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POTENTIAL ROLE OF *Xiphinema americanum* AND *Meloidogyne incognita* IN TRANSMISSION OF *Peach rosette mosaic virus*

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ABSTRACT

Peach rosette mosaic virus (PRMV) was detected in naturally infested grapevine (*Vitis vinifera* cv. Superior) plants growing in the Horticulture Institute Experimental Station Giza Governorate. The virus was found, then to be transmitted by mechanical, nematode *(Xiphinema americanum* and *Meloidogyne incognita)* and graft inoculation. Double antibody sandwich enzyme linked immunosorbent assay (DAS- ELISA) was used to detect the presence of virus and to confirm the transmission. As for *X. americanum*, its effect was more pronounced at the highest nematode inoculum level than in plants infected with virus added to the lowest nematode level. Moreover, on grape plants infected with *M. incognita*, nematode inoculum levels significantly (P≤ o.o5 and 0.01) increased when nematodes were inoculated alone than when nematode and virus were inoculated simultaneously (where virus source and virus-free bait plants were grown simultaneously in the same pot).

Keywords: PeRMV, *Nepovrus*, DAS-ELISA, grapevine, grafting, *Xiphinema americanum* , *Meloidogyne incognita*

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INTRODUCTION

Grapevine suffers from invasion by several graft transmissible diseases caused by viruses and virus like agents (Choueiri *et al*., 1996; El-Banna, 1998). *Peach rosette mosaic virus* (PRMV) causes an economically important disease in vineyards worldwide (Brown *et al.,* 1993). (PRMV) , was firstly noticed in Michigan peach orchards in 1917 and described as pathogen of virus etiology in 1933 (Cation, 1933). PRMV is a member of *Nepovirus* group and vectored by *Xiphinema americanum* (Allen *et al*., 1982 and Brown *et al*., 1993). Also, *X. americanum* was reported on peach and grape in Egypt (Oteifa, 1964). The virus is mechanically transmissible from young leaves of peach or grape tissues extracted in phosphate nicotine buffer, to the indicator hosts viz., *Chenopodium quinon* and *C.amaronticolor*(Dias and Cation 1976). Leaf emergence of infected peach or grape trees is delayed. Then, leaves develop chlorotic mottling. Leaf distortion might follow a chlorotic lesion that forms on one side of the leaf and the leaves formed later in the next season are narrower than healthy ones. A shortening of internodes (Rosette) is a prominent symptoms in late spring and summer. Infected trees are become darker green, stunted and produced little or no fruit (Ramsdell and Myers 1974).

From nematological point of view, it is well known that root-knot nematodes are not virus-transmitted nematode but, under field conditions some plants exhibited concomitant infection with viruses and *Meloidogyne incognita* together. These plants were heavily galled while virus-free plants showed poor root-knot development (Abu Foul, 1984 and Jabri *et al.*, 1985). In some cases, more root-knot nematode populations in soil and roots were recorded in the treatment inoculated with nematode alone than those inoculated with both nematode and virus (Varshney *et al*., 2005).

The aim of this research is to study interaction between PRMV at different inoculum potentials of its vector, *X. americanum* that infecting both grape and tobacco and the role of *M. incognita* as well.

MATERIALS AND METHODS

Source of diseased materials sampling:

Grapevine (*Vitis vinifera* cv. Superior) leaves showing typical symptoms of PRMV (mosaic, malformation, vein banding and vein clearing of leaves) were sampled from Giza Governorate during the growing season (April – May 2004). Obtained samples including leaves of grapevines and soil samples from plant rhizosphere were collected with an auger from trenches 20- 40 cm deep, stored in refrigerator and using to extracted and evaluate population of nematodes.

Isolation and identification of the virus isolate:

PRMV-infected young leaves of grapevines were triturated in 2 ml of 0.05 M potassium phosphate buffer, pH 7.0, containing 1% (v/v) nicotine alkaloid (2 ml/g of tissue) and then rub-inoculated onto five seedlings of *C. quinoa* plants at the first two leaves stage previously dusted with carborundum (600 mesh), Single local lesions (Kuhn, 1964) were used for biological purification of the virus isolate from Grapevine (*Vitis vinifera* cv. Superior) which was used as propagative host plant.

1-Mechanical transmission

Five seedlings from each of 17 plant species belonging to eight families were mechanically inoculated with the virus isolate. An equal number of healthy seedlings of the same age and cultivar were rubbed with buffer to serve as controls.All plants were kept under an insect-free greenhouse at 25-30˚C. The plants were examined daily up to 30-60 days for symptoms development and were checked for virus infection by back inoculation onto the indicator hosts(*C. amaranticolor* and /or *C. quinoa*)and /or by DAS-ELISA.

2-Transmission by graft inoculation:

Bark tissue from young shoots of the natural (PRMV) infested grape (cv. Superior) tree was side grafted on five potted grapes and peach seedlings under greenhouse conditions of virus free symptoms [that is confirmed by ELISA using *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Peach rosette mosaic virus* (PRMV) *Prunus necrotic ring spot virus* (PNRSV) and *Tomato ring spot virus* (TRSV) antisera]. Inoculated rootstocks and scions were tied together with plastic strips. Three to four months later, symptomless plants were checked for virus infection by back inoculation into the indicator host plant and/or by ELISA.

3-Nematode transmission

The dagger nematode, *Xiphinema americanum* population from vineyard at farm of Horticulture Institute, Agricultural Research Center was selected for this study. This population was collected from rhizosphere associated with PRMV-infested and free grape plants. Transmission experiments were conducted using nematodes from soil extracted by wet-sieving method (Cobb, 1918) and decanting method (Seinhorst, 1988) and returned to the soil within 3 hours.

a-Transmission by *Xiphinema americanum* on tobacco

In the first experiment, *Nicotana sylvestris* tobacco seeds were sown in sterilized soil in 25cm-diam. clay pots. Three weeks after germination, plants were thinned to one plant per pot inoculated by the virus isolate and identified population of virus-free *X. americanum* at levels 0, 10, 100 and 1000 individuals/pot were tested for their ability to transmit acquired PRMV (Brown,1986) to tobacco plants in each pot as illustrated diagrammatically in Fig (1A).

b- Transmission by *Xiphinema americanum* on grape

In the second experiment, grape cv. Superior seedlings of 4 months old each was transplanted to sterilized soil in 25 cm-diam. clay pots and inoculated by the virus isolate. Two weeks later, populations of virus-free *X. americanum* at the levels (0, 100,1000 and 2000 individuals/pot) were tested for their ability to transmit acquired PRMV to grape plants, as illustrated in Fig. (1).

c-Interaction between *Meloidogyne incognita* and PRMV on grape

In the third experiment, *Meloidogyne incognita* juveniles at inoculum levels of o, 100, 1000 and 2000 juveniles (J_2) /pot in sterilized soil were tested for their ability to transmit PRMV to grape cv. Superior plants of 4 months old as the previous treatments. The three mentioned experiments were repeated again except that virus source and virus-free bait plants grown simultaneously in the same pot were used (Fig 1B). While other three treatments were inoculated with the different inoculum levels (100, 1000 and 2000 juveniles (J_2 /pot) of the root-knot nematode only. The root-knot nematode was identified by separating mature females from galled roots. Examination of the prepared posterior cuticular patterns of such females was carried out according to the morphological characters described by Taylor *et al*. (1955) and Taylor and Sasser (1978).

Four months later as illustrated in Fig. (1A and B), tobacco or grape plants were uprooted and nematodes were extracted from soil by sieving and decanting methods. *X. americanum* population as indicated by numbers of larvae and female in soil were recorded. The number of *Meloidogyne incognita* juveniles in soil and galls and eggmasses on grape roots were determined. Also, % chlorophyll contents were determined by using chlorophyll meter SBAD 50I for the tested plants. Data were statistically analyzed using the Fisher's Least Significant Difference (LSD) (Carmer and Swanson 1973).

Serological assays using DAS-ELISA technique:

a-Virus detection in different plants:

Leaves and leaf blades of tested hosts were examined serologically using
commercial Kits supplied by SANOFI (Sante Animale, Paris, France). Double –antibody sandwich ELISA (DAS- ELISA) for PRMV (Clark and Adams, 1977).

b- Virus detection in nematodes:

The plates were coated with 100 µ L IgG of PRMV (for 3 hours) at 37˚C and then rinsed three times with PBS-T (phosphate buffer saline pH 7.4, containing 0.5 µ L/ ml Tween 20). Adult nematodes were hand picked from the final water suspensions and frozen. The nematodes were ground in a potter micro blender in the presence of 100 µ L Tris – Hcl (0.2 M , pH 8.2) containing 2 % polyvinyl pyrolidone, 0.8 % Na Cl, 0.05% Tween 20, and carborandum. Homogenates were transferred to the micro titration plate and incubated overnight at 4˚C. After three washing with PBS- T, an IgG-conjugate at 1/20,000 was added. After one hour at 37 ˚C, then second conjugate was added and incubated at 1/10,000 for 30 minutes. Absorbance at 405 nm were measured with a Dynatech MR 7000) 0.5, 1, and 2 hours incubation of the substrate (1mg/ ml p- microphenylphosphate in 0.01 M diethanolamine, pH 9.8(Huss *et al*., 1986 and Esmenjaud *et al*., 1993).

RESULTS AND DISCUSSION

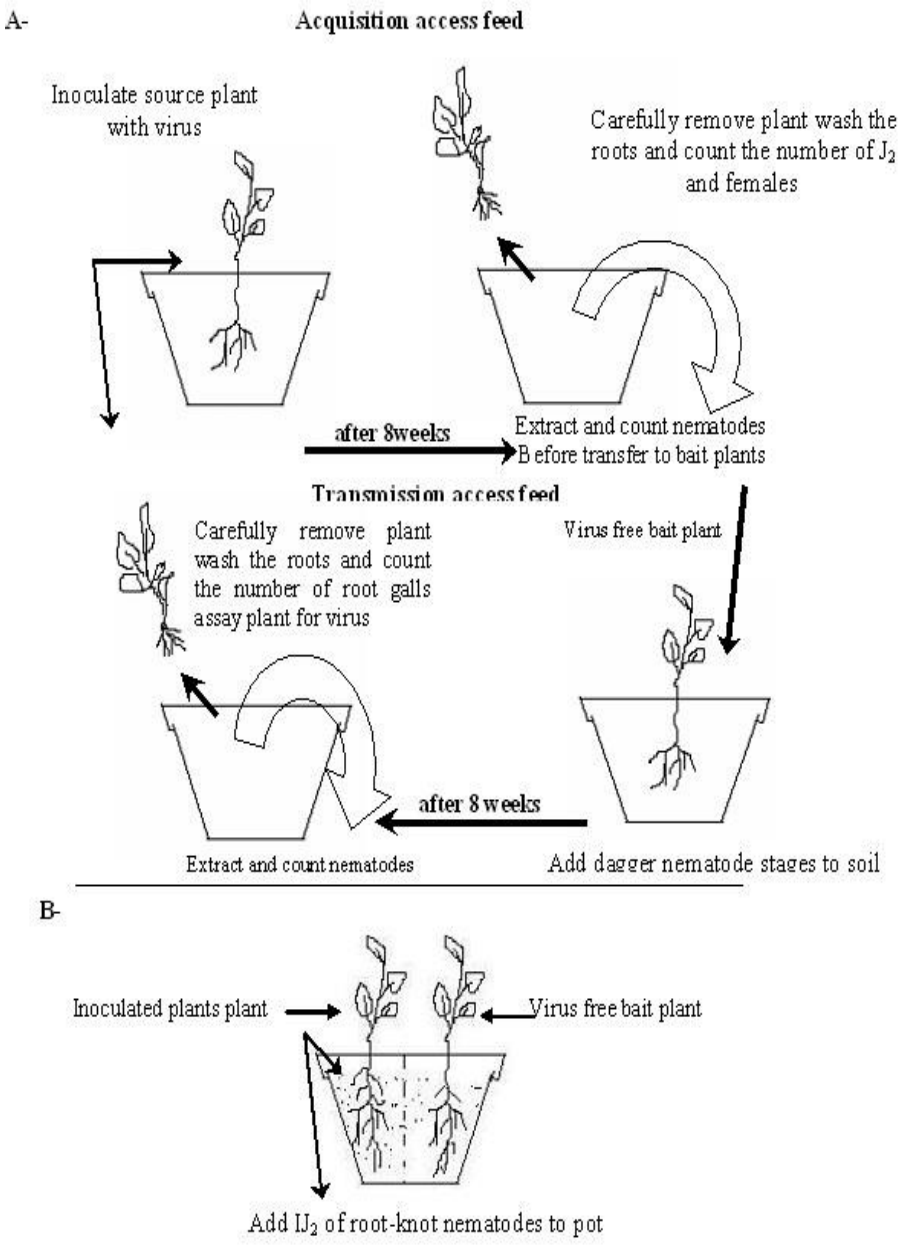
Isolation and Identification:

Virus isolate was obtained from naturally infested grapevine plants cv. Superior showing severe mosaic and malformed leaves (Fig.2 a) were collected from Horticulture Institute Experimental Station, Giza Governorate. After successive single lesion transferred in *C .quinoa, the* resulting virus isolate was propagated in *N. sylvestris*.The symptoms were very similar to those illustrated by Dias (1975) and Dias and Cation (1976).Subsequent work clearly proved that the virus under study is *Peach rosette mosaic virus*. These results were based mainly on symptomatology, host range, modes of transmission and serology.

Mechanical transmission

Reactions of 17 plant species belonging to eight different families to virus infection are shown in Fig (2) and Table (1). The presence of the virus isolate in all tested plants was further confirmed biologically using the indicator host plants or serologically by ELISA. Such results were similar to those reported by Dias (1975), Dias and Cation (1976), Ramsdell *et al*. (1979), Németh (1986) and Ahmed *et al* (2004)).

Fig (1): Outline of procedure using two methods (A and B) for detecting virus transmission by nematodes.



Illustrated diagrammatically by Matthews (1993).

Table (1): Reaction of host plants inoculated mechanically by PRMV.

Infected host	Symptoms	ELISA test
Family: Amranthaceae		
Gompherena globosa L.	L.L.	+
Family:Chenopodiaceae		
Chenopodium amaranticolor Coste & Reyn.	C. L.L.	+
Chenopodium quinoa Wild	C. L.L.	+
Family:Compositeae		
Zinnia elegans L.	M	+
Family:Cucurbitaceae		
Cucumis sativas L. cv.Beta Alfa	0	-
Family: Leguminosae		
Pisum sativum L. cv. Linkolin	0	-
Family:Rosacae		
Prunus persica L. cvs: Soltany*	M	+
Florda prince*	M	+
Family:Vitidaceae		
Vitis vinifera L.cvs: Superior*	M	+
Tomson seedless*	M	+
Flam seedless *	M	+
Family: Solannceae		
Datura metal L.	0	-
Datura stramonium L.	M	+
Nicotiana debneyi L.	0	-
Nicotiana glutinosa L.	mM	+
Nicotiana sylvestris Speg & Comes	M	+
Nicotiana tabacum L. *var. White Burly*	M	+

Mech. = Mechanical transmission. Nema. = Transmitted by Nematod graf. = Transmitted by grafting. (+ Positive, - Negative) in ELISA test .L.L. = Local Lesion. C.L.L. = Chlorotic Local Lesion. M= Mosaic mM = mild Mosaic 0 = no symptoms * = grafting inoculated

Transmission by graft-inoculation:

The different methods of virus transmission may be useful diagnostic criteria. All viruses which are systemic in their hosts can be transmitted by grafting between susceptible and compatible plants. Graft-inoculation was the first widely used technique for the transmission and detection of viruses, especially of woody plants (Németh, 1986) and (Matthews, 1993). Their usefulness may depend on the particular circumstances. Vegetative propagation, which grafting is essentially a form of it, is an important horticultural practice, but it is unfortunately a very effective method for perpetuating and spreading viruses (Dias, 1975 Dias & Cation, 1976 and Ramsdell and Myers, 1978). The virus under study was successfully transmitted from artificially infected grapes to healthy grape and peach seedlings. About four months later, inoculated plants developed the same symptoms as those appeared in naturally infested plants.

Nematode transmission:

Brown and Weischer (1998) divided the nematode transmission of a virus into seven discrete but inter –related processes viz., injection, acquisition, adsorption, retention, release, transfer and establishment. There is specificity in the relationship between nematodes and the viruses they transmit with often an apparent unique association between the virus isolate and the vector species.

In this work, trails have succeeded to transmit PRMV isolate by *X. americanum* only. This result also agree with those reported by Dias (1975), Brown *et al*. (1993), Esmenjaud *et al*. (1993), Anonymous (1996) and Taylor and Brown (1997). The effect of PRMV transmission by *X. americanum* or interaction by *M. incognita* on virus concentration, severity of symptoms, nematode parameters and total chlorophyll in leaves is presented in Tables (2, 3, 4 and 5). It is clearly noticed that as *X. americanum* inoculum levels on tobacco and grape plants increased, number of larvae and females in soil steadily significantly (P≤o.o5 and 0.01) increased. Conversely, reproduction factor (R) of nematodes decreased as the tested inoculum levels is increased. Also, on grape plants infected with *Meloidogyne incognita*, number of galls, gall index, number of eggmasses, eggmass index and number of nematodes in soil increased as nematode inoculum levels increased either when nematodes and viruses were inoculated alone or when nematode and viruses were inoculated simultaneously. On the other hand, it is clearly noticed that the plants inoculated with *M. incognita* only produced more number of galls and eggmasses and number of J2 in soil than the treatments inoculated with both pathogens. In all cases, total chlorophyll contents of leaves decreased as the nematode inoculum levels increased, being the highest decrease at the highest nematode inoculum levels.

Table(2):Relationship between nematode (*X. americanum*) and the concentration of PRMV in *Nicotiana sylvestris* determined by ELISA.

Inoculum levels	V. det.	Time period (8 weeks)							
		1	2	3	4	5	6	7	8
10	Sym.	-	-	-	-	-	-	-	-
	ELISA	0.036	0.049	0.033	0.032	0.045	0.064	0.048	0.072
100	Sym.	-	-	-	-	-	+	++	++
	ELISA	0.042	0.034	0.046	0.039	0.066	0.321	0.761	0.
1000	Sym.	-	-	-	-	+	++	++	++
	ELISA	0.033	0.07	0.050	0.047	0.384	0.655	0.618	0.722

Negative control= (0.040) Positive control= (0.282)
V. = virus det.= determined sym.= symptoms

Table (3): Development and rate of build up of *X. americanum* in *Nicotiana sylvestris* inoculated by PRMV.

Inoculum levels pi N V	Number of juveniles (J_2)	Females	Total Pf	Rate of build up Pf / Pi	Total chlorophyll
0	0	0	0	0	33.9
10+V	15	12	27	2.7	27.5
100+V	80	110	190	1.9	17.6
1000+V	325	500	825	0.83	16.3
L.S.D 5%	32.27	58.70	53.79		2.66
1%	49.04	89.15	81.72		4.04

Values are means of six replicates.
N= nematode V= virus
Rate of build –up = final nematodes population (Pf) / Initial nematodes population(Pi)

Table (4): Reproduction and development of *X. americanum* on *V.vinifera* cv. Superior inoculated by PRMV.

Inoculum levels Pi N V	Number of juveniles (J_2)	Females	Total stages Pf	Rate of build up (pf/pi)	Total chlorophyll
0	0	0	0	0	29.2
100+V	120	112	232	2.3	16.3
1000+V	510	450	960	0.96	14.1
2000+V	1095	700	1795	0.89	10.5
L.S.D. 5%	361.89	82.14	146.53		1.76
1%	549.79	124.77	222.42		2.67

Table (5): Effects of *Meloidogyne incognita* inocula(N) on grape cv. Superior under inoculation by PRMV(V).

Inoculum levels of N	Number of juveniles (J_2) in soil		No. of galls		Gall index		No. of eggmasses		Eggmass index		Total chlorophyll	
	N	N+V	N	N+V	N	N+V	N	N+V	N	N+V	N	N+V
0	0	0	0	0	0	0	0	0	0	0	34.0	30.2
100	1200	1000	70	53	7	7	61	45	7	6	25.2	23.4
1000	3300	2900	135	96	9	8	115	90	9	8	20.1	19.2
2000	4600	4200	184	168	9	9	156	142	9	9	16.9	15.6
L.S.D.5%	501.37	345.07	23.71	12.70			11.33	18.87			4.02	4.64
1%	761.71	524.24	36.02	19.30			17.20	28.66			6.12	7.04

Galls and eggmasses indices according to Sharma *et al*. (1994)

Results in the present study indicate the interaction of *Peach rosette mosaic virus* (PRMV) and different nematode inoculum levels as virus symptoms, severity and concentration increased in leaves of tobacco and grape infected with *X. americanum* and grape infected with *M. incognita.* This effect was more pronounced at the highest nematode inoculum level than those in plants infected with virus alone or with virus plus lower nematode inoculum levels. These findings agree with Mayee *et al*. (1973) who reported that the increase in cowpea chlorotic mottle virus concentration in soybean plants and symptoms severity of tomato leaf curl virus on tomato were related to a high incidence of nematode infestation. On the other hand, numbers of root galls and eggmasses on roots and number of J_2 in soil were much greater in plants infected with nematode alone than those of plants infected with nematode and virus. This might be due to reduced supply of food to the roots caused by virus and *M. incognita* infection as suggested by Varshney *et a*l. (2005). Similar observations were made by Mclauglina *et al*., (1993) working on *Trifolium repens*.

Serological reaction:

Positive reaction obtained using specific antiserum against PRMV at a dilution of 1:500 confirmed the identification of the virus under study. Serological tests, such as ELISA provide rapid and convenient methods for the identification and estimation of plant viruses in leaves and nematode (Ramsdell *et al*., 1979; Németh, 1986 and Esmenjaud *et al*., 1993)

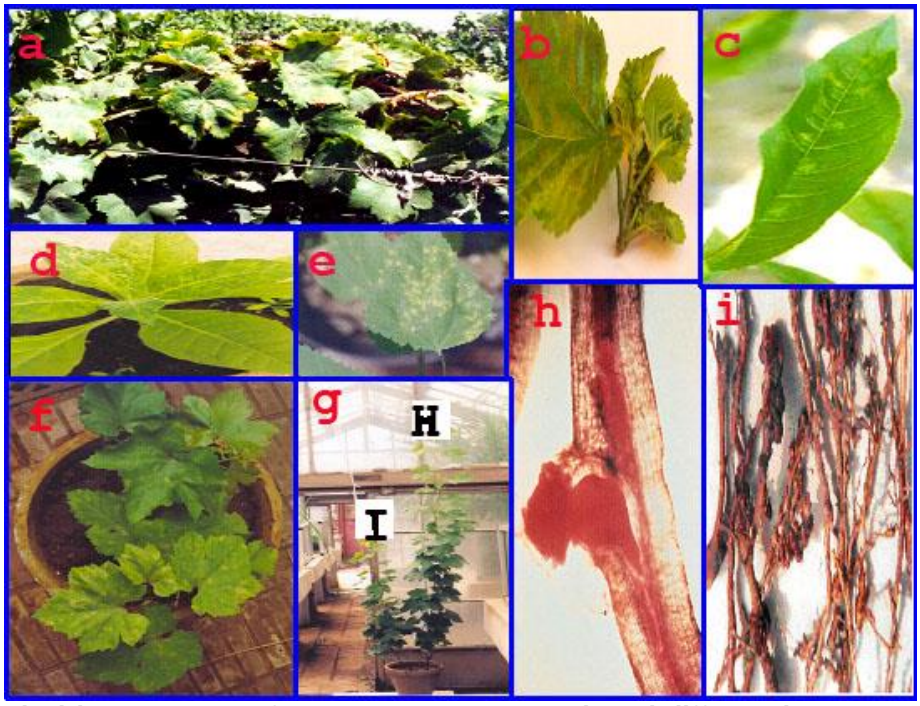


Fig (2): Symptoms of PRMV on grapes, peach and different host range and root knot nematodes in grape roots.

a-Natural infestation of PRMV on grapes (*Vitis vinifera* cv. Suprior) showed leaf malformation and vein clearing.

b-Artificial infection by nematode transmission on (*Vitis vinifera* cv. Suprior) showed typical symptoms (mosaic , malformation and rosetting).

c- Artificial infection by nematode transmitted on (*Prunus persica* L. cv. Soltany) showed systemic mosaic and chlorotic veins.

d – Severe mosaic symptoms appeared on *Nicotiana sylvestris* produced by PRMV transmitted.

e- Local lesion on *Chenopodium quinoa* infected with PRMV.

f- In the same pot the healthy grape plant and infected one but without contact between roots by wood seizor with holes to grant *Meloidogyne incognita* to move.

g- *Vitis vinifera* L cv. Suprior (I) showed delayed bud-break and stunting after graft inoculation with PRMV (H) healthy plant of similar age.

h-Gall with eggmass formed by root- knot nematode on the grape root.

i- Galls of root-knot nematode on grape root.

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الدورالـذى تلعبـه النيمـاتودا الخنجريـة ونيمـاتودا تعقـد الجـذورفى إنتقـال فيـروس موزايك وتقزم الخوخ

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تـم التعـرف علـى فيـروس موزايـك وتقـزم الخـوخ فـى نباتـات العنـب (صـنف سوبريور)المصابة طبيعياً والمنزرعة فى مزرعة التجارب بمعهد بحوث البساتين – مركز البحوث الزراعية – محافظة الجيزة. ولقد تم عزل الفيروس من نباتات العنب ثم دراسة طـرق الأنتقـال (الميكـانيكى و بالنيمـاتودا الخنجريـة *Xiphimenma americanum* ونيمـاتودا تعقد الجذور *Meloidogyne incognita* والأنتقال بـالتطعيم). وتـم إستخدام الاختبار السيرولوجى DAS-ELISA (الأجسام المضـادة المعلمـة بـالأنزيم) للتعرف علـى الفيروس وكذلك لتاكيد نتـائج طـرق الأنتقـال المختلفـة. وبالأضـافة الـى ذلك فلقد تـم إستخدام نيمانودا تعقد الجذور ودراسـة دورهـا فـى انتقـال الفيـروس عـن طريـق التغذيـة علـى النباتـات المصابة ثم النباتات السليمة التـى تنمو فـى نفس المكان.وبالنسبة للنيمـاتودا الخنجريـة الناقلـة للفيروس علـي نبـاتي العنـب والـدخان فقد وجـد أن كـلا مـن اليرقـات والانـاث لهـذة النيمـاتودا زادت بزيادة مستويات العدوي المستخدمة.

وقد أتضح من نتائج التجربة لنبات العنب المصابة بنيماتودا تعقد الحذور أن كثافة النيمـاتودا تزيد بزيادة الأعداد الأولية للنيماتودا في حالة العدوي بالنيماتودا والفيروس معـا أو فـي حالـة العدوي بكل منهما معا.

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