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Acquisition and transmission of *Grapevine fanleaf virus* (GFLV) by *Xiphinema index* and *Xiphinema italiae* (Longidoridae)

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Review Article

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Abstract

Grapevine fanleaf virus (GFLV) is one of the most severe virus diseases of grapevines, causing fanleaf degeneration that is transmitted by *Xiphinema index*. This paper aims to isolate *Xiphinema* species from Tunisian vineyard soil samples and assess their ability to acquire and transmit GFLV under natural and controlled conditions. Based on morphological and morphometric analyses, Tunisian dagger nematodes were identified as *X. index* and *Xiphinema italiae*. These results were confirmed with molecular identification tools using species-specific polymerase chain reaction primers. The total RNA of GFLV was extracted from specimens of *Xiphinema* and amplified based on real-time polymerase chain reaction using virus-specific primers. Our results showed that *X. index* could acquire and transmit the viral particles of GFLV. This nepovirus was not detected in *X. italiae*, under natural conditions; however, under controlled conditions, this nematode was able to successfully acquire and transmit the viral particles of GFLV.

Introduction

Grapevine (*Vitis* spp.) presents a great production of fruits with high economic value worldwide (Myles *et al.* 2011; This *et al.* 2006). Unfortunately, grapes have been susceptible to various pathogens, among them the dagger nematode, *Xiphinema index* (Hewitt *et al.* 1958). This migratory ectoparasitic nematode is considered a major pest in grape-growing countries in Mediterranean environments and temperate climates where grapevine grows (Hao *et al.* 2012; M'rabet Samaali *et al.* 2022). *Xiphinema index* is a nematode vector of nepovirus *Grapevine fanleaf virus* (GFLV), which is the causal agent of grapevine fanleaf degeneration disease (Belin *et al.* 2001; Hewitt *et al.* 1958). GFLV belongs to the genus Nepovirus of the family Comoviridae (Mayo & Robinson 1996). It is one of the most destructive grapevine viruses worldwide (Nourinezhad Zarghani *et al.* 2012). Upon infestation of host roots, the nematode perforates the cell wall with the stylet, followed by salivation and ingestion of the cell cytoplasm. This results in hypertrophy and necrosis at feeding sites. As a result, the root tips progressively swell and gradually transform into a terminal gall (Weischer & Wyss 1976; Wyss 1977). The transmission of GFLV by *X. index* is non-circulative and semi-persistent (Brown & Weischer 1998; Mc Farlane 2003; Taylor and Brown 1997). GFLV could be acquired from infested plants and transmitted to recipient plants within 1 to 10 min (Wyss 2000). GFLV does not replicate within the nematode and has no negative impact on the reproduction of *X. index* (Das & Raski 1969).

Other Longidorus species, such as *X. diversicaudatum* Thorne, *X. vuittenezi* (Luc et *al.*, 1964), and *X. italiae* Meyl, are known or suspected of being vectors of nepoviruses (Wang *et al.* 2002). Vector *Xiphinema* species could retain GFLV adhered to the surface of its cuticular lining in a specific region of the esophagus. Later, the nematode releases this nepovirus when its stylet is inserted into the parenchyma tissue of growing root tips (Demangeat *et al.* 2005; Wang *et al.* 2002; Van Ghelder *et al.*, 2015). Several methods, including immunoassays, reverse transcriptase polymerase chain reaction (RT-PCR), and real-time RT-PCR, are used to detect viruses in nematodes (Deng *et al.*, 2003; Osman *et al.* 2015). Grapevine chrome mosaic virus and *Arabis mosaic virus* were vectored by *X. vuittenezi* and *X. diversicaudatum*, respectively (Andret-Link *et al.* 2004; Digiaro *et al.* 2017; Van Ghelder *et al.* 2015). Besides, *X. italiae* has also been reported as a vector of GFLV (Cohn *et al.* 1970). Controversially, other studies have reported that *X. italiae* does not act as a specific vector of GFLV (Brown *et al.*, 1995; Catalano *et al.*, 1992; Martelli, 1975).

In this study, we aimed to detect GFLV from *Xiphinema* species. To this end, for each *Xiphinema* specimen, the posterior body half of the nematode was used for identification, and the anterior body half was used to detect GFLV. Furthermore, the ability of *X. index* and *X. italiae* to transmit GFLV when feeding on grapevine roots was studied.

Table 1. Geographical location of soil sampling sites

Region of sample collection	GPS coordinates	Number of soil samples collected
Rafraf	37° 11' 34.8756'' N 10° 11' 54.0348'' E	70
Grombalia	36° 18' 33.764" N 10° 24' 32.724" E	70
Takelsa	36° 47' 32.42"N 10° 37' 46.23"E	70
Mornag	36° 40' 43.10"N10° 17' 16.46"E	50

Material and methods

Soil sampling and nematode isolation

During the spring season, 260 rhizosphere soil samples were collected from four Tunisian grapevine-growing regions, including Rafraf, Grombalia, Takelsa, and Mornag (Table 1). Soil samples were collected at a depth of 30 – 80 cm from vineyards showing typical GFLV symptoms. Then, nematodes were extracted from each soil sample according to Cobb's decanting and sieving methods (Brown & Boag 1988; Flegg 1967). Nematode specimens were mounted on glass slides with sterile distilled water and visualized under a light microscope (Olympus C40, model SZX-ILLK200, Japan). The morphometric features reported by Luc and Dalmasso (1975), Siddiqi (1974), and Cohn (1977) served to identify *Xiphinema* species. The built-in software (Nikon Eclipse 50i) was used to measure these morphometric characters.

Nematode molecular identification

Twenty specimens each of *X. index* and *X. italiae* were cut transversely into two fragments using a sterilized scalpel. The posterior body half was transferred to a sterilized tube (1.5 mL) containing 20 µL of RNase-free water and stored at –20°C for ulterior molecular analysis. The anterior part was placed in a sterilized Eppendorf tube (1.5 mL) containing 10 µL of RNA later™ (Qiagen, Germany) and stored at –20°C for GFLV detection (Kulshrestha *et al.* 2005).

Total genomic DNA was extracted from each single nematode according to the modified protocol described by Wang et al. (2003). The posterior body half of the nematode was added to Eppendorf microtubes containing 2 µL of proteinase K (60 µg/mL) and 2 µL of Taq polymerase buffer 10X (Invitrogen). Then, the mixture was crushed gently using a sterilized cone and incubated for 1 h at 60° C and 10 min at 95°C. The obtained DNA lysate was stored at –20°C. For molecular identification of *Xiphinima* sp, the Internal Transcribed Spacer 1 (ITS1) sequences spanning the 18S and 5.8S ribosomal DNA were amplified using primer sets: S-ITS1 (5′-TGATTACGTCCCTGCCCTTTGTAC-3′) and A-ITS1 (5′-CG AGCCTAGTGATCCACCGCTTAG-3′). For the specific molecular identification of *X. index* and *X. italiae* species, specific PCR primers were used to amplify ITS1 gene sequences. To identify *X. index*, primers my-I27 (5′CGTTAGTACACACGGCGACGAA3′) and myA-ITS1 (5′CGAGCCTAGTGATCCACCGCTTAG3′) were used, whereas primer sets my-ITA26 (5′CCGTCGGTTTCGAAGGTC TG3′) and myA-ITS1 (5′CGAGCCTAGTGATCCACCGCTTA G3′) were used to identify *X. italiae*. These species-specific primers were newly designed.

Each PCR was performed in a 25 µL total volume, containing: 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.8 pmol for each primer, 0.5 units Taq polymerase (Bioron), 5 µL of the genomic DNA, and distilled water adjusted at 25 µL.

All PCR reactions were carried out using a thermocycler (Qiagen, Germany), programmed as follows: initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 45 s at 56°C (for my-V18/myAITS1) or 58°C (for S-ITS1/A-ITS1, and my-ITA26/ myAITS1) or 60°C (for my-I27/myAITS1), and an elongation at 72°C for 60 s. The final extension was performed at 72°C for 10 min. PCR products were purified and sequenced by Genome Express services (Neylan, France). The Netprimer software was used to determine the annealing temperature of each primer set.

Phylogenetic analysis

The obtained sequences were deposited in the GenBank database using the basic local alignment search tool (BLASTn) of the National Centre for Biotechnology Information and were aligned using the ClustalW software implemented in MEGA 7 (Tamura *et al.* 2011). The likelihood method was used to calculate the trees using the Jones-Taylor-Thonnton evolutionary model in 1000 bootstrap replications (Jones *et al.* 1992). The genetic distances among and within groups were determined by the same software. *Meloidogyne Luci* (LN713294) was used as the outgroup taxa.

Extraction of GFLV RNA from the nematode

Demangeat *et al.* (2005) reported that GFLV resides in the anterior region of the nematode. The GFLV-RNA extraction protocol from the anterior part of *Xiphinema* species was applied using the RNesay kit (Plant Mini Kit) and the QIAcube HT extractor (Qiagen, Germany), as described by Kulshrestha *et al.* (2005).

Synthesis of complementary DNA

To linearize RNA, a mixture of 10 µL of TNA, 1 µg/µL random primers (Invitrogen Corporation, USA), and 1.5 µL RNase-free water was heated at 95°C for 5 min. Next, RT was carried out by adding to the prepared mixture: enzyme buffer Fs 5xcc (Invitrogen, USA), 0.1M DTT (Invitrogen, USA), 10 mM dNTPs (Promega, USA), and 200 U/µL reverse transcriptase enzyme *Moloney Murine Leukemia Virus* M-MLV (Invitrogen, USA). Then, the mixture was incubated at 39°C for 60 min, followed by 70°C for 10 min in a thermocycler (Qiagen, Germany). The obtained DNAc were stored at –20°C.

PCR

PCR amplification was performed using a mixture of 2.5 µL of DNAc, Taq polymerase buffer 10X, 1.5 mM $MgCl_2$, 10 mM dNTPs, 10 µM of each primer, and 5 U/µL of Taq polymerase. GFLV-specific primers were used for RT-PCR, GT1076 (5′-CCAAGG ATTGCCAGGCA-3′) and GT1826 (5′-TCCATAGTGTCCC GTTCC-3′) (Saamali M'rabet et al. 2018). PCR cycling conditions used were as follows: an initial denaturation step at 94°C for 4 min, followed by denaturation at 94°C for 30 s (35 cycles), annealing at 57°C for 45 s and elongation at 72°C for 60 s. A final extension was performed at 72°C for 7 min. PCR products were separated on 0.5% TAE (Tris–acetic acid–EDTA) buffered agarose gel stained with ethidium bromide.

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Acquisition and transmission of GFLV by X. index *and* X. italiae *under controlled condition*

To evaluate the acquisition and the transmission of GFLV by *X. index* and *X. italiae*, for each nematode population, 20 *Muscat d'Alexandrie* grapevines (infected with GFLV), rooted in pots, were inoculated with nematode suspension (100 nematodes suspended in 10 mL of distilled water).

GFLV-carrying nematodes were obtained from grapevines showing GFLV symptoms. The presence of GFLV in grapevine samples was detected using DAS-ELISA (Double-Antibody Sandwich) according to the Bioreba protocol. DAS-ELISA was carried out using 1:1000 dilution of polyclonal antiserum anti-GFLV IgG. The optical density was measured at 405 nm using an automatic microplate reader (Multisacan Ascent, Labsystems USA). The positive signal threshold was set at twice the mean of healthy controls. A total of 20 healthy *Muscat d'Alexandrie* (free GFLV), taken from cuttings, were rooted in (2-L) pots and placed under greenhouse conditions.

The French population of *X. index* was reared on *Ficus carica* and used as a positive control. Fig plants were not found to be infected by *X. index*, which was not viruliferous (Esmenjaud *et al.* 1993). Fig cuttings were cultivated in black plastic bags (2 L) using sterilized soil as described by Demangeat *et al.* (2004) and maintained in greenhouse conditions. Healthy grapevine plants that were not inoculated with nematodes were used as a negative control.

Six weeks after inoculation, the infected grapevines were carefully removed from pots. Next, the soil in the pot of each grapevine-infected sample was used to retransplant the healthy *Muscat d'Alexandrie* (free GFLV). Six weeks after incubation, the transmission of the virus to these healthy plants by nematodes was checked using RT-PCR. The bioassay was performed under greenhouse conditions.

To evaluate the transmission of GFLV from nematodes to grapevine plants, the virus was detected in the root, leaf, and stem of each inoculated plant using RT-PCR. To evaluate the acquisition of GFLV, nematodes were extracted from each grapevine root, and re-identified molecularly using the PCR method as mentioned previously. Subsequently, for each nematode population (*X. index*-TN, *X. italiae*-TN, and *X. index*-FR), 20 specimens of nematode were used to detect the eventual presence of GFLV using RT-PCR.

Results

Molecular identification of nematodes

For DNA extracted from the posterior half of nematode, using primer pairs of my S-ITS1 and my A-ITS1, the approximate sizes of the amplified products obtained were 1.1 – 1.2 kbp. The findings indicated that the nematode populations used belong to the genus *Xiphinema* (Figure 1). The amplified PCR product, using specific primers (my-I27 and my-A-ITS), was 250 bp, corresponding to *X. index* (Figure 2A). Similarly, using specific primers (my ITA26 and my A-SIT), a PCR product of the expected size (900 bp) was obtained, corresponding to *X. italiae* (Figure 2B). The molecular identification was confirmed with morphological characterization.

Phylogenetic analyses

The phylogenetic analyses were performed in "MEGA7" to reconstruct the evolutionary history of gene sequences of the Tunisian

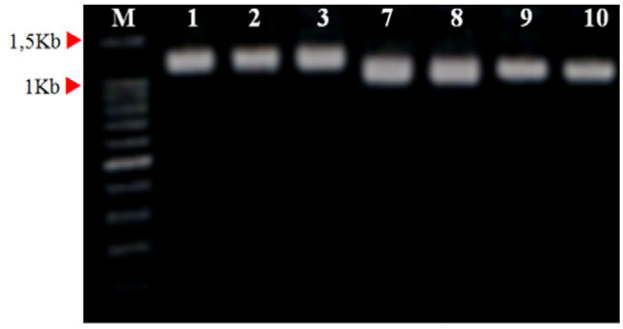

Figure 1. PCR products obtained using primers my A-ITS1 and my S-ITS1. M: 100 bp DNA marker. Lanes 1, 2, and 3 correspond to the amplification of the genome of the posterior part of three individuals of *X. index.* Lanes 7, 8, and 9 correspond to the amplification of the genome of the posterior part of three individuals of *X. italiae.* Lane 10 corresponds to the amplification of the genome of *X. index* French population.

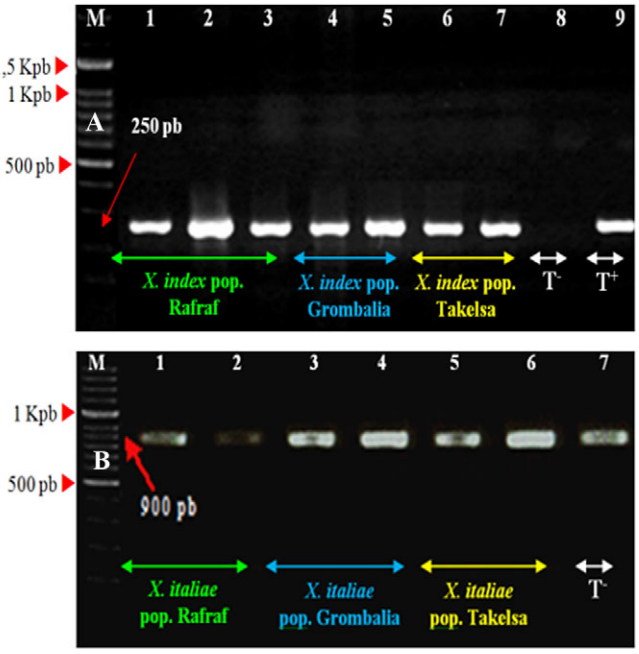

Figure 2. (A) Electrophoresis of the amplification product from DNA isolated from posterior parts of *X. index* of the three geographical regions (Rafraf, Grombalia, and Takelsa). M: 100 bp DNA marker. Lanes 1 – 2 and 3: *X. Index* from Rafraf. Lanes 4 – 5 *X. index* from Grombalia. Lanes 6 – 7: *X. index* from Takelsa. Lane 8: Negative control and Lane 9: positive control (*X. index* French population). (B) Electrophoresis of the amplification product from DNA isolated from posterior parts of *X. italiae* from the three geographical regions (Rafraf, Grombalia, and Takelsa). M: 100 bp DNA marker. Lanes 1 – 2: *X. Italiae* from Rafraf. Lanes 3 – 4 *X. italiae* from Grombalia. Lanes 5 – 6: *X. italiae* from Takelsa. Lane 7: Positive control.

populations of *X. index* as well as *X. italiae.* The Tunisian populations of *X. index* matched well with gene sequences deposited in GenBank, being 99% – 100% similar with the accessions AY430175, AJ437026, AY584243, HM921334, and JF37918, originating from Belgium, France, Italy, Spain, and Chile, respectively (Table 2). The phylogenetic tree showed that *X. index* isolates from different geographical regions are dispersed in the tree and do not form a distinct group, suggesting a low level of divergence among the different taxa (Figure 3). A clear separation between *X. index* isolates and *X. italiae* was observed, revealing that the two species of *Xiphinema* were genetically distinct. The Tunisian isolates of

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Table 2. Nucleotide identity (%) of the Tunisian sequences of *X. index* in comparison with the sequences deposited in GENBANK

	JF437918 Chile	AY584243 Italy	AY430175 Belgium	AJ437026 France	HG969306 Hungary	HG969305 Hungary	HM921334 Spain	MH427542 Tunisia	MH427547 Tunisia	MH427549 Tunisia	MH427548 Tunisia	MH427550 Tunisia
JF437918 Chile	ID											
AY584243 Italy	100	ID										
AY430175 Belgium	100	100	ID									
AJ437026 France	100	100	100	ID								
HG969306 Hungary	99	100	100	99	ID							
HG969305 Hungary	99	99	99	99	100	ID						
HM921334 Spain	99	99	99	100	99	99	ID					
MH427542 Tunisia	98	98	**100**	98	97	97	98	ID				
MH427547Tunisia	98	99	99	**99**	98	98	**99**	99	ID			
MH427549 Tunisia	97	97	97	98	97	97	97	96	99	ID		
MH427548 Tunisia	98	98	98	98	97	97	98	97	99	97	ID	
MH427550 Tunisia	**99**	**99**	**99**	**99**	98	98	**99**	99	99	98	99	ID

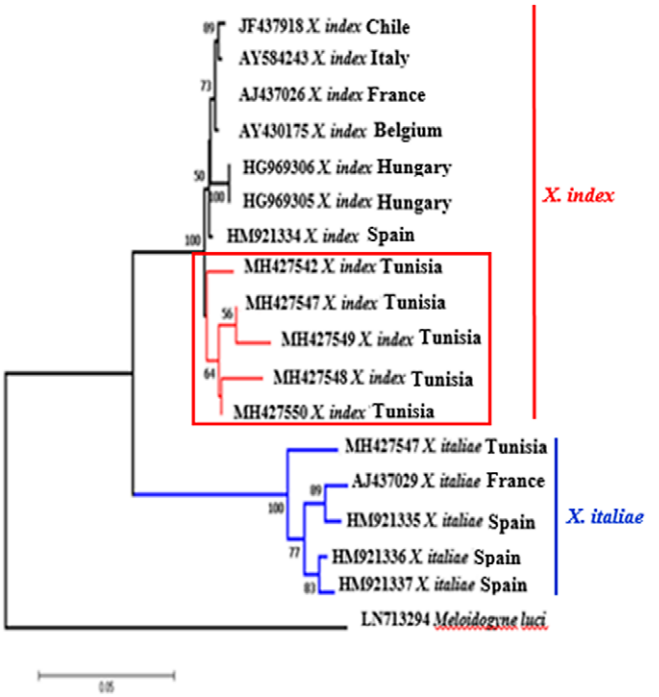

Figure 3. Phylogenetic tree linking the different Tunisian populations of *X. index* (red rectangle) according to the likelihood method. The Tunisian populations of *X. index* are grouped together in the same clade, illustrated in red with the other foreign populations. The foreign populations of *X. italiae* are grouped together in another clade, illustrated in blue. The bootstrap values determined by the MEGA 7 over 100 replications are indicated near the nodes. The 0.05 bar represents the genetic distance.

X. italiae showed 88% – 94% similarity to the Tunisian isolate (KX062698) (Guesmi-Mzoughi *et al.*, 2017), the Spanish isolates (KX244936 and KX244937), and the French isolate (AJ437029) (Table 3). The 12 Tunisian populations of *X. italiae* formed a monophyletic clade with each other. However, the Tunisian isolate (KX062698) formed a separate clade with the Spanish and French isolates, which was distinct from the other 12 Tunisian populations (Figure 4).

Acquisition of GFLV by Xiphinema species under natural conditions

Total RNA of GFLV was extracted from all *X. index* populations and amplified based on RT-PCR, using virus-specific primers to detect the virus in its vector. The expected gene fragments (750 bp) were visualized and electrophoresed in a 1.5% agarose gel. The detection of GFVL was recorded at three localities in Tunisia: Rafraf, Grombalia, and Takelsa, with prevalences of 32%, 12%, and 12%, respectively. However, GFVL was not detected in *X. italiae* (Table 4).

Acquisition and transmission of GFLV by X. index and X. italiae under controlled conditions

All grapevine plants inoculated with *X. index, X. italiae*, and the French population of *X. index,* were found to be 100% infected by GFLV. Both DAS-ELISA and RT-PCR successfully detected GFLV in root, leaf, and stem samples from each grapevine plant used in the experiment (Figure 5 and Figure 6). Thus, the acquisition and transmission of GFLV from healthy to infected grapevines were

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Table 3. Nucleotide identity (%) of the Tunisian sequences of *X. italiae* in comparison with the sequences deposited in GENBANK

	MK318835 Tunisia	**MK318831 Tunisia**	MK318830 Tunisia	**MK318829 Tunisia**	MK318834 Tunisia	**MK318828 Tunisia**	MK318833 Tunisia	MK318837 Tunisia	MK318827 Tunisia	**MK318836 Tunisia**	MK318832 Tunisia	**MK318826 Tunisia**	**KX062698 Tunisia**	**HM921335 Spain**	KX244936Spain	KX244935Spain	AJ437092 France
MK318835 Tunisia	ID																
MK318831 Tunisia	**93**	ID															
MK318830 Tunisia	**99**	**93**	ID														
MK318829 Tunisia	**99**	**93**	**99**	ID													
MK318834 Tunisia	**99**	94	98	98	ID												
MK318828 Tunisia	**99**	94	**99**	**99**	**99**	ID											
MK318833 Tunisia	95	94	94	95	94	95	ID										
MK318837 Tunisia	**99**	**93**	**99**	99	**99**	**99**	95	ID									
MK318827 Tunisia	94	94	94	94	94	95	96	94	ID								
MK318836 Tunisia	98	97	98	97	97	98	97	97	97	ID							
MK318832 Tunisia	**99**	**92**	98	98	**99**	**99**	94	**99**	94	97	ID						
MK318826 Tunisia	**99**	94	98	98	98	**99**	95	**99**	94	98	98	ID					
KX062698 Tunisia	92	**88**	92	**93**	92	92	91	**93**	90	**93**	92	**93**	ID				
HM921335 Spain	93	**88**	93	93	93	93	91	93	90	93	93	93	97	ID			
KX244936 Spain	93	**88**	93	93	93	93	92	93	91	**94**	93	93	97	100	ID		
KX244935 Spain	93	89	93	93	93	93	92	**94**	91	**94**	93	93	97	100	100	ID	
AJ437029 France	93	**88**	93	93	93	93	91	93	90	93	93	93	98	97	97	97	ID

Table 4. Molecular characterization of GFLV in the anterior part of the nematode isolated directly from the rhizosphere of vines naturally infected with GFLV

Region of Tunisia	Xiphinema index			Xiphinema italiae		
	Raf Raf	Grombalia	Takelsa	Raf Raf	Grombalia	Takelsa
Number of soil samples collected	25	25	25	25	25	25
RT-PCR results	08/25	03/25	3/25	0/25	0/25	0/25
% of acquisition	32	12	12	0	0	0

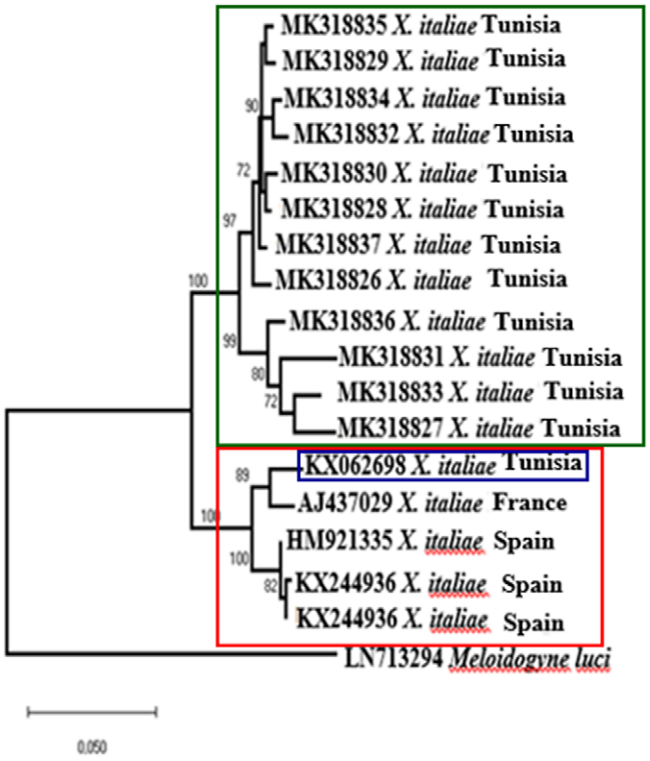

Figure 4. Phylogenetic tree linking Tunisian populations of *X. italiae* according to the likelihood method. The Tunisian populations of *X. italiae* are grouped together in a single clade illustrated in green. The foreign populations of *X. italiae* are grouped together in another clade illustrated in red. The Tunisian population of *X. italiae* population of the olive tree illustrated in blue belongs to the clade which gathers the foreign populations. The bootstrap values determined by the MEGA 7 over 100 replications are indicated near the nodes.

confirmed by both *X. index* and *X. italiae*, under controlled conditions (Figure 7).

Discussion

Xiphinema is one of the most varied genera of plant ectoparasitic nematodes, with more than 280 species, belonging to the family Longidoridae (Cai *et al.* 2020; Archidona-Yuste *et al.* 2016). Numerous species of *Xiphinema* are distributed worldwide, especially in different agricultural regions, such as *X. index* that is present in most if not all vineyards around the world, where it has been presumably introduced via grapevine plants from Mediterranean vineyards (Esmenjaud 2008, Handoo *et al.* 2016). Also, *X. italiae* has been broadly found in grapevine plots (Van Ghelder *al.* 2015). In our study, these dagger nematodes were isolated from Tunisian vineyards. *X. italiae* was the most prevalent species in the surveyed vineyard plots. This is consistent with previous studies indicating that *X. italiae* is among the most widely distributed species in the Mediterranean region (Dalmasso 1970; Gutiérrez-Gutiérrez *et al.* 2011; Martelli *et al.* 1966). *Xiphinema* species are characterized by substantial intra- and interspecific homogeneity of the morphometric characters used for species discrimination (Cai *et al.* 2020; Archidona-Yuste *et al.*, 2016). The Tunisian population of *X. italiae* matched the morphological identification by Luc and Dalmasso (1975) and Cohn (1977). Similarly, the morphometric characteristics of the Tunisian population of *X. index* are consistent with those of Luc and Dalmasso (1975) and Siddiqi (1974). Raski *et al.* (1983) and Nguyen *et al.* (2021) have reported that *X. index* males are rare and females reproduce parthenogenetically, which is consistent with our result. Similarly, for *X. italiae*, no male was found, suggesting a parthenogenetic reproduction of this species (Dalmasso & Younes 1969). The use of molecular tools based on

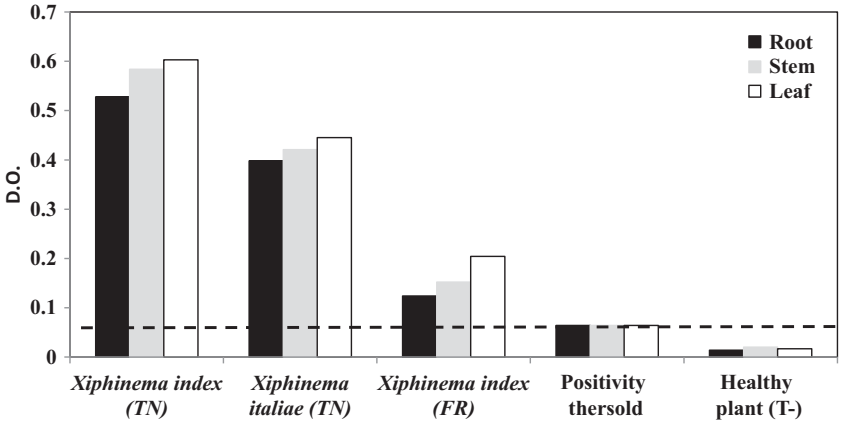

Figure 5. GFLV titer in root, stem, and leaf samples measured by DAS-ELISA. Optic density values (nm) are shown.

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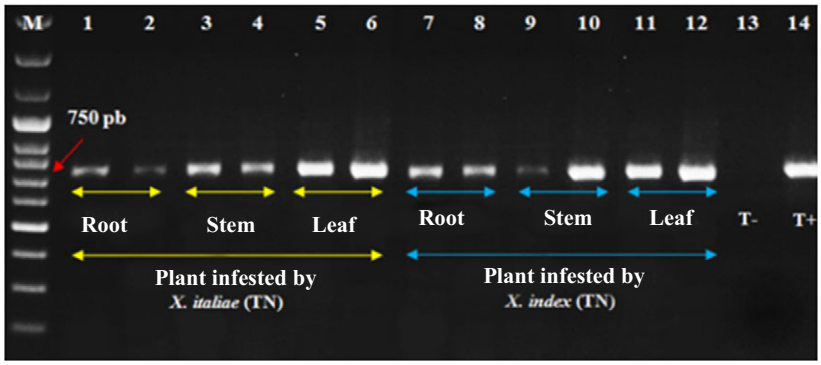

Figure 6. Agarose gel analysis of GFLV obtained by RT-PCR (using GT1076/GT1826 primers) from grapevine leaf samples that were infested with *X. index* and *X. italiae.* M: 100pb Marker (S304105, Bioron). T-: negative control. T+: positive control

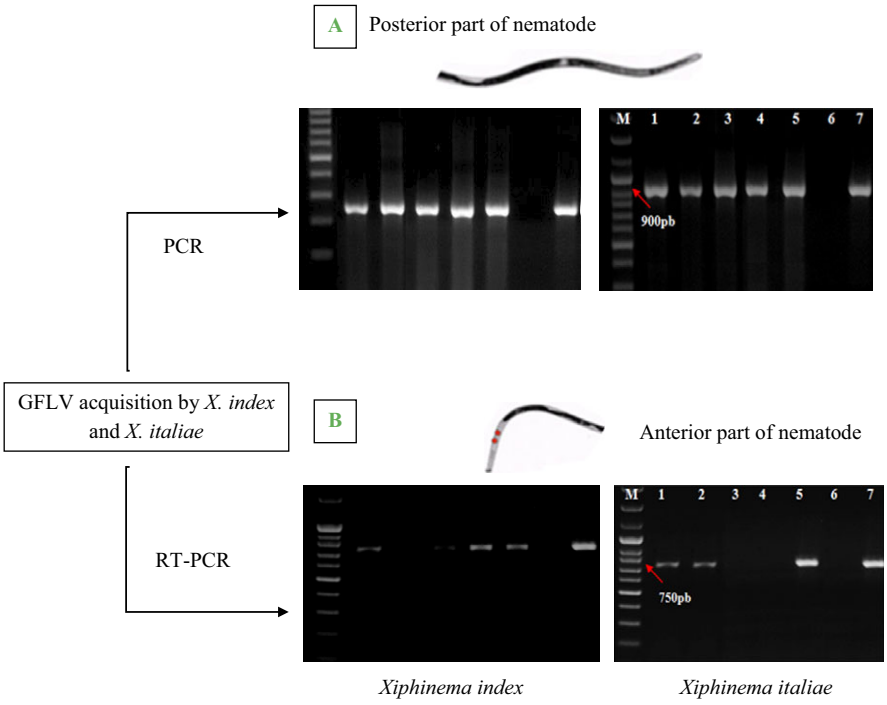

Figure 7. Visualization of PCR products obtained from *Xiphinema index* and *X. italiae* that were isolated from soil after transmission tests. M: marker size 100 bp (S304105, Bioron). Lanes 1 – 5 correspond to *X. index* Tunisian population. Lanes 6 – 7 correspond to the negative and positive controls, respectively. Lanes 1 –5 correspond to *X. italiae* Tunisian population. Lanes 6 – 7 correspond to the negative and positive controls, respectively. Molecular detection of GFLV from the anterior part of *X. index* was isolated from the soil after the transmission test. M: marker size 100 bp (S304105, Bioron). Lanes 1 – 5 correspond to the GFLV detected in the anterior part of the *X. index.* Lane 2 corresponds to the absence of GFLV in the anterior part of *X. index.* Lanes 6 – 7 correspond to the negative and positive controls, respectively (GFLV was isolated from the leaves). Lanes 1 – 2 and 5 correspond to the GFLV detected in the anterior part of *X. italiae.* Lanes 3 – 4 correspond to the absence of GFLV in the anterior part of *X. italiae.*

PCR methods has been applied for *Xiphinema* diagnostics because of their specificity and sensitivity compared with traditional methods (Oliveira *et al.* 2005; Wang *et al.* 2003). In our study, species-specific PCR primers, including my-I27/myAITS1, and my-ITA26/myAITS1 were designed for molecular identification of *X. index* and *X. italiae*, respectively. The phylogenetic analyses revealed that the Tunisian population of *X. index* is closely related to French, Spanish, and Italian populations.

On the other hand, *X. index* transmits GFLV, which is the most severe grapevine virus disease worldwide (Hewitt *et al.* 1958; Van Helden *et al.* 2011). The current study showed that the Tunisian populations of *X. index* are able to acquire and transmit the viral particles of GFLV. The transmission process is mediated by the ability of *X. index* to ingest GFLV particles from a virus source grapevine, retain virions at specific retention sites within its feeding apparatus, and subsequently infect a recipient vine by the release of virus particles from the retention sites (Demangeat *et al.* 2005; Schellenberger *et al.* 2011). Esmenjaud *et al.* (2013) reported that there was no variability in GFLV transmission between seven isofemal populations of *X. index* collected from five different countries with 87.5% – 96% efficiency, whereas other studies revealed differential transmission of nepoviruses associated with *longidorid* nematodes of different geographical locations (Brown & Trudgill 1983; Brown 1985, 1986; Taylor & Brown 1997). GFVL was not detected from Tunisian populations of *X. italiae* under natural conditions. Thus, GFLV could not be naturally vectored by *X. italiae.* However, under controlled conditions, GFLV virus particles were successfully acquired and transmitted by *X. index* and

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X. italiae. In areas where grapevines are grown, *X. italiae* is frequently observed. Its potential as a GFLV vector is controversial because the transmission reported for a Middle East population was not experimentally reproduced with any other population of this species (Catalano 1992; Cohn *et al.* 1970; Lamberti and Roca 1987). This association has never been confirmed by other studies. It is therefore very unlikely that it could be a specific vector of GFLV (Demangeat 2007; Martelli & Taylor 1990; Taylor & Brown 1997).

Brown and Weischer (1998) demonstrated that specific associations between nematodes and viruses are constantly evolving, possibly resulting in some viruses losing their vector transmissibility or some vectors losing their ability to transmit viruses, while concurrently new virus and vector associations are becoming established.

Conclusion

Xiphinema species have caused a serious problem in viticulture worldwide, particularly *X. index,* which is the main vector of GFLV. In conclusion, this paper revealed the morphometric and molecular characteristics of Tunisian populations of *X. index* and *X. italiae* compared with foreign populations. Additionally, this study reported that the population of *X. index* was capable of acquiring and transmitting the viral particles of GFLV. This nepovirus was not found to be naturally retained by *X. italiae.* However, under controlled conditions, GFLV was successfully acquired and transmitted by this dagger nematode. The acquisition and transmission of GFLV by *Xiphinema* species still need further studies notably in Tunisian grapevines.

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Ethical standard. This contains no studies with human participants or animals performed by any of the authors.

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