Nematode survey on grape and other important crops in Morocco

Abbad Andaloussi F.[1], Di Vito[2] M., De Luca[2] F. and Alaoui[3] Y.

1. Institut National de la Recherche Agronomique, BP 415 RP, Rabat, Morocco;
2. Istituto per la Protezione delle Piante, CNR, 70126 Bari, Italy.
3. Université Mohamed V- Agdal, Faculté des Sciences, Rabat, Morocco.

Abstract

A survey of phytoparasitic nematodes associated with grape and other important crops in Morocco was undertaken in 2004, 2005 and 2006. The root-knot nematodes, Meloidogyne incognita and M. javanica were very common on grape, peach, banana, common bean and pea. Xiphinema index, X. pachtaicum and Mesocriconema xenoplax were found on grape. Tylenchulus semipenetrans, Hemicycliophora arenaria and Xiphinema sp. were found on Citrus. Aphelenchoides ritzemabosi and A. fragariae were found on strawberry. Pratylenchus vulnus was found on peach and almond, and P. thornei was common on chickpea. Grapevine FanLeaf Virus (GFLV) was detected in association with X. index in four locations in Morocco.

Key words *: GFLV, grape, Morocco, phytoparasitic nematodes, survey.*

نيماتودا العنب ونباتات أخرى بالمغرب

عباد أندلوسي فؤاد، دي فيتو مورو، دي لوكا فرنسيسكا والعلوي ياسمينة

ملـخص

تم مسح ميداني خلال السنوات 2004/2005/2006 من أجل كشف أهم النيماتودا المتصلة بالعنب ونباتات أخرى بالمغرب. أدى هذا البحث إلى التعرف على النيملتودات التالية: نيماتودا التعقد الجدري *Meloidogyne incognita* و*M. javanica* كانت موجودة بكثرة في زراعات العنب، الخوخ، الجوز، الفاصوليا والبسلة. وتم وجود كذلك النيماتودا *Xiphinema index* ، *X. pachtaicum* و*Mesocriconema xeneplax* في زراعة العنب. النيماتودات *Tylenchulus semipenetrans* و*Hemicycliophora arenaria* و*X. sp* وجدت متصلة بزراعة الحمضيات. أما زراعة الفرولة فقد كانت مصابة بنيماتودات *Aphelenchoides ritzemabosi* و*A. fragaria* النيماتودا *Pratylenchus vulnus* كانت موجودة في حقول الخوخ واللوز أما P. thornei فهي منتشرة في حقول الحمص.
فيروس التواء ورق العنب(GFLV) تم العثور عليه مصاحب لنيماتودا *X.index* في أربعة مناطق.

الكلمات المفتاح :GFLV، العنب، المغرب، نيماتودا، مسح

Prospections sur les nématodes de la vigne et d'autres cultures au Maroc

Résumé

Des prospections ont été menées durant les années 2004, 2005 et 2006 en vue de l'identification des principaux phytonématodes associés à la vigne et d'autres cultures au Maroc. Les nématodes à galles, Meloidogyne incognita et M. javanica ont été très fréquents sur les cultures de vigne, pêcher, bananier, haricot et petit pois. Les nématodes Xiphinema indes, X. pachtaicum et Mesocriconema xenoplax ont été associés à la culture de la vigne. Tylenchulus semipenetrans, Hemicycliophora arenaria et Xiphinema sp. ont été trouvés dans les vergers d'agrumes. Aphelenchoides ritzemabosi et A. fragariae ont été associés au fraisier. Pratylenchus vulnus a été rencontré dans les vergers de pêcher et d'amandier, et P. thornei a été très commun au niveau des champs de pois chiche. Le virus de l'enroulement des feuilles de la vigne (GFLV) a été détecté en association avec X. index dans quatre sites.

Mots clés: GFLV, vigne, Maroc, nématodes phytoparasites, prospections.

Introduction

Phytoparasitic nematodes cause severe damage to grape and some other important crops such as vegetables, citrus, banana and stone fruits in many countries especially in the Mediterranean basin where the agriculture is very diversified. Root-knot nematodes (*Meloidogyne spp.*), lesion nematodes (*Pratylenchus spp.*), *Tylenchulus semipenetrans* and *Xiphinema* spp. are major pests in the area. The last group of nematodes assumes a particular importance because the *species X.* index can transmit Grapevine FanLeaf Virus (GFLV) to grape. However, information on the distribution and occurrence of this nematode in Morocco is limited. Therefore, a survey was conducted in some areas of the country during 2004-2006 to ascertain the nematodes associated with grape and other important crops.

Material and methods

i) Survey and morphological nematode identification

The survey, in the framework of the project "Studi sulla distribuzione, identificazione e dannosità dei nematodi del colture in Marocco ed utilizzo di marcatori per la identificazione rapida di questi parassiti" of Italian Foreign Minister "Programma Esecutivo Accordo Cooperazione Culturale, Scientifica e Tecnologica Marocco/ Italia" for the years 2004-2006, was undertaken mainly in the major grape areas and occasionally in other important crop areas of Morocco (14, 26 and 17 samples in 2004, 2005 and 2006, respectively) (Table I; Fig. 1). Root and soil samples were taken at the root system level from the different crops. In the case of strawberry, five more samples of aerial parts were collected. Nematodes were extracted from 500 cc soil samples by the Baermann funnel method, and from roots or aerial parts of the plants using the wet incubation method (Young, 1954). Nematodes in water suspension were then counted, fixed and preserved in 5% hot formalin solution. Nematodes destinated to identification were selected using a stereomicroscope and then transferred to water agar for temporary mounts (Esser, 1986) and measured under a light microscope with camera lucida. Perineal patterns were also prepared for the identification of root-knot nematodes (*Meloidogyne spp.*) (Hartman and Sasser, 1985).

ii) PFCP analysis for *Xiphinema* species identification

Nematodes were handpicked and placed individually in 3 µl of the lysis buffer (10mM-Tris-HCl pH 8.4, 50 mM KCl, 15 mM $MgCl_2$, 0,045% NP40, 0,045% Tween 20, 0.1% gelatine with 90 µg/ml proteinase K) and cut into small pieces by using a sterilized syringe needle under a dissecting microscope. The suspension was recovered and transferred into a cold 0.5 ml microcentrifuge tube. Samples were incubated at 60°C for 1 hour and then at 95°C for 10 minutes. The crude DNA extracted from each individual nema-

tode was directly amplified by diluting the sample to 100 µl such that it contained 0.2 mM of each dNTP, 20 pmols of each primer and 2.5 units of Taq DNA polymerase (Roche, Germany). Oligonucleotides used were the universal 18S-26S primers spanning from the 3'end of the 18S rDNA to the 5' end of the 26S rDNA and including the ITS1 and ITS2 regions and the 5.8S rDNA (Vrain et al., 1992).

The PCR conditions used for amplification were: an initial denaturation at 94°C for 2 minutes and then 35 cycles of 95°C/50 seconds, 55°C/50 seconds and 72°C/1minute 30 seconds followed by a final step at 72°C for 7 minutes. The size of amplification products was determined by comparison with the molecular marker Ladder 100 (Fermentas, St. Leon-Rot, Germany) after the electrophoresis of 10 µl on a 1% agarose gel.

The PCR products, containing the ITS region, were digested at 37°C overnight with the following restriction enzymes: Alu I, Dde I, Eco RI, Nde II, Pvu and Rsa I. The digested DNA fragments were loaded onto 2.5% agarose gel and visualized by ethidium bromide staining. All gel images were stored digitally.

iii) Virus identification on grape

Young leaves with viral symptoms, such as yellowings, were collected from different grape (*Vitis vinifera L.*) fields for direct processing in the laboratory. Leaves were crashed and the juice was used for GFLV identification according to the Enzyme Linked Immunosorbent Assay test (ELISA) with the Bioreba kit.

Results

i) Survey and morphological nematode identification

A total of sixty one soil and root samples were collected in fields of grape and other important crops, and five more aerial plant part samples of strawberry (*Fragaria vesca L.*) (Table I). Tree root-knot nematode species were identified and out of them the most common species was *Meloidogyne incognita* (Kofoid et White) Chitwood which occurred in eleven fields. One sample was infested by *M. javanica* (Treub) Chitwood. Four soil samples collected at Ain Taoujdate were infested by the nematode vector of GLFV, *Xiphinema index* Thorne et Allen and five soil samples at Bouznika and Khemisset were infested by X. *pachtaicum* (Tulaganov) Kirjanova. Two, one and two soil samples at Kenitra, Bouznika and Khemisset, respectively, were infested by *Mesocriconema xenoplax* (Raski) Luc et Raski.

Eleven soil and root samples were collected in fields of banana (*Musa paradisiaca L.*) at Boulaouane and Manasra (Table I). All of them were infested by the root-knot nematode *M. javanica*.

A total of eight soil and root samples were collected in fields of orange (*Citrus sinensis* (L.) Osbeck.) at El Menzeh. All, five and five samples were infested by *Tylenchulus se-*

mipenetrans Cobb, *Hemicycliophora arenaria* Raski and *Xiphinema sp.*, respectively. One sample per field was collected in one field of each of the crops, almond (*Amygdalus communis* L.), peach (*Prunus persica* (L.) Batsch.) and fig (*Ficus carica* L.) at Ain Taoujdate. Almond was infested by *Pratylenchus vulnus* Allen et Jensen, peach by *P. vulnus* and *M. xenoplax*, and fig by *M. incognita* and *X. index*.

In annual crops, one root sample each was collected from a field of pea (*Pisum sativum L.*) and a field of common bean (*Phaseolus vulgaris* L.) at Manasra, and both were infested by M. *javanica*.

Five aerial plant part and root samples of strawberry (*Fragaria vesca* L.) were collected at Manasra. Out of them, two, three and three samples were infested by *Aphelenchoides* ritzemabosi (Schwartz) Steiner, A. *fragariae* (Ritzema Bos) Christie and *M. hapla* Chitwood, respectively.

Finally, one root sample of chickpea (*Cicer arietinum* L.) collected at Marchouch was infested by P. *thornei* Sher et Allen.

Table I. Nematodes in the soil and root samples collected during the survey in Morocco from 2004 to 2006;

(The last column shows the results of the GFLV detection tests in the grape fields)

Location	Crop	Samples collected	Samples infested with*													GFLV** (ELISA)
			Ar	Af	Ha	Mes	M	Mj	Mh	Pt	Pv	Ts	Xi	Xp	Xsp	
Boulaouane	Grape	2				1	1	1								-
Oulad Frej	"	3											1			+
Bouznika	"	3				1								2		-
Skhirat	"	1														-
Marchouch	Chickpea	1								1						
Khemisset	"	8				2	3							3		
El Hajeb	Grape	2						1					1			+
Meknes	Grape	2						1					1			+
Ain Taoujdate	Grape	4					4						4			+
	Almond	1									1					
	Peach	1				1	1				1					
	Fig	1					1						1			
Sidi Kacem	Grape	4					2						2			-
Kenitra	Grape	6				2	2									-
El Menzeh	Orange	8			5							8			5	
Manasra	Strawberry	5	2	3					3							
	Pea	1						1								
	Banana	7						7								
	Common bean	1						1								
Total		61	2	3	5	7	13	12	3	1	2	8	10	5	5	

*Ar = *Aphelenchoides ritzemabosi*, Af = *A. fragariae*, Ha = *Hemicycliophora arenaria*, Mes = *Mesocriconema xenoplax*, Mi = *Meloidogyne incognita*, Mj = *M. javanica*, Mh = *M. hapla*, Pt = *Pratylenchus thornei*, Pv = *P. vulnus*, Ts = *Tylenchulus semipenetrans*, Xi = *Xiphinema index*, Xp = *X. pachtaicum*, Xsp = *Xiphinema sp.*

** GFLV ELISA detection: + positive; - negative.

ii) RFCP analysis for *Xiphinema* species identification

Three samples, one grape sample from Sidi Kacem (n° 427) one sample from grape (n°433) and one sample from fig (n°435) from two different sites in Ain Taoujdate, were characterized at the molecular level. The samples from grape from Sidi Kacem (n°427) and from fig from Ain Taoujdate (n°435) exhibited mixed populations of *Xiphinema* and three different size classes of amplified fragments were obtained.

The populations from the samples from grape from Sidi Kacem (n°427) and from fig from Ain Taoujdate (n°435) amplified two fragments of 1.8 kb and 1.4 kb, and 2.0 kb and 1.4 kb, respectively. The population from grape from Ain Taoujdate (n°433) amplified a single product of 2.0 kb. The ITS-RFLP analyses of the 2.0 kb fragments, amplified in all three populations, produced identical restriction patterns. The digestion of the 1.4 kb and 1.8 kb fragments produced peculiar restriction patterns (Fig. 1).

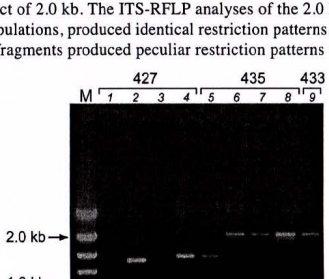

Fig. 1. Electrophoresis of the amplified products from DNA isolated from individual nematode. (M=100 bp size marker; lanes: 1 and 3 specimens belonging to population n°427 from grape sample from Sidi Kacem, *Xiphinema pachtaicum*; lanes: 2, 4 and 5 specimens belonging to populations n°427 from grape sample from Sidi Kacem and n°435 from fig sample from Ain Taoujdate, *Xiphinema sp.*; and lanes 6, 7, 8 and 9 specimens belonging to population n°433 and n°435 from grape and fig sample from Ain Taoujdate respectively, *X. index*.

According to the size of the amplified fragments and the RFLP results, the specimens showing the amplified products of 2.0 kb, could be easily identified as *X. index* when compared with the patterns already determined in our laboratory (De Luca et al., unpublished). For those specimens that amplified the 1.8 kb fragments, the restriction with the enzymes Alu I, Bam HI and Rsa I produced patterns similar to those reported for *X. pachtaicum* (Ye, 2003). Because the size of the amplicons and the restriction patterns corresponded to those of *X. pachtaicum*, the population from Morocco could be identified as *X. pachtaicum* too. The specimens with the 1.4 kb fragments generated two

classes of restriction patterns that did not correspond to known patterns already reported in literature.

iii) Virus identification on grape

Typical symptoms of GFLV were observed on leaves of grapes in the regions of Oulad Frej, Meknes, Ain Taoujdate and El Hajeb. The ELISA test confirmed the presence of GFLV virus in samples collected from those regions. According to nematode identification, *X. index* was also associated with virus presence in the tissue of leaves of grape (Table I).

Discussion and conclusions

The survey confirms that several root-knot nematodes (*Meloidogyne* spp.), *T. semipenetrans and Xiphinema* spp. are important nematodes of grape, citrus, banana and other major crops of Morocco. Among them, *M. incognita* and *M. javanica* are the most common in sandy soil fields of grape, banana, common bean and pea. They cause severe damage to banana, pea (Photo 1) and common bean (Photo 2) (Sasser, 1979; Sikora et al., 2005). *Xiphinema* index, the nematode vector of GFLV on grape is apparently very common and associated with the virus symptoms (Photo 3) especially in fields with clay or clay loam soil and the roots infested plants showed the typical root galls (Photo 4). In fact, this nematode was never found in the sandy soil fields. The presence of GFLV was confirmed by ELISA test in four samples. *Xiphinema pachtaicum* is also very common on grape in Morocco and our results confirmed that this nematode is wide spread in the Mediterranean Basin (Brown and Trudgill, 1989). In samples collected on citrus at El Menzeh, several specimens of *Xiphinema sp.* that are close to *X. brevicollum* Lordello and da Costa were found. It is highly probable that these individuals belong to a new species. In the same area we found the sheath nematode *H. arenaria* associated with infested root showing the typical apical coral galls (Photo 5).

The presence of A. ritzemabosi and A. fragariae in strawberry samples confirms results obtained in a previous survey conducted in Larache region (Abbad Andaloussi, unpublished data). In this study, grouping 52 samples, the two nematodes were found in more than 15% with the dominance of A. fragariae in 75% of the cases. In the fields, symptoms were very characteristic (Photo 6). However, in this previous study, we didn't identify any galls of M. hapla in roots. Strawberry crop has been recently introduced in Morocco and stolons were brought from USA and Spain where those nematodes (*Aphelenchoides* and *M. hapla*) are very common.

Pratylenchus vulnus, *M. xenoplax* and the root knot nematodes were found on some stone fruit (*Prunus spp.*) such almond and peach, and on fig. The presence of *P. thornei* on chickpea confirmed that this species is very common in Morocco and in the Mediterranean basin (Di Vito et al., 1994; Abbad Andaloussi F. and Bachikhi, 1996, Di Vito and Greco, 1999; Troccoli and Di Vito, 2002).

The association of GFLV and *X. index* may cause very serious damage on grape in *Morocco*. Therefore, it need, more investigation on the occurrence and distribution, and

we suggest to the farmers to use virus-free plants and to grow grape in fields without the nematode. In the case of replant, we suggest to maintain the field free of nematode host plants at least for three years and to treat the field with nematicides before planting

More studies are necessary to investigate on the populations of *Xiphinema sp*. found on Citrus and to identify the corresponding nematode species.

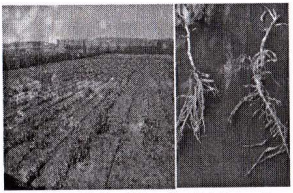

Photo 1. A field of pea infested by *Meloidogyne javanica* (Left); roots with typical galls (Right).

Photo 2. A root of common bean infested by *Meloidogyne javanica*.

Photo 3. A field of grape with symptoms (yellowings) of GFLV.

Photo 4. Root of grape infested by *Xiphinema index*; with high proliferation of new rootlets at the feeding sites of the nematode.

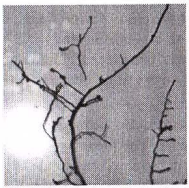

Photo 5. Root of orange infested by *Hemicycliophora arenaria*; note the typical apical and coral galls along the root.

Photo 6. A field of strawberry infested by *Aphelenchoides* spp.

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