identification of the present phytoplasma.

Expected length amplicons were obtained with R16F2n/R2 primers from about half of the tested samples and after RFLP analyses with *Tru*I, the majority of positive samples resulted to contain phytoplasmas affiliated to 16SrXII ribosomal group. These phytoplasmas are also referred to as stolbur phytoplasmas (23), and reported to be associated to the Bois Noir disease worldwide (31, 35). However, in some of the samples phytoplasmas referable to 16SrIX group (*Candidatus* Phytoplasma phoenicium) were identified as mixed infection with the 16SrXII phytoplasmas. 16SrIX phytoplasma was identified in one of the samples tested was subjected for further molecular characterization analysis. The obtained sequence was aligned producing a sequence of 1,063 bp deposited in the GeneBank under ID HQ714331 and showing 99% identity Pigeon pea witches broom phytoplasma group and all other phytoplasmas assigned to group 16SrIX as well as to the sequenced strains of '*Ca.* P. phoenicium'. Comparison of the obtained sequence with those available in GenBank for 16SrIX group phytoplasmas allow to verify that the sequence show fine mismatch with other sequences having a 99% homology, and also show a unique *Rsa*I restriction site. The 16SrIX group phytoplasmas are severely infecting stone fruit trees (almond, apricot) in countries around Turkey so its identification in grapevine for the first time indicates the susceptibility of the species to this pathogen and the urgent need to further verify its presence in grapevine to avoid its possible epidemic spread.

Vectors of Grapevine yellows phytoplasmas: Vectors of the BN phytoplasma is *Hyalestes obsoletus* (Cixiidae) a polyphagous vector and seldom and erroriously feeds on grapevine(6, 10, 32). It has been detected in Erzurum plato in potato fields(19). *H. obsoletus* isolate was collected from vineyards of Diyarbakır and all of the indivuals were negative for the presence of the BN phytoplasma according to the results of PCR and PCR-RFLP in our researchs. Another vector of BN was *Dictyophara europaea* (27) which is also present and detected in the vineyards located in Ankara and Denizli, Turkey. They were also negative in PCR and PCR-RFLP analysis and free of infection.

Another phytoplasma infection which was detected in vineyards of Turkey was *Flavescense doree* (FD) phytoplasma which shows similar symptoms on the infected vines (13). It was detected only in Thrace and Çanakkale so far but its vector *Scaphoideus titanus* (26) were not present in Turkey although it is present in our neighbours such as Bulgaria (7).

Pome fruit phytolasmas and vectors: Another phytoplasma problem is Apple Proliferation (AP) and Pear Decline (PD) phytoplasmas of pome fruits in Turkey (11, 12). They are both belong to 16SrX group phytoplasma and called as *Candidatus Phytoplasma mali* (AP), *Candidatus* Phytoplasma pyri (PD). AP phytoplasma is show typical symptom of enlargement of the leaflets and trasmitted by *Cacopsylla melanoneura and C. picta,* PD cause severe redness in pear leaves and late blossom in fall and trasmitted by *Cacopsylla pyri* (9). All those vectors are present in Turkey and all belong to Psyllidae family of Hemiptera order. PD was detected from Bursa and Ankara whereas AP was present in Ankara and Isparta provinces (12, 11). AP vectors also overwinter on

conifers and migrate to fruit trees in the orchards in early spring (37).

Stone fruit phytoplasmas and vectors: Most important phytoplasma infection of stone fruits is European stone fruit yellows phytoplasma (ESFY) (*Candidatus* Phytoplasma prunorum) infection. The disease is of high economic importance, causing decline of apricot (apricot chlorotic leaf roll), Japanese plum (plum lectonecrosis) and peach (peach decline). It is a member of 16SrX group phytoplasma. It was also detected in wild *Prunus* species especially *Prunus spinosa* ve *Prunus serratina* which are the principal hosts for the vector, *Cacopsylla* pruni. The only known of ESFY is *C. pruni* Scopoli and like the AP vectors, *C. pruni* hibernates on conifers and return to their feeding hosts in early spring. The summer generation also acquires the phytoplasma from infected trees in the orchards and distributes it. The pathogen was detected in Bursa (37, 38) and very recently in Ankara.

Stolbur phytoplasma in tomato and potato: Stolbur phytoplasma is also associated with diseases of tomato and potato with great economic importance (2, 18). Since *H. obsoletus* is involved as a vector, epidemiologic cycle is similar to BN. The causal agent belongs to 16 SrXII group, *Candidatus* phytoplasma solani. Stolbur phytoplasma causes great yield loss in potato and tomato cultivation and main symptoms are production of very small potato tubers and flower sterility on infected tomato plants. Stolbur phytoplasma on potato is a quarantine pathogen in Turkey and also in European Union countries. The infection is common in the potato fields located in Erzurum Plato (2) and in tomato fields in everywhere in Turkey.

Sesame phyllody in sesame: Another phytoplasma infection is sesame phyllody which is found in Western and Southern Anatolian region of Turkey (20). The infection is transmited by several Hemipterean vectors. Various symptoms occur according to growing stage and time of infection on the infected seasme plants. Its prevalent symptom is flower phyllody (floral parts are modified into leafy structures bearing no fruit), stunting and which is followed by significant yield reduction. The disease belongs to 16SrII group, *Candidatus* phytoplasma aurantifolia.

In conclusion, phytoplasma diseases are new threats for Turkish viticulture and pome and stone fruit some of the field crops production, and presence of their vectors indicates that they can be epidemic in the near future. Identification of vectors and natural source of inoculum is a prerequisite for the development of control stratejies. Recently only a few universities are involved in researches of present phytoplasma infections, identification of the causal agents and vectors involves. Since many agents associated by phytoplasma diseases are vector transmitted, controlling of the vectors, eliminating the infected plant material can be carried out for the control of epidemic outbreaks of phytoplasma infections. Recently, also the possibility of phytoplasma transmission by seeds was reported for several spesies of alfalafa, corn and tomato seeds (10). These results represents new pathways for phytoplasma dissemination. There is no doubt that new vectors will be included associated for phytoplasma dissemination, giving valuable information on the epidemiology of new phytoplasma diseases, as the researches on phytoplasmas progress.

Acknowledgement

This article was presented at 1st National Symposium on Vectors and Vector-Borne with International Participation, 9-10 September, 2012, Avanos, Cappadocia, Nevsehir, Turkey.

References

1. **Anonymous** (2008): *Tarımsal Yapı ve Üretim.* Başbakanlık İstastistik Enstitüsü (TUIK).
2. **Açıkgöz S** (1989): *Erzurum yöresinde Mikoplazma Benzeri Organizma (MLO)'lar ile enfekteli patates bitki ve yumru dağılımları ve oranları üzerine araştırmalar.* Türk Tarım ve Ormancılık Dergisi (Doğa*)*, **13,** 459-466.
3. **Angelini E** (2010): *Field Assestment and Diagnostic Methods for Detection of Grapevine Phytoplasmas.* 247-255. In: Delrot S, Medrano HOE, Bavaresco L, Grando S (Eds), Methodologies and Results in Grapevine Research. Springer.
4. **Angelini E, Clair D, Borgo M, Bertaccini A, Boudon-Padieu E** (2001): *Flavescence doree in France and Italy-occurence of closely related phytoplasma isolates and their near relationships to Palatinate Grapevine Yellows and Alder Phytoplasma.* Vitis, **40(2),** 79-86.
5. **Ahrens U, Seemuller E** (1992): *Detection of DNA of plant pathogenic Mycoplasmalike organisms by a Polymerase Chain Reaction that amplifics a sequence of 16S rRNA Gene*. Phytopathology*,* **82,** 828-832.
6. **Alma A, Soldi G, Tedeschi R, Marzachi C** (2002): *Role of Hyalestes obsoletus Signoret (Homoptera Cixiidae) in transmission of grapevine bois noir in Italy.* Petria, **12(3)**, 411-412.
7. **Avramoz Z, Gillet J, Laginova M** (2008*): First Detection of Stolbur Phytoplasma in Grapevines (Vitis vinifera* cv. *Merlot) Affected with Grapevine Yellows in Bulgaria.* J Phytopatol, **156(2),** 112-114.
8. **Berger J, Schweigkofler W, Kerschbamer C, Roschatt C, Dalla Via J, Baric S** (2009): *Occurence of stolbur phytoplasma in the vector Hyalestes obsoletus herbaceaus host plants and grapevine in south Tyrol.* Vitis, **48(4),** 185-192.
9. **Bertaccini A** (2007): *Phytoplasmas, Diversity, Taxonomy And Epidemiology.* Front Biosci, **12**, 673-689.
10. **Calari A, Paltrinieri S, Contaldo N, Sakalieva D, Mori N, Duduk B, Bertaccini A** (2011): *Molecular evidence of phytoplasmas in winter oilseed rape, tomato and corn seedlings.* Bull. of Insectology, **64,** 157-158.
11. **Canik D, Ertunc F** (2007): *Distribution and molecular characterization of Apple Proliferation phytoplasma in Turkey.* Bull Insectol**, 60(2),** 335-336.

12. **Canik D, Dikilitas M, Ertunc F** (2009): *Molecular detection of Pear Decline Phytoplasma in pear trees and their biochemical responses.* 87. In: Proceedings 21st Int Conference on Virus and Other Graft Trannsmissible Diseases of Fruit Crops, July, 5-10, Neustadt, Germany.
13. **Canik D, Ertunç F, Paltrinieri S, Contaldo N, Bertaccini A** (2011): *Identification of different phytoplasmas infecting grapevine in Turkey*. Bull. of Insectology**, 64(Suppl),** 225-226.
14. **Constable FE** (2010): *Phytoplasma Epidemiology. Grapevine as a model.* 188-213. In: Weintraub PG, Jones P (Eds), Phytoplasmas Genomes, Plant Hosts and Vectors, CabI.
15. **Çelik H, Kunter B, Soylemezoğlu G, Ergül A, Çelik H, Karataş H, Özdemir G, Atak A** (2010): *Bağcılığın Geliştirilmesi Yöntem ve Üretim Hedefleri*. 23. In: Ziraat Mühendisliği 7. Teknik Kongresi*,* 11-15 Ocak, Ankara.
16. **D'Amelio R, Marzachi C, Bosco D** (2007): *Double infection of 'Candidatus Phytoplasma asteris' and "flavescence doree" phytoplasma in the vector Euscenlidius variegatus*. B Insectol, **60(2)**, 223-224.
17. **Duduk B, Tian J, Contaldo N, Fan X, Paltrinieri S, Chen Q, Zhao Q, Bertaccini A** (2010): *Occurence of phytoplasmas related to stolbur and to 'Candidatus Phytoplasma japonicum' in woody host plants in China*. J Phytopathol, **158(2),** 100-104.
18. **Fialova R, Valova P, Balakishiyeva G, Danet JL, Safarova D, Foissac X, Navratil M** (2009): *Genetic variability of stolbur phytoplasma in annual crop and wild plant species in South Moravia.* J Plant Pathol, **91(2),** 411-416 .
19. **Guclu S, Ozbek H** (1988): *Some studies on the biology of Hyalestes obsoletus Signoret (Homoptera:Cixiidae)in the conditions of Erzurum.* Turk Entomol Derg, **12,** 103-111.
20. **Ikten C, Yol E, Çatal M, Uzun B** (2011) **:** *Frequency distribution of sesame phyloody infected by phytoplasmas in Antalya, Turkey.* 25. In: COST Action FA0807, Emerging phytoplasma diseases of stone fruits and other crops and their possible impact on EU countries, 1-2 December, İstanbul.
21. **Karimi M, Contaldo N, Mahmodi B, Duduk B, Bertaccini A** (2009): *Identification of stolbur related phytoplasma in grapevine showing decline symptoms in Iran*. 208-209. In: ICVG.
22. **Kuzmanovic S, Martini M, Ermacora P, Ferini F, Starovic M, Tosic M, Carraro L, Osler R** (2008): *Incidence and molecular characterization of flavescence dorée and stolbur phytoplasmas in grapevine cultivars from different viticultural areas of Serbia*. Vitis, **47(2),** 105-111.
23. **Lavina A, Battle A, Larrue J, Daire X, Clair D, Boudon-Padieu E** (1995): *First report of grapevine Bois Noir phytoplasma in Spain*. Plant Disease, **79(10)**, 1075.
24. **Lee IM, Gundersen DE, Hammond RW, Davis RE (**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(6),** 559-566.
25. **Lee IM, Davis RE, Gundersen-Rindal DE** (2000): *Phytoplasma: phytopathogenic mollicutes.* Annu Rev Microbiol, **54**, 221-255.
26. **Lessio F, Alma A** (2004): *Seasonal and daily movement of Scaphoideus Titanus Ball (Homoptera: Cicadellidae).* Environ Entomol, **33(6),** 1689-1694.
27. **Lessio F, Alma A** (2008): *Host Plants and seasonal presence of Dictyophara Europaea in the vineyard agro-ecosystem*. B Insectol, **61(1),** 199-200.
28. **Maixner M** (2011): *Recent Advances in Bois Noir Research*. 17-32. In: Book of Abstract, 2nd European Bois Noir Workshop, 27 Feb-1st March, Castelbrando, Venice.
29. **Martini M, Murari E, Mori N, Bertaccini A** (1999): *Identification and epidemic distribution of two Flavescence Doree related phytoplasmas in Veneto (Italy)*. Plant Disease, **83(10),** 925-930.
30. **Martini M, Botti S, Marcone C, Marzachi C, Casati P, Bianco PA, Benedetti R, Bertaccini A** (2002): *Genetic variability among Flavescence doree phytoplasmas from different origins in Italy and France.* Mol Cell Probes, **16(3),** 197-208.
31. **Mori N, Pavan F, Bondavalli R, Reggiani N, Paltrinieri S, Bertaccini A** (2008): *Factors affecting the spread of "Bois Noir" disease in North Italy vineyards*. Vitis, **47(1),** 65-72.
32. **Mori N, Reggiani N, Bacchiavini M, Pavan F, Paltrinieri S, Bertaccini A** (2010): *Cixiid presence and distribution of in Lambrusco vineyards affected by Bois Noir*. 737-739. In: INMF-2010.
33. **Pasquini G, Ferretti L, Bagnoli B, Gentili A, Gargarini E** (2010): *Epidemiological investigation on Bois Noir disease in central and southern Italy*. 64. In: COST-2010.
34. **Prince JP, Davis RE, Wolf TK, Lee IM, Mogen BD, Daily EL, 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(10),** 1130-1137.
35. **Sabate J, Lavina A, Legorburu J, Fortanete JJ, Perez Deobanos JL, Erez Marin P, Reyes J, Batlle A** (2007): *Incidence of "Bois Noir" phytoplasma in different wine-growing regions of Spain and its relation* to *Hyalesthes obsoletus*. B Insectol, **60(2),** 367-368.
36. **Seemuller E, Marcone C, Lauer U, Ragozzino A, Goschi M** (1998): *Current status of molecular classification of the phytoplasmas*. J Plant Pathol, **80,** 3-26.
37. **Ulubas-Serce C, Kaya K, Gazel M, Caglayan K, Sauvion N** (2012): *Psyllid vectors of AP Group (16SrX) phytoplasmas infecting grapevine in Turkey*. 104. In: Book of Abstracts, 22th International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops, 3-8 June, Rome.
38. **Ulubas-Serce C, Gazel M, Caglayan K, Bas M, Son L** (2006): *Phytoplasma diseases of fruit trees in germplasm and commercial orchards*. EPPO Bulletin, **36,** 216-217.
39. **Weintraub PG, Beandland L** (2006): *Insect vectors of phytoplasmas*. Annu Rev Entomol, **51**, 91-111.
40. **Weintraub PG, Jones P** (2010): *Phytoplasmas, Genomes, Plant Hosts and Vectors*. Cab International, United Kingdom.

Geliş tarihi: 18.01.2013 / Kabul tarihi: 20.03.2013

Address for correspondence:
Filiz Ertunc
Ankara University, Faculty of Agriculture,
Department of Plant Protection,
06110, Diskapi, Ankara, Turkey.
email: ertunc@agri.ankara.edu.tr