African Journal of Microbiology Research Vol. 5(33), pp. 6013-6017, 31 December, 2011
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMRX11.021
ISSN 1996-0808 ©2011 Academic Journals

Review

Phytoplasmas associated with *Almond* witches' broom disease: An overview

Maryam Ghayeb Zamharir

Phytobacteriology laboratory (PPDRI), Plant Pathology Research Department, Iranian Research Institute of Plant Production, Tabnak street, Evin, Tehran, 19395/1454 Iran.
E-mail: zamharir2005@yahoo.com. Tel: +98 (21) 22406793. Fax: +98 (21) 22403691.

Accepted 22 November, 2011

Almond is affected by several phytoplasma diseases but almond witches' broom (AlmWB) is the most destructive one. The disease spread rapidly, killed about a hundred thousands trees in recent years. Association of phytoplasma with AlmWB disease that belonged to the pigeon pea witches'-broom (PPWB) group has been established and the name '*Candidatus* Phytoplasma phoenicium' (16sIX-B) is proposed for it. The phytoplasma was graft-transmissible to almond, plum and peach seedlings. A specific PCR test was developed to distinguish the almond phytoplasma from others in the PPWB group. The specific PCR will be particularly useful for identification of both the insect vector(s) and reservoir plant(s) of the almond phytoplasma. For the moment only very few data is available on the extent of the disease, on its biology and epidemiology. However, this disease could represent a serious threat for almond-producing countries.

Key words: Almond witches broom, phytoplasma, peach, almond.

INTRODUCTION

Almond (*Prunus dulcis*, syn. *Prunus amygdalus* Batsch, *Amygdalus communis* L., *Amygdalus dulcis* Mill.) is a species of *Prunus* belonging to the subfamily Prunoideae of the family Rosaceae. Botanically, the almond is not a nut, but a fruit. The almond is native to Iran (Germplasm Resources Information Network, 2007). Global production of almonds is around 1.7 million tons, with a low of 1 million tons in 1995 and a peak of 1.85 million tons in 2002. Major producers are the USA (715623 t, 41%), Spain (220000 t, 13%), Syria (119648 t, 7%), Italy (112796 t, 6%), Iran (108677 t, 6%) and Morocco (83000 t, 5%). Algeria, Tunisia and Greece each account for 3%, Turkey, Lebanon and China each account for 2% (Fao, 2010).

Almond phytoplasma diseases include Almond brown line and decline (Peach yellow leaf roll phytoplasma), (Guinchedi et al. 1982), Almond kernel shrivels (Peach yellow leaf roll phytoplasma), (Morvan, 1977; Blomquist and Kirkpatrick 2002), European stone fruit yellows (European stone fruit yellows phytoplasma) and Western X disease (Western-X phytoplasma) (APS, 2002; Lorenz et al., 1994; Bernhard et al., 1977). In 1990s, almond witches broom has been reported from Iran and Lebanon (Choueiri et al., 2001; Salehi et al., 1995; Bove et al., 1999). The disease spread rapidly, killed about a hundred thousands trees in 10 years in Lebanon (Abou-Jawdah et al., 2002). Almond trees were assayed for presence of phytoplasma by PCR amplification of 16S rDNA gene sequence. A phytoplasma was detected in naturally diseased plants. This has been confirmed by electron microscopy (Verdin et al., 2003). Murray and Schleifer (1994) described the rules for the description of putative taxa of uncultured bacteria and proposed 'Candidatus Phytoplasma phoenicium' for the phytoplasma associated with almond witches'-broom (Verdin et al., 2003, 2002).

It has been shown that almond brooming in Iran and almond witches'-broom in Lebanon may be caused by the same phytoplasma species but recent studies indicate that different types of phytoplasma in almond induce witches broom. Consequently, detailed information

on population structure is essential for understanding the strategies to reduce the impact of almond witches broom disease.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Witches'-broom of cultivated and wild almond has been reported for the first time in Khafr and Meimand in Fars province of Iran in 1993 (Salehi et al., 1995; Bove´ et al., 1999). Based on the disease symptoms, graft and dodder transmission and positive reaction to diene's stain, it has been suggested that the causal agent of cultivated and wild almond is a mycoplasma like organism (Salehi et al., 1995). Trunk injection has been used to study the effectiveness of oxytetracycline to control almond witches' broom. Incomplete suppression of symptoms has been observed by injection of 0.25 g tetracycline. The Results have confirmed that phytoplasmal etiology of Almond brooming (Salehi et al., 1998).

During 1990 decade, an epidemic of almond witches'-broom (AlmWB) has caused devastating losses in almond production in Lebanon (Abou-Jawdah et al., 2003). It has shown almond witches broom disease symptoms have been found in various orchards of northern and southern areas in Lebanon (Verdin et al., 2003; Choueiri et al., 2001). Surveys on almond witches broom disease in 13 provinces in Iran have shown that almond witches broom phytoplasma have been detected in all almond varieties in various orchards of Iran (Ghayeb, 2007). All almond varieties in almond growing areas of Iran and Lebanon have been affected but some varieties (e.g. Alwani and Awja in Lebanon and Sangi in Iran) were highly susceptible and have developed severe witches'-brooms, leading to rapid death of tree; other varieties (e.g. Kachabi) were less affected, so that in orchards where all trees of other varieties were dying of the disease, the witches'- brooms symptoms were limited to one part of the canopy and only a few trees were affected (Verdin et al., 2003; Choueiri et al., 2001; Ghayeb, 2007).

Symptoms

Infection sweet almond trees exhibiting symptoms of cholorosis, little leaf witches'- brooms arising originally from the main trunk and roots, early flowering, stunted growth, leaf resetting, dieback, off-season growth, internodes' shortening, and proliferation of slender shoots. Such symptoms are often associated with phytoplasma infections (Figure 1) (Abou-Jawdah et al., 2003; Choueiri et al., 2001; Ghayeb, 2007; Abou-Jawdah et al., 2002).

In bitter almond the symptoms induced by KAlmWB (phytoplasmas from Khafr of Iran) consisted of severe proliferation, internodes' shortening and leaf size reduction (Salehi et al., 2006 b).

Causal agent and host range

Almond witches broom disease causes by 'Candidatus Phytoplasma phoenicium', Phytoplasma cells have been restricted to the sieve tubes and have been found in leaf petioles and midveins of symptomatic, but not healthy almond trees. Cells had the typical phytoplasma pleomorphic ultra structure, with predominantly filamentous and branched forms 0.1 to 0.2 mm in diameter. Phytoplasma cells were surrounded by the triple layered cytoplasmic membrane characteristic of the class Mollicutes (Verdin et al., 2003).

The main hosts of AlmWB are the cultivated and wild almond (Salehi et al., 1995, 2006 b; Bove´ et al., 1999). Recently, '*Candidatus* Phytoplasma phoenicium' has been reported in association with phytoplasma diseases on nectarine and peach trees in south of Lebanon (Abou-Jawdah et al., 2009) and west of Iran (Ghayeb et al., 2010).

Transmission

In Iran (Fars province), *Frutioidea bisignata*, *Zigina discolor*, *Psamotettix striants* and an unidentified planthopper species (captured infrequently) were carrier of '*Candidatus* Phytoplasma phoenicium' (Siampour et al., 2004). The phytoplasma was detected in saliva of *F. bisignata*. Since *F. bisignata* is consistently found on almond trees and also 'Candidatus Phytoplasma phoenicium' was present in its body and salvia, it is suspected as a potential vector of almond brooming phytoplasma in Fars province of Iran (Siampour et al., 2004). In Lebanon, the most frequently found leafhopper on stone fruits is *Assymetrasca decedens* (Paoli). Preliminary transmission tests in insect-proof cages, using this leafhopper were not successful, indicating that an unknown vector may be present in low population densities or may live on other hosts and use stone fruits as a transient host (Abou-Jawdah et al., 2003). However, only one positive peach tree among many affected almond trees, and no affected plum trees, which may indicate that the insect vector is preferentially attracted by almond trees (Verdin et al., 2003). Further studies are needed, in particular on possible insect vectors (Choueiri et al., 2001; Abou-Jawdah et al., 2002).

The Lebanon AlmWB, KAlmWB and NalmWB phytoplasmas could be experimentally transmitted by graft-inoculation of infected almond shoots into seedlings of almond (*Prunus amygdalus*), peach (*Prunus Persicae* GF305) and plum (*Prunus mariana* GF8-1) (Verdin et al., 2003; Salehi et al., 2006a). Symptoms developed after one month in the inoculated *Prunus* species, and consisted of auxiliary bud proliferation similar to that

Figure 1. Almond witches broom disease symptoms, A: yellows and extensive witches broom. B: witches Broom.

observed in naturally infected almond trees. It has been reported that the most apparent symptom on almond, nectarine, and peach has been the development of bushy growth at the base of the stem (rootstock) at or below the soil level. The stems were succulent, with shortened internodes, and the leaves were small and light green in color. New growth above the scion, in almond, peach, and nectarine, was considerably weaker than in their respective controls; internodes were shorter, leaves were lighter green and smaller and showed high susceptibility to infection by powdery mildew. On apricot and plum, no symptoms were observed in the parts above the grafting buds (Verdin et al., 2003).

KalmWB phytoplasma was transmitted to periwinkle and eggplant and from experimentally infected periwinkle to almond by dodder. It was also transmitted from eggplant to eggplant, ornamental eggplant and tomato by grafting. Under similar test conditions, NAlmWB was not transmitted to herbaceous plants by dodder (Salehi et al., 2006 a).

General and specific detection of the almond phytoplasma by PCR

AlWB phytoplasma grow and reproduce in the phloem of host plants and in their vector insects, so Koch's postulates may not be fulfilled. However, demonstration of the presence of phytoplasmas only in infected almond trees by electron microscopy and PCR is a strong indication that the phytoplasma is the causal agent of the disease (Verdin et al., 2003; Choueiri et al., 2001; Abou-Jawdah et al., 2002; Abou-Jawdah et al., 2003).

Almond witches broom phytoplasma diagnostics and phylogenetics have historically been based on the 16S rRNA gene and the 16S–23S rRNA spacer region. P1 and P7 are universal primers that could amplified a part of 16S rRNA gene and the 16S–23S rRNA spacer region from all phytoplasma phylogenetic groups (Smart et al., 1996; Firrao et al., 2005). Analysis of the 16S rRNA gene has resulted in the comprehensive and widely used 16Sr group classification system for phytoplasmas, which is based on restriction enzyme digest patterns of the 16S rRNA PCR products. In this system, universal primers are used to amplify a specified region of the 16S rRNA gene (Ahrens et al. 1993; Zak et al., 2011), and the PCR product is digested with specific restriction enzymes and the profiles analyzed using agarose or polyacrylamide gel electrophoresis. The RFLP patterns are then compared, and specific profile used to classify phytoplasmas into groups and subgroups (Lee et al*.,* 1998).

In order to distinguish the almond phytoplasma from others in the PPWB group, A forward primer, AlmF1 (5'-CCTTTTTCGGAAGGTATG- 3') within the 16S rRNA gene (position 170) and a reverse primer, AlmR1 (5'-GATAACACGCTTAAGACG-3') within the 16S–23S spacer region (position 1682), have been defined (Verdin et al., 2003). These primers can serve as effective tools for identification of both the insect vector(s) and reservoir plant(s) of the almond phytoplasma as well as vectors and reservoirs of other phytoplasmas in the PPWB group (Verdin et al., 2003, 2002).

Sequence similarity and phylogenetic relationships

Lebanese and Iranian almond phytoplasmas (NalmWB) belong to pigeon pea witches'-broom (PPWB) group. PPWB include the phytoplasma infecting the wild lettuce and periwinkle plants and all phytoplasmas of the PPWB group have more than 97.5% identity with the almond phytoplasma and at this stage cannot be described as separate *Candidatus* species, because, a new species may be described when a 16S rDNA sequence (>1200 bp) has less than 97.5% identity with any previously described '*Candidatus* Phytoplasma' species (IRPCM 2000; IRPCM, 2005), even though they have been found in different host plants (Verdin et al. 2002). Analysis of the complete sequence of P1/P7-amplified fragments for two different Lebanese almond phytoplasma isolates, from southern and northern regions, and NalmWB (phytoplasmas from Neyriz of Iran) have been revealed that two almond phytoplasmas from Lebanon were almost identica l and differed by only 4 nt (99.7%

similarity) from the sequence of NalmWB phytoplasmas, showing that almond brooming in Iran and almond witches'-broom in Lebanon may be caused by the same phytoplasma species (Verdin et al., 2003).

Lebanese and Iranian almond phytoplasmas (NalmWB) represent a new lineage in the PPWB group defined by Schneider et al. (1995) or in the 16S rIX-A group defined by Lee et al. (1998) and a new subclade in the '*Candidatus* Phytoplasma' phylogenetic tree (Lee et al., 1998; Seemuller et al., 1998). All of the organisms in PPWB group have more than 97.5% similarity with phytoplasmas in other subclades (Lee et al., 1998; Seemuller et al., 1994). Almond witches broom phytoplasmas are different from phytoplasmas infecting other *Prunus* species in Europe or in the United States. Its 16S rRNA gene sequence has 90.4% similarity to European stone fruit yellow (ESFY) phytoplasma and 94.3% similarity to Western X phytoplasma. They are also different from apple proliferation (AP) phytoplasma (with 90.35% similarity) and pear decline (PD) phytoplasma (with 90.84% similarity) (Verdin et al., 2003).

Salehi et al. (2006) has reported KAlmWB phytoplasma (phytoplasmas from Khafr of Iran) as a new phytoplasma of AlmWB disease. Based on phylogenetic and putative restriction site analyses and sequence homology, KalmWB (phytoplasmas from Khafr of Iran) was closer to the *Knautia arvensis* phyllody (KAP) agent. Clustering of KAlmWB with KAP has been confirmed by analysis of full length 16S rDNA sequence. On the basis of host range, dodder transmission, symptomatology and molecular analyses of 16S rDNA and spacer region (SR), two different phytoplasmas related to PPWB group were associated with AlmWB disease in Iran (Salehi et al., 2006 a).

Control

Tetracycline antibiotics are effective against phytoplasmas, the mechanism is that they inhibit protein synthesis by binding to the 30S ribosomal subunit. By Injection of antibiotic solutions into almond infected plant tissue the symptoms were diminished, but did not heal the plants completely (Salehi et al., 1998). The use of antibiotics not only causes the problems on food crops, but also symptom remission is usually temporary, as tetracycline only persists in plants for 1 to 4 months (McCoy, 1982; Kaminska and Silwa, 2003).

Since the vector is unknown, it is not possible to control the natural spread of the disease and roguing may not always be successful (Choueiri et al., 2001; Abou-Jawdah et al., 2002).

Research activities would concentrate on the breeding of resistant cultivars. At present, resistant rootstocks are the most efficient way to control the disease (Aps, 2002). Until resistant almond cultivars become available, apricot, plum, or cherrie, may serve as replacement crops in AlmWB-infested areas. AlmWB poses a great threat to almond, nectarine, and peach production because it seems to be effectively transmitted by one as yet unidentified vector (Abou-Jawdah et al. 2003).

CONCLUSION

Witches' broom (AlmWB) is a destructive disease in Almond. Its ability to cause epidemics and our difficulty in controlling them should be noted as a high priority for *Prunus* disease researches. Tow distinct types of phytoplasma has associated with almond witches broom diseases that are distinguishable by putative restriction site analysis of SR. These phytoplasmas have been detected in different almond leafhoppers but it has not been clarified whether they have the capacity to transmit these phytoplasmas or not. For the moment only very few data is available on the disease etiology, on it's vectors and disease cycle. However, as extensive tree mortality is reported, it was felt that this disease could represent a serious threat for almond-producing countries and host status of other *Prunus* species. The future will offer many research opportunities for those working with phytoplasma diseases of Almond. None of the genomes of phytoplasmas which infect Almond have been completely sequenced and it's genome is expected to be full sequence and that will contribute significantly to our attempts to devise sustainable disease management strategies.

REFERENCES

Abou-Jawdah Y, Karakashian A, Sobh H, Martini M, Lee IM (2002). An epidemic of almond witches'-broom in Lebanon: classification and phylogenetic relationships of the associated phytoplasma. Plant Dis., 86(5): 477-484.

Abou-Jawdah Y, Dakhil H, El-Mehtar S, Lee IM (2003). Almond witches'-broom phytoplasma: a potential threat to almond, peach, and nectarine. Can. J. Plant Pathol., 25: 28–32.

Ahrens U, Lorenz KH, Seemüller E (1993). Genetic diversity among mycoplasma like organisms associated with stone fruit diseases. Mol. Plant Microbe In., 6: 686–691.

APS (2002). Compendium of Nut Crop Diseases, 1st Ed. APS Press.

Bernhard R, Marénaud C, Eymet J, Séchet J, Fos A, Moutous G (1977). Une maladie complexe de certains *Prunus* : le dépérissement de Molières. C. R. Acad. Agric. Fr., 178–189.

Blomquist CL, Kirkpatrick BC (2002). Identification of phytoplasma taxa and insect vectors of peach yellow leaf roll disease in California. Plant Dis., 86: 759–763.

Bove JM, Danet JL, Bananej K, Hassanzadeh N, Taghizadeh M, Salehi M, Garnier M (1999). Witches' broom disease of lime (WBDL) in Iran. In Proceedings of the 14th Conference of the International Organization of Citrus Virologists (IOCV), Edited by da Grac JV ¸ Lee RF and Yokomi R. K. Riverside, CA: IOCV, pp. 207–212.

Choueiri E, Jreijiri F, Issa S, Verdin E, Bové J, Garnier M (2001). First report of a phytoplasma disease of almond (*Prunus amygdalus*) in Lebanon. Plant Dis., 85(7): 802.

Fao (2010). http://www.fas.usda.gov/htp.

Firrao G, Gibb K, Streten C (2005). Short taxonomic guide to the genus '*Candidatus* phytoplasma'. J. Plant Pathol., 87: 249–263.

Germplasm Resources Information Network (2007). http://www.ars-grin.gov.

Ghayeb Zamharir M (2007). Detection of Almond witches broom

phytoplasma in important almond growing areas of Iran. In: proceedings of the International symposium on viruses of ornamental and temperate fruit crops, pp. 16.
Ghayeb Zamharir M, Sirjani M, Sahragard N, Mohammadipour M, Esmaelzadeh HAS, Salehi M (2010). Detection of Almond witches broom phytoplasma in important almond growing areas of Iran (AREO, Tehran, Iran.). Final report of project no. 2-009-10000-02-0000-85036.
Guinchedi L, Poggi-Pollini C, Credi R (1982). Susceptibility of stone fruit trees to the Japanese plum-tree decline causal agent. Acta Hort., 130: 285–290.
IRPCM (2000). Report of the working team for Consultations of the International Research Program of Comparative Mycoplasmology (IRPCM) of the International Organization for Mycoplasmology (IOM). Fukuoka, Japan: IOM.
IRPCM (2005). Short Taxonomic Guide to the Genus '*Candidatus* Phytoplasma'. J. Plant Path., 87(4): 249-263.
Kaminska M, SilwaH (2003). Effect of antibiotics on the symptoms of stunting disease of *Magnolia liliiflora* plants. J. Phytopathol., 151: 59–63.
Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk IM (1998). Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int. J. Syst. Bacteriol., 48:1153–1169.
Lorenz KH, Dosba F, Poggi-Pollini C, Llacer G, Seemüller E (1994). Phytoplasma diseases on *Prunus* species in Europe are caused by genetically similar organisms. Z. Pflanzenkr. Pflanzenschutz, 101: 567–575.
McCoy RE (1982). Antibiotic treatment for control of tree diseases associated with mycoplasma-like organism. Rev. Infectious Dis., 4:157–161.
Morvan G (1977). Apricot chlorotic leaf roll. Eppo Bull., 7: 37–50.
Murray RGE, Schleifer KH (1994). Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. Int. J. Syst. Bacteriol., 44: 174–176.
Salehi M, Izadpanah K (1995). Almond brooming. In: proceedings of the 12th Iranian plant protection congress, pp. 238.
Salehi M, Izadpanah K (1998). Tetracycline treatment of almond trees against almond brooming. In: proceedings of the 13th Iranian plant protection congress, pp. 248.
Salehi M, Izadpanah K, Heydarnejad J (2006 a). Characterization of A New Almond Witches' Broom Phytoplasma in Iran. J. Phytopath., 154(7-8): 386-391.
Salehi M, Izadpanah K (2006 b). Detection and Identification of Almond witches broom phytoplasma in wild almond trees in Fars province. In: proceedings of the 17th Iranian plant protection congress, pp. 323.
Schneider B, Seemuller E, Smart CD, Kirkpatrick C (1995). Phylogenetic classification of plant pathogenic mycoplasma like organisms or phytoplasmas. In Molecular and Diagnostic Procedures in Mycoplasmology, Edited by S. Razin & J. G. Tully. New York: Academic Press. 1: 369–380.
Seemuller E, Marcone C, Lauer U, Ragozzino A, Goschl M (1998). Current status of molecular classification of the phytoplasmas. J. Plant Pathol., 80: 3–26.
Seemuller E, Schneider B, Maurer R (1994). Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. Int. J. Syst. Bacteriol., 44: 440–446.
Siampour M, Izadpanah K, Afsharifar A, Salehi M, Taghizadeh M (2004). Study of piercing-sucking insects from almond groves in relation to transmission of almond brooming phytoplasma. Iranian J. Plant Path., 36 (3-4): 390.
Smart CD, Schneider B, Blomquist CL, Guerra LJ, Harrison NA (1996). Phytoplasma-specific PCR primers based on sequences of 16S–23S rRNA spacer region. App. Environ. Microb., 62: 2988–2993.
Verdin E, Salar P, Danet JL, Gélie B, Bové JM, Garnier M, Choueiri E, Jreijiri F, El Zammar S (2002). Phylogentical Characterization and PCR Detection of a New Phytoplasma in Almond (*Prunus amygdalus*) and Peach (*Prunus Persicae*) in the Mediterrsnen Area. Acta Hortic., 657: In : XIX International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops - Fruit Tree Diseases.
Verdin E, Salar P, Dane JL, Choueiri E, Jreijiri F, El Zammar S, Gelie B, Bove´JM, Garnier M (2003). Candidatus *Phytoplasma phoenicium'* sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. Int. J. Syst. Evol. Micr., 53: 833–838.
Zak LF, Yáñez-Morales M J, Alanis-Martínez I, González-Pérez E (2011). New hosts of 16SrI phytoplasma group associated with edible *Opuntia ficus-indica* crop and its pests in Mexico. Afr. J. Microbiol. Res., 5(8): 910-918.