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NEW OR UNUSUAL DISEASE REPORTS

Isolation and pathogenicity of *Xylella fastidiosa* from grapevine and almond in Iran

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Summary. Symptoms similar to those of Pierce's disease (PD) of grapevine and leaf scorch of almond were observed in vineyards and almond orchards in several provinces of Iran. Grafting of scions from symptomatic almond trees onto seedlings of a local almond (cv. Mamaee) under greenhouse conditions resulted in the transmission of the leaf scorch agent. A number of symptomatic samples from orchard and greenhouse plants were positive for presence of *Xylella fastidiosa* when tested by DAS-ELISA and PCR with *X. fastidiosa* specific antibodies and primers. A Gram-negative bacterium similar to *X. fastidiosa* was isolated on 'periwinkle wilt' (PW) medium. Selected isolates induced symptoms similar to those caused by *X. fastidiosa* when inoculated on *Nicotiana tabacum*, seedlings of almond and grapevine under greenhouse conditions. DAS-ELISA and PCR confirmed the identity of the isolated bacteria. On the basis of disease symptoms, graft transmission, isolation on specific *X. fastidiosa* culture medium, pathogenicity tests and positive reactions in DAS-ELISA and PCR, *X. fastidiosa* is associated with almond leaf scorch and Pierce's disease in grapevine in Iran. This is the first report on the presence of *X. fastidiosa* in the Middle East and western Asia.

Key words: leaf scorch; Pierce's disease.

Introduction

Xylella fastidiosa is a Gram-negative, xylem limited, plant pathogenic bacterium (Wells *et al*., 1987) that causes important diseases such as Pierce's disease (PD) of grapevine, almond leaf scorch (ALS), citrus variegated chlorosis (CVC), oleander leaf scorch (OLS) and coffee leaf scorch (CLS) (Hopkins and Purcell, 2002; Almeida and Purcell, 2003; Hernandez-Martinez *et al*., 2006). This pathogen is also the causal agent of several other leaf scorch diseases in crops, ornamental plants and forest and urban trees (Hopkins and Purcell, 2002; Costa *et al*., 2004; Montero-Astúa *et al*., 2007). It has been reported in the United States of America and Brazil, but there are also reports of the occurrence of *X. fastidiosa* from Taiwan on pear and grapevine (Leu and Su, 1993; Su *et al*., 2013), and from Europe (Berisha *et al*., 1998; Güldür *et al*., 2005, Saponari *et al*., 2013) based detection with double antibody sandwich-enzyme-linked immunosorbent assays (DAS-ELISA) and PCR. *Xylella fastidiosa* has been regarded as a quarantine pathogen in Iran.

A number of strains have been recognized for *X. fastidiosa*. Strains causing OLS belong to *X. fastidiosa* subsp. *sandyi*, PD strains belong to *X. fastidiosa* subsp. *fastidiosa*, while ALS strains from almond are of two general types belonging either to *X. fastidiosa* subsp. *multiplex* or *X. fastidiosa* subsp. *fastidiosa*. The ALS strains assigned to *X. fastidiosa* subsp. *multiplex* are of two different genotypes (ALS I and ALS II) below the subspecies level (Hernandez-Martinez *et al*., 2006). The OLS strains do not infect grape or almond. Pierce's disease strains produce diseases in grape, alfalfa, almond, and some weeds, but they

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do not infect oleander, oak, peach, or citrus. Strains causing ALS that belong to *X. fastidiosa* subsp. *multiplex* do not produce disease in grape (Hernandez-Martinez *et al*., 2006).

Xylella fastidiosa in infected tissue has been identified by *in vitro* cultivation. However, cultivation of *X. fastidiosa* in the laboratory is difficult and time consuming. More recently, PCR-based specific detection of the bacterial DNA has become popular (Ledbetter and Rogers, 2009; Janse and Obradovic, 2010). Several specific PCR primer sets are currently available for *X. fastidiosa* detection, including the most thoroughly tested RST31/33 primer set (Minsavage *et al*., 1994), based on RNA polymerase sigma 70 factor (*rpoD*) genomic locus. Additional primer sets, based on the 16S rRNA gene (Chen *et al*., 2005), have often been used as important tools for taxonomic classification of *X. fastidiosa* (Qin *et al*., 2001).

Grapevine and almond are two economically important and widely grown fruit crops in Iran, with estimated areas of 328,000 ha of grapevines and 223,000 ha of almonds. Symptoms of leaf scorch, yellowing, and dieback have been observed in many vineyards and almond orchards throughout the country.

In this study, we report the isolation and pathogenicity of *X. fastidiosa* strains from symptomatic almond and grapevine plants in Iran. This is the definitive record of the pathogen from this country. A preliminary report of this study has been published (Sahragard *et al*., 2010).

Materials and methods

Field survey

The main Iranian almond and grapevine growing provinces of Chahar Mahal-va-Bakhtiari, Fars, West Azerbaijan, Qazvin, Hamedan, Semnan, Khorasan Razavi, Alborz and Isfahan were visited, and plants exhibiting ALS and PD symptoms were sampled during summer and autumn of 2009–2012. Samples consisted of stems and leaves. The samples were placed in individual plastic bags and transferred to the laboratory and tested by DAS-ELISA for infection by *X. fastidiosa* (Schaad *et al*., 2001).

Grafting experiments

Seeds of almond (cv. Mamaee) were planted under greenhouse conditions. Forty five seedlings testing negative for *X. fastidiosa* by DAS-ELISA were used in graft inoculation experiments. Scions from almond trees showing leaf scorch symptoms and being DAS-ELISA positive for *X. fastidiosa* were grafted by the T-budding method onto almond seedlings. Twenty seedlings were graft inoculated each with two buds. Twenty seedlings were also cleft grafted using 5–8 cm long bud woods. Controls consisted of five ungrafted seedlings. Data on disease symptoms were recorded during the late spring and early summer of the next year. The presence of *X. fastidiosa* in symptomatic tissues above the scion of each grafted plant was assessed by DAS-ELISA, PCR and isolation in culture, as described below.

Isolation of *Xylella fastidiosa*

Since the symptoms on almond and grapevine were suggestive of *X. fastidiosa* infection, attempts were made to isolate the bacterium on specific culture media.

Two to three cm long pieces of almond and grapevine petioles and midribs from plants exhibiting ALS and PD symptoms were transferred to sterile plastic bags, surface sterilized by soaking for 3 min in 1% sodium hypochlorite and 4 min in 70% ethanol, and rinsed three times each for 3 min in sterile distilled water. The tissues were transferred to new plastic bags in 3 mL sterile succinate-citrate-phosphate buffer (1 g L^{-1} disodium succinate, 1 g L^{-1} trisodium citrate, 1.5 g L^{-1} K_2HPO_4, 1 g L^{-1} KH_2PO_4, pH 7). After a 20-min incubation period at room temperature, 50 μL of the liquid were streaked on the media PD3 (Davis *et al*., 1980), PW (Schaad *et al*., 2001), *X. fastidiosa*-D1 (Almeida *et al*., 2004) and nutrient agar (Schaad *et al*., 2001). Alternatively the petioles and midribs were crushed in the plastic bags using a pair of sterile pliers and 30 μL of extract were streaked on culture medium. The plates were incubated at 28°C for 4 weeks and examined with a stereomicroscope at weekly intervals for the presence of *Xylella*-like colonies (Wells *et al*., 1987; Chen *et al*., 2007). Seven to 20 d after plating on PD3 and PW media, small white colonies, if found, were re-streaked three times onto PW medium to ensure the purity of the strains. Bacterial cells were Gram stained and observed at 1000× magnification using phase contrast microscopy (Schaad *et al*., 2001).

DAS-ELISA

DAS-ELISA was used to study the presence of *X. fastidiosa* in symptomatic samples of almond and grapevine. One gram of petiole or midrib tissue from each sample was surface sterilized by soaking for 3 min in 70% ethanol, rinsed twice for 3 min in sterile distilled water and transferred to a plastic bag with 3 mL of extraction buffer (Agdia, Inc.,) and crushed with a pestle at room temperature. The sap was loaded into strips coated with *X. fastidiosa* -specific antibodies using the *PathoScreen* kit, and processed according to the manufacturer's instructions (Agdia, Inc.). Bacterial isolates cultured on PW and bacterium cells suspended in extraction buffer were also used as antigen. The results were analyzed by using a plate reader (Bio-Tek KC4, v.3.1) at 630 nm. Petiole and midrib tissues of healthy almond and grapevine plants were processed similarly as negative controls. All test plates included three negative controls. A suspension of cells of a *X. fastidiosa* type-strain included in the ELISA kit was used as the positive control. Samples with absorbance values above the average absorbance values of the known negative samples plus three times the standard deviation were considered positive for *X. fastidiosa* (Sutula *et al*., 1986).

DNA extraction

Total DNA was extracted from fresh almond and grapevine samples using the Qiagen Plant DNAeasy minikit (Qiagen, Inc) according to the manufacturer's instructions. The cetyltrimethylammonium bromide (CTAB) method (Ausubel *et al*., 1992) was used for DNA preparation from bacterial cells. Bacterial cells were scraped from the PW agar plates after 10 d incubation at 28°C, washed twice in TAS buffer (50 mM Tris/HCl, 50 mM EDTA, 150 mM NaCl, pH 8) and resuspended in 500 μL of the same buffer. SDS (1% final concentration) and proteinase K (150 μg mL^{-1} final concentration) were added and the tubes were incubated for 1 h at 50°C. Proteins and other cellular components were removed using CTAB and chloroform extraction. The DNA present in the aqueous phase was precipitated by adding absolute ethanol followed by washing with 70% ethanol. The pellet obtained was dissolved in distilled water. The DNA was subjected to electrophoresis in a 1% agarose gel. The gels were stained with ethidium bromide and observed with ultraviolet light to estimate DNA concentration. The DNA samples were stored at -20°C.

PCR detection of *Xylella fastidiosa* in total DNA, xylem fluid, and bacterial colonies

Three primer sets: RST31/RST33 (Minsavage *et al*. 1994) targeting the *rpoD* gene, 272-1-int/272-2-int (Pooler and Hartung, 1995) and Dixon454fa/Dixon1261rg (Chen *et al*. 2005) targeting the 16S rRNA gene, were utilized for amplification of parts of the *X. fastidiosa* genome, and the resulting products were sequenced. PCR reactions were carried out using the TaKaRa TaqTM (Hot Start Version, Takara Bio Inc.) in 25 μL volumes. The components for the PCR included Master Mix (2 × Premix) 12.5 μL, 0.1 μM of each primer (1 μL) and 8 μL of water. Templates consisted of 300 ng of the total plant DNA, extracted bacterial DNA or a small portion of the bacterial colony suspended in distilled water for whole-cell PCR. The amplification programme consisted of an initial denaturation step at 95°C for 10 min; 40 cycles of 95°C for 45 s, an annealing temperature of 55°C for the 16S rRNA gene, 59°C for the *rpoD* gene for 30 s, and 72°C for 30 s; and a final elongation step of 72°C for 10 min. PCR products were separated by electrophoresis on 1% agarose gels run at 5 V cm^{-1} for 1 h, stained with ethidium bromide (10 μg L^{-1}), and visualized over UV light. All the PCR products were sequenced by Macrogen. Sequences obtained from the *rpoD* and 16SrRNA genes were analyzed and two sequences were deposited in the GenBank database under accession numbers KF463301 for isolates from almond and KF463302 for isolates from grapevine.

Pathogenicity studies

Pathogenicity tests were conducted on seedlings of almond (cv. Mamaee), grapevine (cv. Bidaneh Qazvin) and *Nicotiana tabacum* (cv. White Burley) under greenhouse conditions. Two strains of *X. fastidiosa*, one each from almond and grapevine, were grown in liquid PW medium at 28°C with shaking for 8 d. The bacteria were centrifuged to obtain a pellet, and the pellet was resuspended in succinate-citrate buffer (ca. 10^6 bacterial cells mL^{-1}). Each of the isolated bacteria was inoculated into the host from which it was isolated. For each inoculation, a cell suspension drop was placed on the young stem of a healthy almond or grapevine plant, near a petiole, followed by prick-

ing the stem five times through the cell suspension using a syringe needle, the suspension was absorbed into the xylem. For *N. tabacum*, bacterial suspension of both strains was injected into midribs and petioles. Control plants were treated in the same way except that distilled water was used instead of bacterial suspension. Five plants each of almond, grapevine and *N. tabacum* were inoculated with each tested isolate. All plants were kept in the greenhouse and fertilized with irrigation water. Symptom development was closely monitored and recorded on a weekly basis for 4 months (Chang and Donaldson, 2009).

Leaves of inoculated and control plants were assayed for *X. fastidiosa* infection 1 week (for tobacco), 3 months (for grapevine) or 4 months (for almond) after inoculation, using DAS-ELISA. Leaves were also used for re-isolation of *X. fastidiosa*. Bacterial colonies were tested by PCR using RST31/RST33 primers (Ledbetter and Rogers, 2009).

Results and discussion

Field symptoms

In vineyards of Chahar Mahal-va-Bakhtiari, Fars, Qazvin, Hamedan, Khorasan Razavi, Alborz and Isfahan provinces, the first symptoms resembling those of PD were observed in early summer. The disease was often localized and confined to one or a few plants in affected vineyards (Figure 1A). An early sign was sudden drying of part of a green leaf which then turned brown, while adjacent tissues turned yellow or red (Figure 1B). Leaf discoloration began at the margin and moved toward the midrib. Gradually, more yellowing occurred and leaves shriveled and dropped. Some vines showed "matchstick" symptoms in which the leaves dropped from the plant, while petioles remained attached. Symptoms spread upward along the canes which sometimes died back. Stems failed to mature resulting in green islands of tissue (Figure 1C). Some grapevines showed dehydration of fruit clusters. Occasionally, grapevines died 2–3 years after the first symptoms were observed.

In almond orchards in Chahar Mahal-va-Bakhtiari, West Azerbaijan and Semnan provinces, symptoms of almond scorch appeared in midsummer as leaf discoloration. The tip and margins of leaves initially turned light gray-green. Scorching appeared with progress of the season (Figure 2A). The symptoms were fully developed by early September. Symptoms appeared first in single branches or limbs and spread throughout the canopy in subsequent years. The entire canopies of the affected trees showed symptoms of leaf scorch in 3–4 years. These trees showed striking yellow colour known as "golden death", since they eventually died (Figure 2B) (Mircetich *et al.*, 1976).

Figure 1. A. Grapevine plant with symptoms similar to PD in a vineyard. B. Grapevine leaves with marginal chlorosis and necrosis. C. Green island on a grapevine twig.

Isolation of *Xylella fastidiosa*

Xylella fastidiosa was cultured from four of 73 almond and eight of 84 grapevine samples of field and greenhouse tested (Table 1) in the three selected media. The number of bacterial colonies varied from three to more than 40 per plate (Figure 4). Colonies appeared 1–4 weeks after plating. Prolonging the incubation period to 40 d did not result in colony formation in other plates. The PW culture medium was more suitable for bacterium isolation, PD3 was a good growing medium, while growth of bacteria was very weak on X. fastidiosa-D1 medium, and some isolates from almond did not grow on this medium. Isolates of *X. fastidiosa* did not grow on nutrient agar.

DAS-ELISA and PCR

Fifty-four percent of grapevine samples and 32% of almond samples of field and greenhouse tested positive for *X. fastidiosa* by DAS-ELISA (Table 1). When DAS-ELISA positive samples were tested by the 3 PCR protocols, seven of 23 almond samples and six of 39 grapevine samples reacted positively. Samples that were positive for *X. fastidiosa* by PCR had large DAS-ELISA readings.

Fragments of 840 bp were amplified by PCR with primer pair Dixon454fa/ Dixon1261rg, 733 bp with primer pair RST31/RST33, and 600 bp with primer pair 272-1-int/272-2-int. By sequencing we obtained identical sequences for the *rpoD* and 16SrRNA genes.

BLASTN alignment of the sequence obtained with the primer pair RST31/RST33 revealed 99% identity with the *rpoD* gene of *X. fastidiosa*, for isolates from grapevine, and the sequence obtained with the primer pair Dixon454fa/ Dixon1261rg revealed 99% identity with 16S ribosomal RNA gene of *X. fastidiosa*, for isolates from almond.

Pathogenicity tests

The grapevines showed symptoms of PD about 4 months after inoculation with bacterial cells. Leaf scorch symptoms appeared on almond seedlings grafted with buds and shoots of infected trees under greenhouse conditions 8 months after grafting. Five of 20 almond seedlings grafted with buds and 11 of 20 seedlings grafted with shoots developed leaf scorch symptoms. Almond seedlings inoculated with bacterial cells of *X. fastidiosa* showed disease symptoms 5 months after inoculation under greenhouse conditions (Figure 2C).

Figure 2. A. Typical leaf scorch symptoms on an almond tree, caused by *X. fastidiosa.* B. Almond tree with symptoms of "golden death". C. Almond leaf scorch symptoms on an almond seedling 5 months after inoculation.

Table 1. Comparison of *Xylella fastidiosa* detection methods in symptomatic almond and grapevine plant samples from Iran[a].

Plant	Number of plant samples	Sampling month	Sampling province	Assay result		
				DAS-ELISA (positive/tested)	PCR (positive/tested)	Culture (positive/tested)
Almond	4	August 2011	West Azerbaijan	0/4	-	-
	67	August–October 2011	Chahar Mahal-va- Bakhtiari	11/67	2/8	0/23
	2	October 2011	Semnan	0/2	-	-
	32	August–October 2012	Chahar Mahal-va- Bakhtiari	17/32	1/5	2/24
	28	2011–2012	Greenhouse (grafted)	15/28	4/10	2/26
Grapevine	9	August 2011	Qazvin	5/9	0/3	-
	45	August–October 2011	Chahar Mahal-va- Bakhtiari	14/45	1/7	0/12
	7	October 2011	Fars	2/7	-	0/5
	7	July 2012	Hamedan	2/7	-	0/4
	16	July 2012	Qazvin	9/16	-	1/10
	7	July 2012	Alborz	5/7	-	0/5
	2	August 2012	Esfahan	0/2	-	0/2
	8	August 2012	Fars	7/8	1/3	0/6
	21	September 2012	Khorasan Razavi	16/21	1/7	2/16
	6	September 2012	Qazvin	5/6	1/4	1/4
	24	August–October 2012	Chahar Mahal-va- Bakhtiari	15/24	0/8	1/10
	12	2011–2012	Greenhouse (inoculated)	8/12	2/7	3/10

[a] The PCR results were determined to be positive or negative by the presence of products of the correct size on agarose gels. The bacterial culture column refers to samples that yielded *X. fastidiosa*-like colonies when cultured.
-, Not tested.

Grapevine and almond isolates of *X. fastidiosa* induced chlorosis symptoms at injection sites around the main veins in tobacco 8 d after inoculation (Figure. 3A). Chlorotic and necrotic areas appeared at leaf margins (Figure 3B) and expanded until they engulfed whole leaves 3 weeks after inoculation. As symptoms progressed, leaves became totally necrotic 3 months after inoculation (Figure 3C). Presence of *X. fastidiosa* in symptomatic leaves was confirmed by DAS-ELISA and PCR. No symptoms appeared in control plants.

The main purpose of this investigation was to study possible association of *X. fastidiosa* with leaf scorch symptoms in almond and grapevine plants in Iran. A number of plants showing leaf necrosis, defoliation, and shoot dieback were positive for *X. fastidiosa* as demonstrated in DAS-ELISA, PCR and culturing assays. These results provide strong evidence for association of *X. fastidiosa* with the leaf scorched almond and grapevine plants. The data indicated that *X. fastidiosa*-infected almonds and grapevines

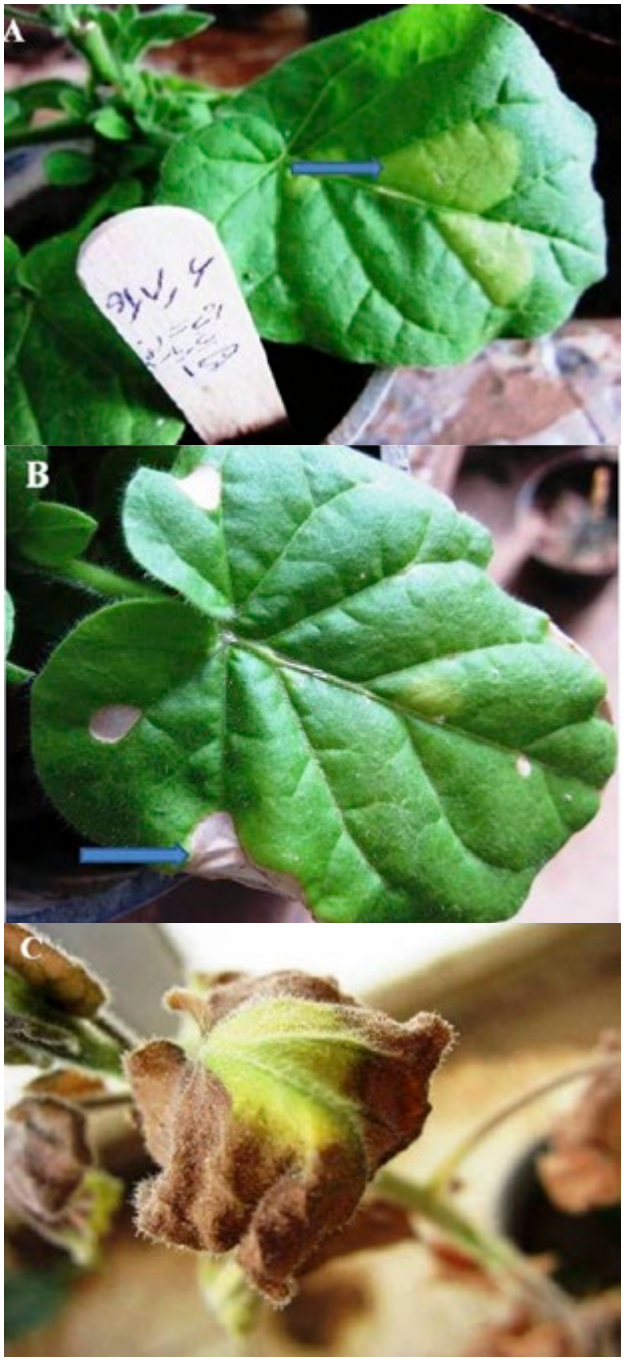

Figure 3. Progressive development of tobacco leaf symptoms after inoculation with *Xylella fastidiosa*. (A) Chlorosis of vein areas (inoculation site) 8 d after inoculation. (B) Chlorotic area at leaf margin, 3 weeks after inoculation. (C) Necrosis of a leaf 3 months after inoculation.

are widely distributed in Iran. Symptoms of disease in almond and grapevine were similar to those described for *X. fastidiosa* infections in other regions of the world (Raju and Wells, 1986; Sanderlin and Heyderich-Alger, 2000). Typically in this group of diseases, necrosis develops at leaf margins during mid- to late summer, and expands toward the midribs and bases of affected leaves. Lesions are often bordered by zones of dark reddish-brown tissue. The leaves absciss prematurely. The pathogenicity test indicated that cultures of two *X. fastidiosa* isolates, one each from almond and grapevine, were pathogenic to *N. tabacum*. In previous investigations, *N. tabacum* cv. SR1 and *N. benthamiana* were shown to be suitable hosts for *X. fastidiosa* assays, in particular for PD and almond leaf scorch strains (Francis *et al.*, 2008). Our investigations have demonstrated that *N. tabacum* cv. White Burley is also a suitable indicator host for biological studies of *X. fastidiosa*.

Based on disease symptoms, the transmission efficiency of the pathogen in almond seedlings grafted with shoots of infected trees was greater than in those grafted with buds. This may be due to non-uniform distribution of *X. fastidiosa* inside plant tissues and greater amounts of xylem in shoots than in buds (Sanderlin and Melanson, 2006).

Some of the samples of field and grafted that showed high DAS-ELISA values were also positive in culturing assays. However, not all DAS-ELISA positive samples were positive by PCR or culturing.

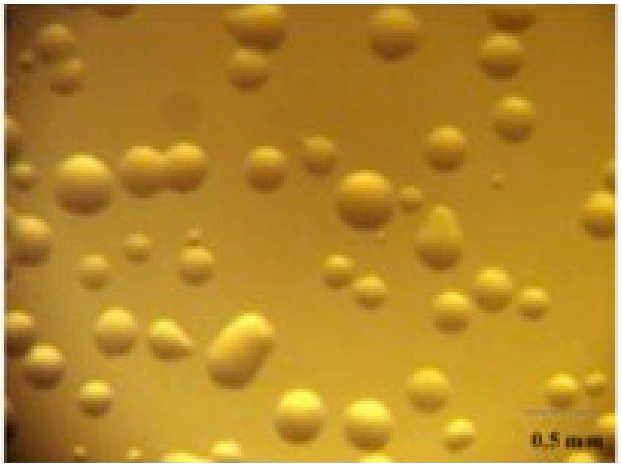

Figure 4. Colony morphology of a *Xylella fastidiosa* strain isolated from grapevine on PD3 agar medium after incubation at 28°C for 10 d. Scale bar = 0.5 mm.

Table 2. Double antibody sandwich-enzyme-linked immunosorbent assay (DAS- ELISA) results for *Xylella fastidiosa* from grapevine and almond samples (absorbance at 630 nm).

Ultimate absorbance	Positive control	Number of samples (positive/tested)		Bacterial cells isolated from (positive)	
		Grapevine (88/164)[a]	Almond (43/133)	Grapevine (8/8)	Almond (4/4)
Max[b]	2.63	2.04	1.33	2.21	2.3
Min	1.74	0.71	0.65	0.86	0.91
X+3SD (Negative control)		0.53	0.61	0.46	0.46

[a] Number of plant samples tested.
[b] Maximum and minimum absorbance at 630 nm samples tested, respectively.

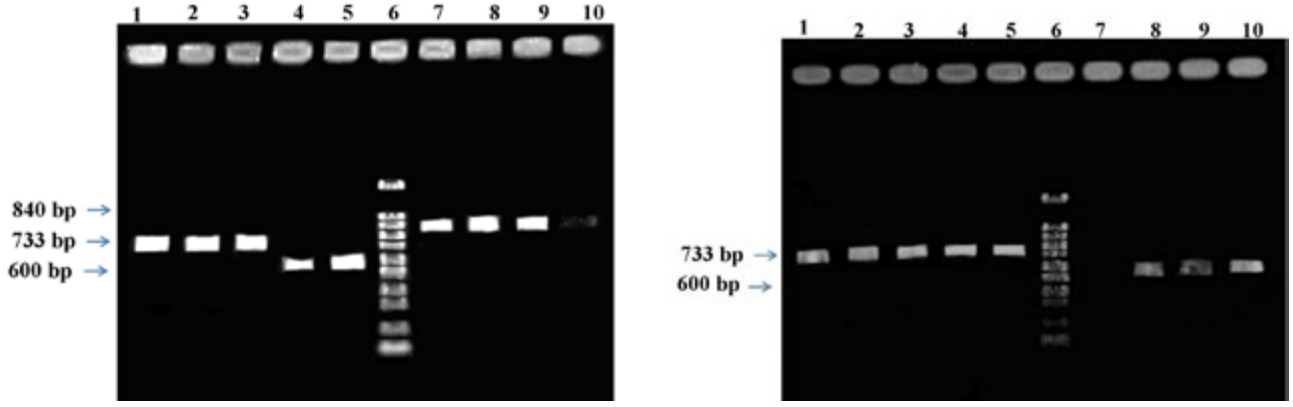

Figure 5. Gel electrophoresis pattern of PCR amplification products using *Xylella fastidiosa* specific primers. **Left**: PCR products from bacterial colonies isolated from almond samples amplified with primers RST31/RST33 (lanes 1, 2, 3), 272-1-int/272-2-int (lanes 4, 5), Dixon454fa/ Dixon1261rg (lanes 7, 8, 9); lane 10, PCR product from petioles of almond amplified with Dixon454fa/ Dixon1261rg primer pair; lane 6, 100 bp DNA ladder. **Right**: Lanes 1, 2, 3, 4 and 5, PCR products from bacterial colonies isolated from grapevine amplified with RST31/RST33 primer pair; lane 6, 100 bp ladder; lanes 7 and 8, PCR products from petioles of grapevine amplified with Dixon454fa/ Dixon1261rg (not amplification) and 272-1-int/272-2-int primer pairs, respectively; lanes 9 and 10, PCR products from bacterial colonies isolated from grapevine amplified with primer pair 272-1-int/272-2-int.

Similar results have been obtained by other investigators (Minsavage *et al.*, 1994, Costa *et al.*, 2004). This may be due to the nature of *X. fastidiosa* antibodies (Carbajal *et al*., 2004). Thus, DAS-ELISA results are not always reliable and may show false positives (Almeida *et al*., 2004; Carbajal *et al*., 2004). Although DAS-ELISA continues to be practical for screening large numbers of plant samples, additional analysis of positive plant samples with other methods is necessary to eliminate the possibility of false positives with DAS-ELISA, and to identify the strains of the pathogen that are present (Costa *et al*., 2004). However, in our studies, DAS-ELISA was as effective for detecting the pathogen in leaf tissue samples as the other methods used (grafting, PCR and culturing).

The failure of all methods to detect *X. fastidiosa* in a large number of symptomatic plants may be due to the fastidious nature of the bacterium or to low concentrations of the organism in the affected plants. Alternatively, the involvement of other biotic or abiotic factors causing similar symptoms should not be overlooked (Sherald, 2001). Leu and Su (1993) showed that the bacteria causing pear leaf scorch in Taiwan were serologically distinct from the PD

agent, and they are genetically very different (Su *et al.*, 2012).

The results of the present study confirm the occurrence of *X. fastidiosa* in almond and grapevine in Iran. The bacterium has been previously reported from Europe (Berisha *et al*., 1998) and recently detected in Italy (Saponari *et al.*, 2013). There is also a report on the presence of almond leaf scorch in India (Jindal and Sharma, 1987). To our knowledge, this is the first report of the pathogen in the Middle East and western Asia.

Transmission of the almond leaf scorch pathogen in orchards of Iran is likely to be by insect vectors, because the occurrence of the disease is scattered and symptoms usually start in an almond orchard on one or two branches over 8 years. Based on the available information, there is, as yet, no report of on the presence of potential vectors of *X. fastidiosa* in Iran.

This report is the first account of the occurrence of *X. fastidiosa* in Iran. It is of prime importance to investigate the mode of the pathogen transmission including insect vectors, detailed molecular phylogeny to better characterize *X. fastidiosa* isolates from Iran, and the possibility of disease induction by *X. fastidiosa* in other important plant species in this country.

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