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Detection and phylogenic relationships of *Xylella fastidiosa* strains from different host plants

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Abstract

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This study aimed to examine and analyze the distribution and phylogenetic relationships of the *Xylella fastidiosa* strain, a Gram-negative bacterium that impacts plant xylem and is responsible for various diseases in multiple plant hosts. Samples were collected from various plant hosts, including grapevines, pistachios, almonds, apples, pears, elms, cherries, and citrus fruits, across 12 provinces in Iran from 2019 to 2022. Disease incidence was detected in grapevine, pistachio, and almond orchards across six provinces, including Kerman, Yazd, Semnan, Chaharmahal and Bakhtiari, West Azarbaijan, and Qazvin. Tracking and diagnosis of the disease in plant extracts were carried out by ELISA and PCR methods using RST31/33 primer. The efficiency of conventional PCR in disease diagnosis was found to be less than 1%. In contrast, a nested PCR method using the ALSC491-F/ALSC491-R primer, which targets a smaller portion of the RNA polymerase sigma-70 factor gene, demonstrated 55% effectiveness in detecting DNA extraction from plants. Additionally, the common FD2/RP1 and specific XF1-XF6 primers were used to amplify the 16S rDNA gene in a limited number of samples. Our results from nested PCR analysis detected *X. fastidiosa* as the causative agent of pistachio and grapevine Pierce disease. Furthermore, *X. fastidiosa* subsp. *fastidiosa* infection was detected in almond and pistachio hosts.

Keywords: almond; Grapevine; *Xylella fastidiosa*; pistachios; phylogenic; nested PCR

Introduction

Xylella fastidiosa is a Gram-negative bacterium that is found exclusively in plant xylems, causing various diseases in a wide range of plant hosts (Wells et al., 1987). It was first identified as the causative agent of Pierce's disease in the grapevines of California vineyards (Giménez-Romero et al., 2022). However, it was not possible to culture this pathogen until the late 1970s (Davis et al., 1978). Currently, this bacterium is considered one of the top 10 plant pathogens, though ongoing studies continue to shed light on its pathogenic characteristics (Mansfield et al., 2012). Recent studies have identified more than 600 plant species, particularly perennial species, as the host range of this bacterium, though not all *X. fastidiosa* species are necessarily pathogenic. Some of them merely colonize plant tissue and act as endophytes (Chatterjee et al., 2008), sometimes exhibiting commensalism. The latter case is true for most of the hosts, and that's why these bacterial strains are considered benign commensals (Castro et al., 2021). Nevertheless, some *X. fastidiosa*

species infect a wide range of plant species, leading to the disease and death of host plants (Cornara et al., 2019). Currently, the risk of pathogenicity is extremely high because of the diversity in subspecies, crops, as well as ecological and ornamental landscapes found worldwide (Castro et al., 2021; Ahmadi, 2022; Kheyrodin et al., 2022; Salehi-Sardoei et al., 2022). Some of the known diseases caused by this bacterium include Pierce's disease of grapevines (Davis et al., 1978), almond leaf scorch (Mircetich et al., 1976), alfalfa dwarf disease (Wells et al., 1987), citrus variegated chlorosis (Chang et al., 1993), olive quick decline syndrome (Barnard et al., 1998), and leaf scorch in a number of plant hosts including almond (Leu et al., 1993), oleander leaf scorch, olive quick decline syndrome (Luvisi et al., 2017), and pistachio leaf scorch (Amanifar et al., 2019).

Based on a polyphasic approach (Su et al., 2016), a novel species *Xylella taiwanensis* (*Xt*) causing pear leaf scorch disease was proposed. Subsequently, all recognized *Xylella* spp. were divided into two speciesnamly *X. fastidiosa (Xf)* and *X. taiwanensis (Xt)*. The *Xf* species itself has the following 6 subspecies: 1) *X. fastidiosa* subsp. *fastidiosa,* the pathogen of grapevines, citrus, coffee, alfalfa, almond, maple, Nerium oleander, rosemary, etc. (Buzombo et al., 2006); 2) the *X. fastidiosa* subsp. *sandyi*, infecting the Nerium oleander as the host (Yuan et al., 2010); 3) the *X. fastidiosa* subsp. *Multiplex,* the pathogen of almond, plum, peach, apricot, and oak hosts hosts (Hernandez-Martinez et al., 2006); 4) the *X. fastidiosa* subsp. *tashke* the pathogen of the ornamental Chitalpa tree (Randall et al., 2009); 5) *X. fastidiosa* subsp. *pauca,* the disease agent of citrus hosts (CVC), olive quick decline syndrome, coffee, Nerium oleander and almond (de Souza et al., 2020), and 6) *X. fastidiosa* subsp.*morus*, as the cause of white mulberry, red mulberry, heavenly bamboo, and Nandina plants diseases (Nunney et al., 2014). There are also reports available on the synergistic effect of *X. fastidiosa* subsp. *pauca*, with living and non-living agents. For example, in the Apulia region in southern Italy, the interaction and association of *Xf* subspecies *pauca* strain CoDiRO with pathogenic fungi *Phaeoacremonium* spp. has caused an outbreak of "olive quick decline syndrome" (OQDS) (Cariddi et al., 2014).

Nevertheless, the potential synergistic effect of microorganisms other than *X. fastidiosa* subsp. *pauca* cannot be excluded (Scortichini et al., 2020). In this respect, OQDS has been reported in Brazil (Safady et al., 2019) and Jordan (AbuObeid et al., 2020). Except for America, where this disease is endemic, *X. f.* subsp. *pauca* (*Xfp*), *X. fastidiosa* subsp. *multiplex* (*Xfm*), and *X. fastidiosa* subsp. *fastidiosa* (*Xff*) have been identified in other parts of the world including Europe and Taiwan (Jacques et al., 2016; Su et al., 2016). In Iran, the presence of *X. fastidiosa* subsp. has been reported in grapevine, almond (Amanifar et al., 2014) and pistachio orchards (Amanifar et al., 2019).

Due to the symptoms of *X. fastidiosa* closely resembling those of drought and salinity stress, accurate diagnosis of this bacterial strain is crucial. This research, conducted over the past three years across various provinces of Iran, traced the disease agent from different hosts in multiple regions. Despite utilizing standard high-quality culture media, the bacterial isolations across various media yielded disappointing results. Moreover, direct PCR method (conventional PCR) showed low efficiency in disease diagnosis. Therefore, from the beginning, two primer pairs, including RST31/33 (Minsavage et al., 1994) and ALSC491 (This study), which was designed based on the inner part of the RST31/33 primer pair, were used in the nested PCR method. Despite the presence of two earlier reports from Iran (Amanifar et al., 2019) the dimensions of this issue had not been investigated at this level a fact which has made the implementation of this research quite necessary.

Accurate identification of the disease agent and its plant hosts is crucial for classification, genetic characterization, management, and disease quarantine. Additionally, identifying contamination centers is of utmost importance in this context (Azevedo et al., 2016). Given the history of the disease in Iran, it was anticipated that the other objectives of this study would be achieved quickly. However, this was not the case in practice. Therefore, the study aimed to track and detect the *X. fastidiosa* strain, the disease agent, in various plant hosts across 12 provinces of Iran over a period of three years.

Material and Methods

Sample collection and characterization of X. fastidiosa infection

Between 2019 and 2022, more than 330 samples were collected from various hosts, including grapevines, pistachios, almonds, apples, pears, elms, cherries, Plantanus, and citrus fruits. Though sampling was primarily conducted by observing visible disease symptoms, symptom-free hosts were also collected from plants in suspicious areas. Sampling took place in 12 provinces of Iran, including Kerman, Qazvin, Yazd, Tehran, Semnan, Fars, East Azerbaijan, West Azerbaijan, Mazandaran, Chaharmahal and Bakhtiari, Razavi Khorasan, and Kurdistan (Figures 1 and 2). Plant samples were collected primarily from fresh branches and leaves. The labeled samples were promptly transferred to the laboratory in plastic bags and stored in an ice container for isolation on specific selective culture media.

Fig. 1. A) Symptoms of leaf scorch on grapevine as the host, B) Irregular immaturity symptoms in infected branches of grapevines, C) Symptoms of leaf scorch on pistachio tree, and D) Symptoms of branch scorch on pistachio tree
Source: Authors' own elaboration

Fig. 2. Sample collections suspected from 12 provinces of Iran
Source: https://www.ecoi.net/en/file/thumb/2073790.jpj

The assessment of disease incidence

The assessment of disease incidence was performed based on the following formula;

Disease incidence (DI) = number of infected plants × 100/total number of plants assessed.

Cultivation of plant samples on selective culture media

The specific culture media which were used for cultivation of plant samples included BCYE, PWG, PW, and PD2 media (Yao et al., 2023). To prepare the sample suspension, 0.5 g of sample was collected from the petiole and main leaf veins exhibiting typical symptoms of the disease. The samples were subsequently washed with water, dried, and disinfected in a 5% sodium hypochlorite solution for 3 minutes. It was then placed in a 70% ethanol solution for 3 minutes. Finally, it was washed in sterile distilled water for 5 minutes, repeating this process three times. After culturing the prepared samples on selective media, the plates were incubated at 28°C in the dark. Fast-growing colonies were excluded as secondary factors. From the second week onward, cultures were observed daily for colony formation. In parallel with the cultivation of plant samples, they were also used for PCR and ELISA analysis.

DAS-ELISA analysis

This method was used to diagnose grapevine samples with typical pierce disease symptoms collected from West Azerbaijan Province. The double antibody sandwich ELISA (DAS-ELISA) procedure was performed on samples, according to the protocol described by Sutula et al. (1986). The presence of *X. fastidiosa* was verified using ELISA plates coated with 100 µl of 1: 1,000 dilutions of related IgGs (Bioreba Inc, Switzerland) in coating carbonate buffer. The samples were ground in an extraction buffer (PBS) (1:5 wt/vol) [3 mM KCl, 3 mM NaN3, 8 mM Na_2HPO_4, 1 mM NaH2PO4, and 0.13 M NaCl containing 2% polyvinyl pyrrolidone (PVP)-24,000 and 0.05% Tween 20, pH 7.4]. After incubation overnight at 4°C the reaction procedure indicated by the manufacturer was followed determining absorbance at 405 nm by the ELISA-reader (SpectraFluor) after 60 min. Each treatment was conducted in duplicate. The negative control, which included the buffer test and Samples with absorbance values higher or equal to three times the average of the negative samples were recorded as positive.

DNA extraction and PCR analysis

Whole plant (pistachio, grapevine and almond) DNA was extracted using cetyltrimethylammonium bromide (CTAB) extraction method as well as DNeasy plant Maxi kit (Cat. No.69104 QIAGEN, based on the manufacturer's guidelines. In the CTAB extraction method, 50 mg sample of the main veins of middle leaves from fresh plant tissue was crushed with liquid nitrogen and subsequently mixed with 5 mL of CTAB buffer (pH 8.0). PCR amplification was performed using four pairs of primers including RST 31/33 primer pairs (Minsavage et al., 1994) to amplify the RNA-Pol sigma-70 factor gene, our designed ALSC491-F/ALSC491-R primer pairs to amplify a smaller part of the RNA-Pol sigma-70 factor gene, FD2/RP1 primer pairs to amplify 16S ribosomal gene, and XF1-XF6 (Weisburg et al., 1991) primer pairs to amplify 16S rDNA gene (Table 1).

The reaction mixture for PCR amplification was included 50-100 ng of DNA, 6 µL of master mix (2 × Premix), 5 µL of nuclease-free water, and 1 µL of each primer (0.1 µM) in a total volume of 13 µL. The amplification program for the RNA-Pol sigma-70 factor gene included an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 8 minutes. In addition, for the amplification of 16S ribosomal DNA, PCR program was set to initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 8 min.. In the nested PCR, 1 µL of the primary PCR product was diluted and homogenized with 19 µL of nuclease-free water in order to be used as DNA template. The reaction mixture for nested PCR amplification involved 1 µL of DNA template solution, 6 µL of the master mix (2 × Premix), (EmeralAmpMaxHS PCR primer Mix TaKaRa-RR330Q or Go Taq-Promega), 5 µL of nuclease-free water, and 1 µL of forward and Reverse Primers of ALSC491-F/ALSC491-R (0.1 µM) in the final volume 13 µL. The application program of the second stage was repeated as in the first stage. The PCR products were run on 1.2% agarose gel at 8 V for 45 minutes, and the specific band size was observed and photographed under UV light. The PCR products molecular weight was estimated using 1 kb DNA ladder (Fermentas, Vilnius, Lithuania). The sequencing of selected positive samples was performed with the same primers of the amplification by the Sanger method at Macrogene (Korea). After assembling, and alignment using ClustalX (36), the sequences obtained were compared with those available in the GeneBank database, using BLAST (version BLASTN 2.2.18) (National Center for Biotechnology Information, Bethesda, MD, USA). Phylogenetic analyses were done with the RNA-Pol sigma-70 factor gene from selected *X. fastidiosa* strains to verify their relationships to the strains detected. Two sequences of *Xylella tiwanensis complex* was included as an out-group and phylogenetic relationships were obtained using the Neighbor-Joining method (37), with 1,000 replicates. The Maximum Composite Likelihood method in MEGA7 (38) was used to compute the evolutionary distances.

Results

Sample collection and characterization of X. fastidiosa infection

Recent studies on *Xylella fastidiosa* plant hosts have revealed that grapevine, pistachio, and almond plants are the most susceptible to bacterial infections. The highest incidence of infection was observed in six Iranian provinces, with Kerman leading at 35%, followed by Qazvin (28%), Yazd (18%), West Azerbaijan (23%), Semnan (9%), and Chaharmahal and Bakhtiari (6%). These findings highlight the significant impact of *X. fastidiosa* on economically important crops in Iran. The bacterium's ability to infect multiple host species poses a serious threat to agricultural production in these regions. Early detection and implementation of effective control measures are crucial to mitigate the spread of *X. fastidiosa* and minimize its economic impact on affected crops (Table 2).

Table 1. List of the specific primers used for detection and differentiation of *X. fastidiosa* strains

Primer type	Sequence (5' to 3')	Target gene	Amplicon size (bp)	Reference
FD2/RP1	5'-AGAGTTTGATCATGGCTCAG-3' 5'- ACGGTTACCTTGTTACGACTT-3'	16S ribosomal DNA	1400	(Firrrao and Bazzi.1994)
XF1-XF6	5'-CAGCACATTGGTAGTAATAC-3' 5'-ACTAGGTATTAACCAATTGC-3'	16S ribosomal DNA	404	(Weisburg *et al*.1991)
RST31/ RST33	5'-GCGTTAATTTTCGAAGTGATTCGATTGC-3' 5'- CACCATTCGTATCCCGGTG-3'	RNA polymerase gene 70S sigma	733	(Minsavage *et al*.1994)
ALSC491-F ALSC491-R	5'-AATGTTGCAGCAGTTTGGTCG-3' 5'-TTGCCCTACACGTGATTAAGCAC-3'	RNA polymerase gene 70S sigma	480	This study

Source: Authors' own elaboration

Isolations of samples on specific culture media

Despite three years of persistent efforts, attempts to isolate *Xylella fastidiosa* bacteria on various selective culture media, including BCYE, PWG, PW, and PD2, was unsuccessful. No *Xf* bacterial strains were isolated during this extensive period of research. This outcome highlights the challenges associated with culturing and isolating this particular bacterium, underscoring the need for alternative or improved isolation techniques in future studies.

DAS-ELISA method

This method was used to diagnose grapevine samples collected from West Azerbaijan Province. Among the analyzed samples, two macerated plant samples encoded XF203 and XF198 with absorbance values of 1.023 and 1.041 at 405 nm, respectively, compared to the control and non-contaminated samples, had the highest XF contamination at 405 nm (Figure 3).

Table 2. Place, time and number of infected samples in different plant hosts surveyed during 2019–2022

Sampling Time	Sampling province	Host	Number of samples		% of infection
			Symptomatic	Asymptomatic	
2019, 2020 (Summer, Autumn)	Semnan	Grapevine	44	6	9%
2019, 2020 (Summer, Autumn)	Qazvin	Grapevine	41	3	28%
2019–2020 (Summer)	Khorasan Razavi	Grapevine	16	3	-
2018–2019 (Summer, Autumn)	West Azerbaijan	Grapevine	17	2	23%
2019 (Autumn)	East Azerbaijan	Grapevine	8	-	-
2019 (Summer)	Kurdistan	Grapevine	9	-	-
2019–2021 (Spring–Summer–Autumn)	Kerman	Pistachio	96	5	35%
2019–2021 (Spring–Summer–Autumn)	Yazd	Pistachio	54	6	18%
2020–2021 (Summer, Autumn)	Chaharmahal and Bakhtiari	Almond	23	3	6%
2018–2019 (Summer)	Fars	Almond	11	-	-
2018 (December)	Tehran	Plantanus	7	1	-
2019 (December)	Mazandaran	Citrus	6	1	-

Source: Authors' own elaboration

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	0.065	0.065	0.063	0.105	0.090	0.081	0.061	0.054	0.054	0.066	0.057	0.074
B	0.079	0.278	0.445	0.331	0.318	0.165	0.207	1.028	0.721	0.568	1.054	0.075
C	0.145	0.316	0.230	0.210	0.205	0.191	0.221	0.239	0.190	0.207	0.220	0.064
D	0.156	0.586	0.418	0.174	0.085	0.090	0.089	0.104	0.138	0.128	0.140	0.079
E	0.102	0.141	0.112	0.108	0.085	0.047	0.063	0.090	0.122	0.117	0.094	0.081
F	0.086	0.079	0.072	0.060	0.059	0.067	0.064	0.081	0.103	0.104	0.088	0.058
G	0.064	0.058	0.056	0.068	0.061	0.067	0.066	0.066	0.062	0.064	0.072	0.063
H	0.078	0.084	0.065	0.085	0.079	0.057	0.058	058	0.053	0.052	0.057	0.059

Fig. 3. DAS-ELSA analysis of grape samples with pierce disease symptoms collected from West Azerbaijan. Plant samples number 23 and 20 in row B (*Xf* 203, *Xf* 198) showed the highest *Xf* contamination at 405 nm, with optical absorption of 1.023 and 1.041, respectively

Source: Authors' own elaboration

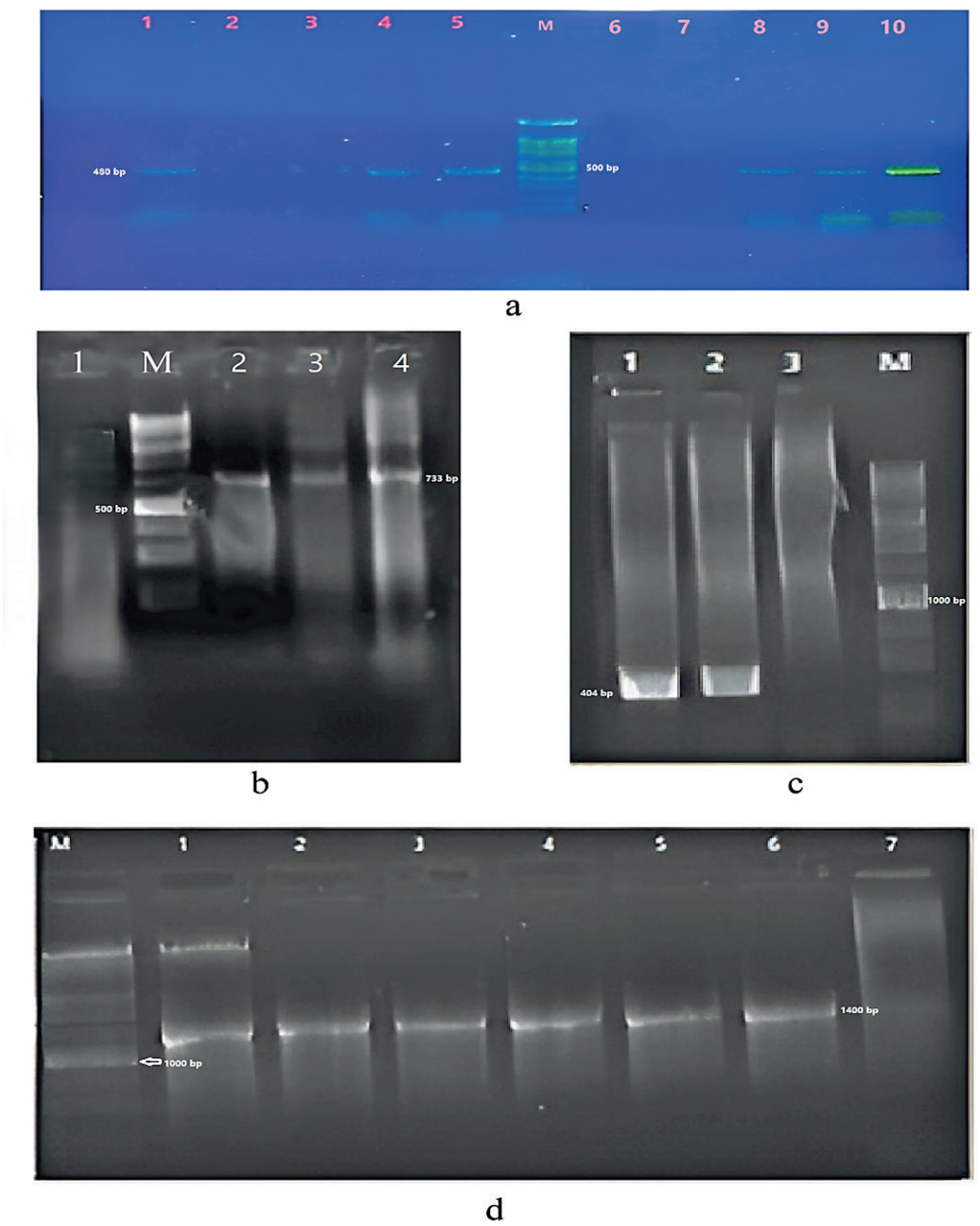

Fig. 4. Agarose gel Electrophoresis of Nested-PCR and PCR products. a) The products amplified using ALSC491-F/ ALSC491-R PCR primer pairs: pistachio plant extractions (lanes 1–5), Ladder (lane M), grapevine plant extractions (lanes 6,8,9,10), negative control (lane 7); b) The products amplified using RST31/33 primer pairs: negative control (lane 1), Ladder (lane M), grapevine plant extraction (lanes 2,3), pistachio plant extraction (lane 4); c) The products amplified using XF1/XF6 primer pairs: almond plant extraction (lane 1), pistachio plant extraction (lane 2), negative control (lane 3), Ladder (lane M); d) The products amplified using FD2/RP1 primer pairs: pistachio plant extractions (lanes 1–3), grapevine plant extractions (lanes 4–6), Ladder (lane M), negative control (lane 7).

Source: Authors' own elaboration

The PCR and Nested PCR-based analyses

Initial ELISA and PCR analysis of the 16S rDNA gene on several samples indicated the presence of *Xylella fastidiosa* infection in specimens displaying typical symptoms. To enhance the accuracy and speed of detecting the disease agent across various samples, a new primer pair was designed and implemented in the Nested PCR method. This advanced approach aimed to improve the sensitivity and specificity of *X. fastidiosa* detection, allowing for more reliable identification of infected plants. The Nested PCR technique, utilizing the newly designed primers, offered a more robust and efficient means of tracking the pathogen in diverse plant samples, potentially facilitating earlier diagnosis and more effective management strategies for *X. fastidiosa* infections.

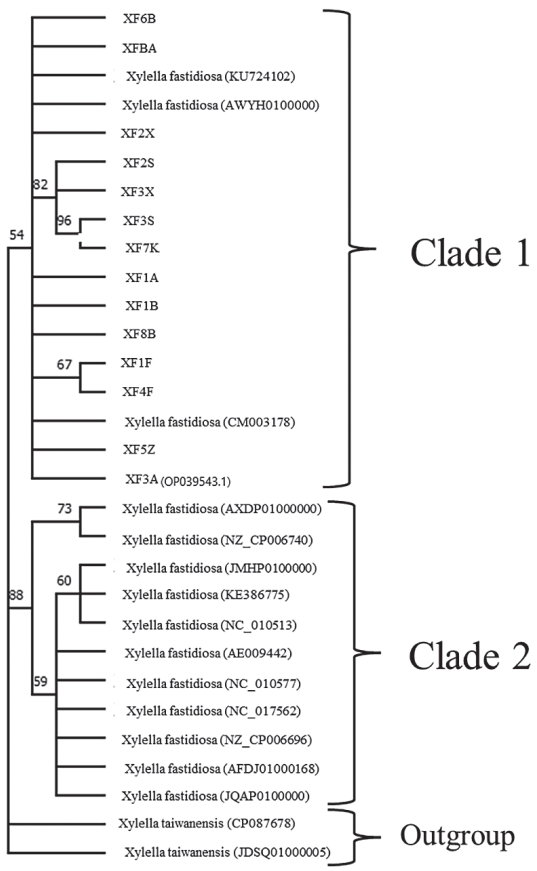

Fig. 5. Phylogenetic tree based on the rpoD gene sequences. Accession numbers provided by NCBI database are shown in parentheses. Bootstrap values (%) are mentioned at the nodes and obtained by repeating the analysis 1000 times. Two sequences of *Xylella tiwanensis complex* was included as an out-group.

Source: Authors' own elaboration

Overall, 14 plant DNA samples were amplified with ALSC491-F/ALSC491-R designed primer pairs, 3 samples with RST31/33 primer pairs, 6 samples with FD2/RP1 primer pairs and 2 samples with XF1-XF6 primer pairs. The length of the amplified fragments from PCR and Nested PCR analyses were 480 bps, 733 bps, 1400 bps, and 404 bps, respectively (Figure 4).

Construction of phylogenetic tree based on the rpoD gene sequences

A phylogenetic tree based on rpoD gene sequences was reconstructed using MEGA7.0.26 software with the Maximum Likelihood method. *Xylella taiwanensis* was used as an outgroup to reconstruct the phylogenetic relationships among 14 selected sequences of *X. fastidi*osa amplified by nested PCR Based on the phylogenetic tree, all Iranian samples in Clade I are allied with three *X. fastidiosa* sequences from GenBank. They formed a clade with 53 % support. The remaining *X. fastidiosa* strain from Genbank in clade II formed a well-supported clade with 88% percent probability, but the phylogenetic relationships among clade I (Iranian samples) and clade II were unresolved (Figure 4).

The sequences XF1F and XF4F showed the highest sequence similarity. These two sequences, together with eight others (XFBA, XF3A, XF1A, XF6B, XF2X, XF1B, XF8B, XF5Z), formed the largest clade with the standard isolate *X. fastidiosa* (CM003178). Four isolates, including XF2S, XF3S, XF3X, and XF7K, showed the least sequence similarity with the above-mentioned isolates. Overall, compared to the standard sequences, all the isolates belonged to the genus *Xylella* (Figure 5).

Discussion

The major symptoms of diseases caused by different strains of *X. fastidiosa* are often in the form of leaf scorch, irregular discoloration, 'tri-color' scorch, dwarf disease, early leaf fall, fruit shriveling, dieback, and plant death. The field investigations revealed that, In contrast to uninfected orchards, the contamination rate in infected areas range from 6% to 35%, so that the highest infection rate was related to pistachio host from Kerman province and the lowest infection belonged to almond host from Chaharmahal and Bakhtiari province. In this research, 330 plant samples from various plant hosts with similar disease symptoms were collected from different parts of Iran. Next, the presence of the disease agent was investigated by various methods, including culture isolation, ELISA,

and conventional and nested PCR methods. As mentioned earlier, pure bacterial culture was not obtained on selective culture media despite employing various standard culture media as well as suspension preparation methods (Anguita-Maeso et al., 2022; Krugner et al., 2022; Zecharia et al., 2022) during three consecutive years. The success rate of *X. fastidiosa* isolation on culture media has been reported as less than 5% in earlier studies (Amanifar et al., 2014). Apart from the isolation problems, the similarity of *Xf*- mediated disease symptoms with those caused by non-living factors added to the problems of tracking this disease agent. However, our results showed that *X. fastidiosa* can be detected in the samples of almonds, grapevines, and pistachios with specific disease symptoms. In addition, no disease agent was detected in other plant samples such as apple, pear, elm, cherry, and citrus trees. This shows that none of the other plant samples studied in the geography of Iran is susceptible to this disease. In addition, the transformation of bacteria into a pathogenic agent or endophyte depends on the defense mechanisms of different hosts against *X. fastidiosa* completely (Ingel et al., 2019). Since the preliminary results of the 16S rDNA gene ELISA and PCR analysis in several samples confirmed the presence of *X. fastidiosa* in samples showing typical disease symptoms, a new primer was designed for use in the nested PCR method to track the disease agent from various samples more accurately and quickly. Phylogeny analysis of 14 selected sequences amplified by nested PCR identified *X. fastidiosa* as the dominant strain in Iran. The two sequences XF1F and XF4F had the most sequence similarity, forming in an independent clade. These two sequences together with other sequences formed the largest cluster with about 88% sequence similarity with each other and with the standard isolate of *X. fastidios*a. Overall, all the isolates were belonged to species of *X.fastidiosa* (Aguilar-Granados et al., 2021; Castillo et al., 2021; Zicca et al., 2020). Implementation of such studies in our country Iran is important from two perspectives. Firstly, it is helpful to trace the disease agent using special primers that are designed based on the genome of native Iranian bacterial isolates; considering highly decisive role of the HOT START master mix in detection, as compared to conventional master mix. Secondly, given the risk of infection spreading among the wide host range of this bacterium all over Iran, the situation could be very dangerous in the near future. This is especially true if the contamination has already been observed and if multiple hosts and vectors have shown increased levels of contamination. Hence, it is crucial to detect pathogens in vectors and weeds in order to manage the disease properly (Shafaati et al., 2022).

Conclusion

Our results on *X. fastidiosa* disease incidence among different native plants in Iran showed the susceptibility of abundant pistachio orchards in Kerman and Yazd provinces, grapevines in Semnan, Qazvin, and West Azerbaijan provinces, and almond orchards in Chaharmahal and Bakhtiari, making these areas the inevitable disease hotspots across the country.

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Conflict of interest

The authors declare no conflict of interest in the reporting of these results. **J. B. –** conceptualization, methodology, writing – original draft**, N. H. –** supervision, review, data analysis, visualisation**, M. Gh. Z. –** supervision, data analysis, **B. H. –** methodology.

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