Scientific Papers. Series B, Horticulture. Vol. LVII, 2013
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NEW HOST PLANT FOR VIRUS VECTOR NEMATODE *XIPHINEMAITALIAE* MEYL, 1953 (NEMATODA: LONGIDORIDAE) IN ROMANIA

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Abstract

Except direct damage to root system, Xiphinema italiae Meyl, 1953 has been reported to be a vector of Grapevine fanleaf virus (GFLV) (Cohn et al., 1958). Soil samples were collected at a depth of 20-40 cm from orchards and vineyards. Xiphinema italiae was identified in rhisosphere of peach orchard. A polymerase chain reaction protocol and the morphological and morphometrical characters has been used for the reliable identification of X. italiae. Morphometrics and illustrations of females are provided. Prunus persica L.is a new host plant for Xiphinema italiae for Romania.

Key words: *Longidoridae, morphology, PCR Multiplex.*

INTRODUCTION

Xiphinema italiae Meyl, 1953 is widespread migratory plant parasitic nematode, species occurring in southern and central Europe: Bulgaria (Peneva and Choleva, 1992, Peneva,1997) France (Wang et al., 2003) Greece (Avgelis & Tzortzakakis, 1997, Tzortzakakis et al. 2006) Hungary (Nagy, 1999), Italy (Martelli et al.,1966), Moldavia (Polinovskij, 1979), Serbia (Barsi & Lamberti, 2003), Slovakia (Liškova et al., 1993) and Spain (Teliz et al., 2007) (Gutiérrez-Gutiérrez et al., 2011).. Outside Europe it was found in Cuba (Dias-Silveira & Herrera, 1995), Egypt (Lamberti et al., 1996), Libya (Siddiqui et al., 1987), Nigeria (Khan et al., 1993) and South Africa (Knoetze et al., 2000). *Xiphinema italiae* has been reported to be a vector of GFLV according to Cohn et al., 1970). In Romania, Romaşcu, 1971 found *X. italiae* in association with grapevines from sandy soil in Platoneşti and Saveni (Ialomiţa county).

MATERIALS AND METHODS

For this study, soil samples were collected from the rhizosphere of peach trees at a depth of 20-40 cm from Valul lui Traian (Constanţa county).

Nematodes were extracted from 200cm^3 soil by a sieving and decanting technique, Nematodes were heat killed at 60ºC for two minutes and fixed in a 4% formaldehyde solution. The specimens were processed to mounted on permanent microscopic glass slides (Seinhorst, 1959).

The morphological and morphometrical observations were made using Leica DMLB microscope fitted with Leica FDC 295 camera.

Multiplex PCR. DNA isolation was carried out by placing 4 nematodes in 10 µL of lysis buffer (1X Platinum Taq DNA polymerase /Invitrogen and 60 µg of proteinase K/mL) between two glass slides and crushed gently. The homogenate was taken up carefully with a pipette, transferred to 0,2 mL Eppendorf tubes and frozen at-80°C for 15 min. After the tubes were incubated at 60°C for 1 h and 95°C for 15 min.

Amplification was carried out in a 25-µl reaction mixture containing the 2,5 µl lysis buffer (nematode lysate as PCR template), 1x Platinum *Taq* DNA polymerase buffer (Invitrogen), 1.5 mM $MgCl_2$ (Invitrogen), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Sigma 10mM), 0.8 pmol each primer, and 0.5 units of Platinum *Taq* DNA polymerase

(Invitrogen). The primers A-ITS 1, I27, D24, V18, ITA26 were used (Wang et al. 2003).
Amplifications were performed in a thermal cycler (Mastercycler Pro S – Eppendorf), with the following cycling conditions: 95°C for 3 min followed by 39 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min 30 s, and ending with 1 cycle at 72°C for 5 min and storage at 4°C.
Amplification product (10 µl PCR product) was separated on 1,5% agarose gel (Sigma) and 0,5X TBE at 100V. The gels were visualized with photo documentation system GENi (Syngene).

RESULTS AND DISCUSSIONS

Xiphinema italiae Meyl, 1953 (Table 1, Figure 3).
Female: Body almost straight, tapering forward and backward, tail end ventrally curved. Cuticle 1,5-1,7µm thick in postlabial region, 2,2-2,7 µm at mid body, 3-4,3 µm. Head end 4,3-5µm convex, clearly separated from adjacent body by a constriction, laterally rounded. Basal bulb measuring 17-18x113-120 µm. Prerectum 451-607µm, rectum 23-31µm. Tail conical, elongated, tapering ventrally and or dorsally before end. Terminus rounded.
Juveniles: The scatter plot diagram based on functional and replacement odontostyle and body length reveals the presence of three juveniles stages (the second, third, fourth) (Figure 1).

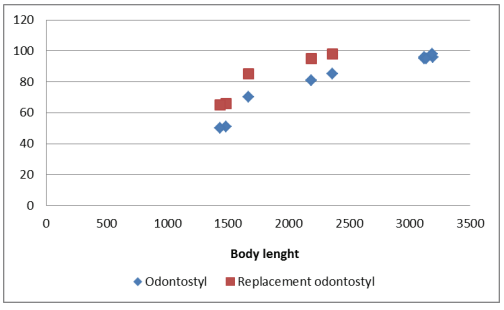

Figure 1. Scatter plot of odontostyle and replacement odontostyle agaist body length of Xiphinema italiae.

Table 1. Measurements of Xiphinema pachtaicum (all in micrometres)

Locality	Valul lui Traian			
Host plant	Prunus persica L.			
Character	Females	J2	J3	J4
n	5	2	1	2
L	3152±35,3	1486,1434	1671	2363,2189
a	98,6±1,6 96,5-101	71, 62	76	95, 87
b	8,3±0,1 8,2-8,4	5,5	6,3	6,6; 6,3
c	35,7±0,3 35,4-36	20,6	21,4	27,8; 27,3
c'	4,4±0,2 4,1-4,6	5,2	5,5	4,8
V%	46,8±1,1 45,5-48	-	-	-
Odontostyle	88,4±1,5 87-90	50,51	70	85,81
Odontophore	59,5±0,7 59-60	40,43	46	60,52
Replacement odontostyle	-	65,66	85	98,95
Oral aperture to guide ring	77,8±2,8 75-81	42,44	60	67,63
Pharynx	378,8±5,8 374	270, 260	265	357, 344
Tail	88,4±1,5 87-90	72	78	85, 80
Length of hyaline part	9,7±0,6 9-10	5,8	-	8,10
Body diameter at: - lip region	10,6±0,2 10,4-11	8; 8,5	8,7	9,4; 9,7
- guiding ring	23,1±0,4 23-23,4	15,6	18	20,21
- base of pharynx	28,5±0,7 28-30	22,5;23	21,5	24
- mid-body	32±0,4 31-33	21,23	22	25
- anus	20,1±0,7 20-21	13,8	14	17,5
- hyaline part	7	3,6	-	5

n=number of specimens; a=body length/greatest body diameter; b=body length/distance from anterior to end of esophageal bulb; c=body length; c´=tail length/anal body diameter; V%=distance of vulva from anterior end

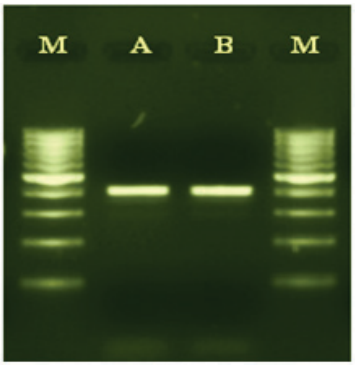

Figure 2. Electrophoresis of the amplification products from DNA isolated from Xiphinema italiae: lane M-100bp DNA ladder (Fermentas), lane A,B – X. italiae

Molecular differentiation showed a single fragment of approximately 414bp was amplified (Figure 2), according to Wang et al., 2003 which correspond to *Xiphinema italiae*.

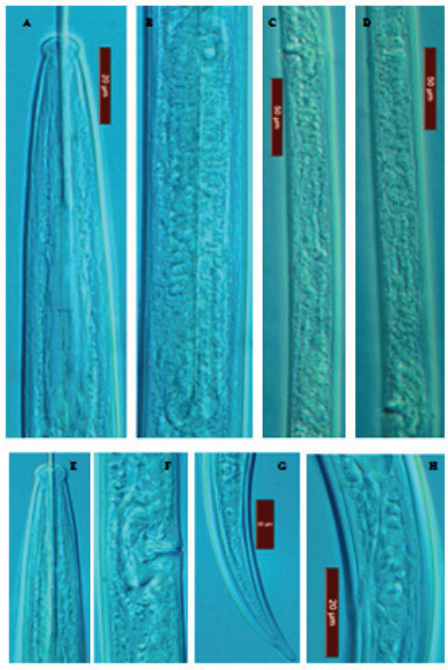

Figure 3. Xiphinema italiae Meyl, 1953: A, anterior region of female with lips region, odontostyle, odontophore, guiding ring; B, oesophageal bulb; C, posterior genital branch; D, anterior genital branch; E, head end; F, vaginal region; G, female tail; H, rectum. Scale bar: 20µm; 50µm.

CONCLUSIONS

Xiphinema italiae was recorded on *Prunus persica* for the first time in Romania.

The monitoring of virus vector nematodes through soil samples, before set up the orchards and vineyards, can assure healthy crop.

Accurate identification of *Xiphinema* spp. is important in regard to their virus transmission capability.

For a reliable diagnosis is necessary to combine identification using morphometric characters and molecular technics.

ACKNOWLEDGEMENTS

This research was carried out with the support of POSDRU/107/1.5/S/76888 project.

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