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Identification and elimination treatment of *Prunus necrotic ring spot virus* (PNRSV) in rose.

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Abstract

Prunus necrotic ring spot Ilarvirus (PNRSV), was isolated from rose shrubs during the survey of rose plantations in Orman Garden, showing *Ilarvirus*-like symptoms. To identify the causal virus, the plants were tested by enzyme-linked immunosorbent assay using antibodies against different Ilarviruses i.e. *Apple mosaic virus* (ApMV), *Prunus* necrotic *ring sopt virus* (PNRSV), *Rose mosaic virus* (RMV) and *Tobacco streak virus* (TSV). Preliminary results revealed the presence of PNRSV in tested rose shrubs. The isolated virus was biologically purified from single local lesions which formed on cucumber cotyledon. Identification of this virus was based on host range, properties in crude sap, transmissibility, and serological tests. PNRSV was able to infect only 12 plant species and varieties from 19 tested by grafting or mechanical inoculation, it has a limited range of experimental hosts. The dilution end-point of infectivity was 10^{-3}, the thermal inactivation point was 54°C, and longevity *in vitro* was of 10-14 h at 25°C. PNRSV was detected by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA). Immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) was used to amplify a 450 bp cDNA fragment from infected rose leaves (*Rosa indica L.*) using the primers Mac1 and Mac2 specific to *Prunus necrotic ring spot virus* (PNRSV) which were designed to amplify of the 3' end of the coat protein gene (RNA-3). Nucleic acid hybridization was useful for the detection of PNRSV in herbaceous and woody plant tissues. In successful attempt to eliminate the virus from infected dormant rose cuttings by heat therapy resulted in 29.6% virus elimination of (PNRSV).

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***Key words*: ELISA, host range, IC-RT-PCR, Nucleic acid hybridization, PNRSV**

Introduction

Rose (*Rosa hybrida* L.) is the most important cut flower roses growing in Egypt .Also; it is considered an important crop in the floricultural industry, important essential oil bearing plants of great demand in national and international markets.

Roses are affected by several diseases of viral etiology that cause serious reduction in the vigour and production of plants (**Abou El-Ela *et al.*, 2004** and **Salem *et al.*, 2004**).

Viruses that infect rose belong mainly to the genera *Ilarvirus* and *Nepovirus*. Among ilarviruses, *Prunus necrotic ringspot virus* (PNRSV) (**Fulton, 1970**) has been isolated in many rose growing regions worldwide. *Prunus necrotic ringspot virus* (PNRSV) is a positive-sense RNA plant virus with a tripartite genome that belongs to the genus *Ilarvirus* of the *Bromoviridae* family (**Fauquet *et al.*,2005**).PNRSV is distributed worldwide, infecting most cultivated *Prunus* spp. and causing symptoms that range from no damage to necrotic spots and shot holes in young leaves to rugose mosaic, all causing lose in yield and vigor (**Uyemoto and Scott, 1992**). PNRSV is easily transmittable by pollen grains and seeds and by routine propagation methods (**George and Davidson, 1963**).

Prunus necrotic ring spot virus (PNRSV) is considered the most common rose viruses and one of the most economically important rose and prunus viruses (**Fulton, 1970; Németh,1986 and Benoit Moury *et al.*, 2001**).

This work was conducted to study in details PNRSV that was identified after isolation from rose grown under local condition. Symptoms in affected rose included mosaic, chlorotic lines, rings and oak leaf patterns on rose (**Bos,1976**).PNRSV is transmitted by grafting in woody plants and mechanically transmitted to several herbaceous plants (**Devergne and Goujon ,1975**).

Studies on heat treatment of cuttings and shoot tips of viruses infected trees gives satisfactory results in virus elimination (**Schmidt, 1974**).

PNRSV has a positive sense tripartite RNA genome with RNA 3, encoding the putative movement protein and the coat protein that is translated from the sub genomic RNA 4. (**Scott *et al.*, 1998**). At the molecular level, sequence data are only available for the genomic RNA 3.

The present work is focused on isolation and identification of PNRSV from the plants was tested by enzyme-linked immunosorbent assay using specific antibody. This diagnosis was confirmed by immunocapture-reverse transcription- polymerase chain reaction (IC-RT-PCR) for detection PNRSV in herbaceous and woody plant tissues. Also, heat treatment of rose cuttings infected with PNRSV was studies for virus elimination.

Materials and Methods

Source of virus isolate and plant materials:

An isolate of PNRSV, was isolated from a naturally infected rose shrub (*Rosa indica* L.) and used in this work. Leaves were sampled in April from PNRSV affected rose shrubs, as determined by ELISA, using antisera against ApMV, PNRSV, RMV and TSV, leaves were ground in 0.1 M sodium phosphate buffer, pH 8.0 and used as antigen in ELISA test. According to **Fulton (1970)**, a pure culture was obtained for this virus isolate by successive single lesion

transfers from *Cucumis sativus* cv Beit Alpha and then was multiplied and maintained on cucumber plants.

Transmission studies:

Mechanical transmission (Host rang and symptomatology):

In preliminary transmission tests; mechanical transmission of PNRSV from infected rose to herbaceous hosts was found to be unsatisfactory. So the following buffers were tested: 0.01 M phosphate buffer, pH 7.2, containing 2.5% nicotine, 0.01 M phosphate buffer, pH 7.2, containing 0.001 M Na-DIECA, 0.01 M phosphate buffer, pH 7.8, containing 0.001 M Na-DIECA and 2.5% nicotine mixed with activated charcoal (100 mg ml-1, w/v), and 0.05 M phosphate buffer, pH 7.2, containing 2% sodium sulfite.

Graft transmission:

Ten seedlings of each rose (*Rosa multiflora* cv.Red Moss), peach (*Prunus persica* cv Earli Gold and cv.GF305) and plum (*Prunus domistica* cv.Clymax) were graft-inoculated from the PNRSV source in May and June 2004. The inoculum consisted of three buds each per each seedling.

Virus stability in crude sap:

Virus properties in plant crude sap, dilution endpoint (DEP), longevity *in vitro* (LIV), and thermal inactivation point (TIP), were determined by procedures described by **Hill (1984)**. *Cucumis sativus* cv Beit Alpha was used as a source plant for the virus isolate, while *Ch. amaranticolor* were used as indicator hosts.Each experiment was repeated twice.

Serological reaction:

Leaf samples were collected randomly from symptomatic as well as asymptomatic rose leaves. Samples were stored at 4°C until processed. The standard double-antibody sandwich enzyme-linked immuno-sorbent assay (DAS-ELISA) was used for detection of PNRSV. IgG and conjugated IgG for four different Ilarviruses were purchased from Sanofi, Sante Animal,Paris,France. Extraction and identification of the infected leaves by ELISA was applied as described by **Clark and Adams (1978).**

Immunocapture - reverse transcription-polymerase chain reaction (IC-RT-PCR):

In the present work, an Egyptian isolate of *prunus necrotic ring spot virus* (PNRSV) previously isolated from Rose(*Rosa indica* L.) was used in IC-RT-PCR(**Salem *et al* .,2004**). Primers Mac1 and Mac2 were designed to amplify of the 3' end of the coat protein gene (RNA-3) of PNRSV-CP according to **MacKenzie *et al.* (1997)**.The sequences of PCR primer was the 25-merMac1(5'ACGCGCAAAAGTGTCG AAATCTAAA-3') and Mac2 (5'-TGGTCCCACTCAGAGCTCAACAA G-3') (**MacKenzie *et al.*, 1997**) corresponding to nt. 1178-1629 of the PE5 isolate of PNRSV(**Hammond and Crosslin, 1995**) and the Titan One Tube RT-PCR system (Roche Diagnostics Corp., Indianapolis, IN, USA). The RT-PCR was performed in a thermocyler with 42°C for 45 min, then the PCR was performed with 92°C for 2 min followed by 35 cycles of denaturation at 92°C for 1 min ; 52 °C for 1 min and 72°C for 2 min. followed by an extension step 10 min at 72°C, 35 repeated cycles. The amplification was preceded in the thermocycler (Uno II, Biometra, Germany).

Gel electrophoresis:

The PCR products were analyzed by 1% (w/v) agarose gel electrophoresis in 0.5x Tris- borate- EDTA buffer (**Sambrook and Russell, 2001**) and photographed using (Gel Doc 2000 Bio-

RAD). 50bp ladder (Sigma) was used to determine the size of RT- PCR amplified DNA products.

Nucleic acid hybridization- Non radioactive method:

cDNA PNRSV probe

The obtained insert from PCR products were labeled following the technique of Boehringer Mannheim GmbH, Mannheim Germany according to the manufacturer's instructions. DNA fragment 450bp of amplified PNRSV coat protein gene was used as template to synthesize a cDNA Dig labeled probe.

Southern blot and Dot blot hybridization:

The capillary transfer of plant DNA from the gel to nitrocellulose membrane support was achieved using the southern technique. For dot blot, leaf tissues 0.3g of both infected and healthy rose (*Rosa indica* L.) and *Chenopodium quinoa*) leave, were placed in microfuge tubes. Dot blots were carried out according to **Loebenstein *et al.* (1997).** 3µl of the supernatant were spotted onto nitrocellulose membrane. The nucleic acid hybridization was carried out as described by Boehringer Mannheium manual protocol.

Heat-therapy:

Five hundred diseased plantes were chosen for treated with hot water from a group inoculated in September (2004).One hundred from each cvs. *Rosa indica* L.; *Rosa setigera* Michx *cv.Queen Bee.*; *Rosa multiflora* Thumb cv.Red Moss; *Prunus persica* cv Earli Gold (peach) and from plum (*Prunus domistica* cv. Clymax). All inoculated plants of each cultivars were showed symptoms of PNRSV then confirmed with ELISA test by the end of March(2005). The diseased dormant cuttings (about 15 to 20 cm.) were divided into 20 lots, each of which contained five plants were immersed in a hot water bath in appending five plants from each cultivar were designated as diseased and treated controls. Treatments were at 45, 50, 55, 60and 65°C for 30, 60, 120and 180 min **(Schmidt, 1974).** After a period of heat adaptation, roses, peach and plum were grown as a scions or rootstocks at temperatures between 37 ± 3 °C with 60 to 80% RH and with a soil temperature of 28 °C. The symptoms were observed and the virus was checked by ELISA.

Results and Discussion

Transmission studies:

Mechanical transmission (Host rang and symptomatology):

Results of host range trials showed that PNRSV had a limited host range **(Table 1** and **Fig 1).** Only twelve of the plant species developed symptoms. They proved to be infection upon back indexing of both inoculated and top leaves. No symptomless infection was detected by DAS-ELISA in either inoculated or top leaves. PNRSV produced chlorotic ring spots on the inoculated leaves and systemic chlorotic mottle on the top leaves of *Chenopodium amaranticolor* and *Chenopodium. quinoa* plants. Also, *Zinnia elegans* reacted to PNRSV with chlorosis at the base of the youngest leaf. Chlorotic and necrotic lesions were observed on cotyledons of *C.sativus* cv. Beit Alpha. Systemic symptoms were consisted of severe mosaic, compact growth, and top necrosis. Among Leguminosae plant species, only *Vigna unguiculata* reacted to PNRSV by forming red necrotic local lesions on the cotyledons, ten days after inoculation. However, **Boulila and Marrakchi**

(2001) showed that *Vigna unguiculata* reacted to PNRSV by forming a chlorotic line pattern and deformation of the leaf lamina. In *Petunia hybrida*, systemic chlorotic lesions formed that eventually developed into necrotic lesions, and systemic mottling was also observed in plants inoculated with PNRSV: *Vinca rosea* and *Nicotiana. clevelendii* exhibited systemic symptoms ranging from mild mottle to severe leaf distortion. Among the different hosts studied, *Gomphrena globosa*, *Cucurbita pepo*, and *Phaseolus vulgaris* were not infected; and the virus could not be recovered from either inoculated leaves or tip leaves, contrary to the results reported by **Civerolo and Mircetich (1972)** who described isolates systemically invading these hosts. *Vigna unguiculata* reacted to PNRSV by forming red necrotic local lesions. The observed differences in the herbaceous host range were probably due to different virus strains or environmental influences, such as different greenhouse temperatures.

One of the four inoculation buffers tested was effective in facilitating the transmission of PNRSV from rose to cucumber cotyledons. This buffer (0.01 M phosphate buffer pH 7.8, containing 0.001 M Na-DIECA, 2.5% nicotine, and 100 mg ml-1 charcoal) was, therefore, used for subsequent studies. Substances such as tannins and cell constituents consisting of proteins, polysaccharides, and enzymes may be involved in the inhibition of virus infection **(Noordam, 1973 and Hill, 1984)**. Oxidation of phenolic materials is an enzymatic process, depending on the activity of polyphenol oxidase **(Fulton, 1966)**. Copper ion is necessary for the activity of the enzyme, and materials such as Na-DIECA, which chelate the copper ion, will stabilize PNRSV infectivity in extracts. Also, nicotine reduced the precipitation and inactivation of viruses by tannins **(Hill, 1984)**. The presence of these components in the inoculation buffer contributed to the successful transmission of PNRSV from *Prunus* spp. to *C. sativus*. Nicotine was not required for the subsequent transmission of this virus from cucumber to other herbaceous plants. PNRSV was mechanically inoculated to nineteen herbaceous hosts from seven botanical families. Inoculated and systemically infected leaves of cucumber provided infective extracts for host range trials. At least four plants of each of the herbaceous indicators were mechanically inoculated with the sap from the source plants (*C. sativus* cv Beit Alpha) diluted 1:1 (w/v) with the inoculation buffer (0.01 M phosphate buffer pH 7.2, containing 0.001 M Na-DIECA and 0.001 M cysteine) and mixed with charcoal (100 mg ml-1, w/v).

Graft transmission:

Typical PNRSV symptoms induced on graft-inoculated seedlings of three different cultivars of rose , also in *P.persica* cv Earli Gold and GF 305 , *P.domestica* cv. Calymax and were generally characteristic of those previously reported for PNRSV **(Németh, 1986)**.

Virus stability in crude sap:

The extracted sap from virus infected cucumbers was infectious by mechanical inoculation to cucumber when diluted to 10^{-3} in inoculation buffer. Infectivity of extracted sap was retained for up to 16 h at room temperature (22°C±3). Sap extracted from virus-infected cucumber remained infectious when heated to 57°C for 10 min. these results were reported by

Salem *et al*(2004) with PNRSV –J infected stone fruit in Jordan.

Serological reaction:

All of the PNRSV-infected samples reacted positively only with antiserum the specific for PNRSV using DAS-ELISA. A darker yellow color developed with the positive samples. In all tests, a visual rating of ELISA plates was in agreement with the ELISA reading.

Immunocapture- reverse transcription-polymerase chain reaction (IC-RT-PCR)

The IC-RT-PCR successfully amplified DNA of the expected size from PNRSV-infected rose (*Rosa indica*), but not from the healthy control plant. Gel electrophoretic analysis of IC-RT-PCR products obtained from PNRSV-infected rose leaves using primers Mac1 and Mac2 showed a fragment with the expected size for amplification (450 bp) **(Fig. 2)**. No reaction was observed in samples of healthy rose leaves. Sensitive and reliable methods are needed in certification programs for detection and characterization of plant pathogens.

An immunocapture - reverse transcription- polymerase chain reaction (IC-RT-PCR) protocol was useful for the detection of PNRSV in herbaceous and woody plant tissues. . **(Salem *et al.*, 2004)**

Serological methods (enzyme-linked immunosorbent assay) are routinely employed for these purposes because they allow sensitive, specific, and simultaneous analysis of many samples in a single microplate or membrane **(Casper, 1973)**. However, molecular methods based on polymerase chain reaction (PCR) amplification of the nucleic acids enable greater sensitivity **(Hadidi *et al.*, 1995.)**.

Non-radioactive DNA hybridization technique was used for detection of designed probe to amplify the 3' end of the coat protein gene (RNA-3) of PNRSV in infected rose.

Southern blot hybridization process was valuable for successful detection of PNRSV in infected rose (*Rosa indica*L.) tissue. The reaction between southern blot products transferred from PCR agarose gel and DNA-probe was demonstrated in **(Fig3)**. These products hybridized strongly with their respective non radioactive-labelled probe coat protein gene (RNA-3). No reaction was observed with healthy sample (negative control)

Southern blot hybridization was used as an effective method to confirm the validation of PCR product of the 3' end of the coat protein gene for PNRSV thought the nucleic acid hybridization PNRSV with the DNA probe coat protein gene **(Saade *et al.*, 2000).**

Positive reaction of dot blot hybridization was observed with extracts of leave of **Rose** (*Rosa indica* L.), and *Chenopodium. quinoa* tissues infected with PNRSV **(Fig 4)**. Also strong signal was obtained with PNRSV-coat protein gene region of DNA used as positive control. No signals were observed in samples of healthy control.

Dot blot hybridization is very sensitive than squash blot and would be used for detection of a small quantity of viral DNA in plant tissues, but it gives a non-specific colored background so it can be used in routine diagnosis **(Loebenstein *et al.*, 1997)**.

Heat-therapy treatement:

The treatments resulted in 148 from 500 shoot cuttings rose, peach and plum plants were survived and virus free plants in ratio 29.6 elimination of PNRSV belonging to 5 cultivars treated

with 45, 50, 55, 60 and 65°C for 30, 60, 120 and 180 min **(Table 2)**.In general, the exposure to 45°C for 180 min; 55°C and 60°C for 60 min and 65°C for 30 min gave the best result of virus elimination. Also, the diseased control plants and the plants treated in hot water but not cured, all died during the summer and were discarded.

Schmidt (1974) and **Uyemoto *et al.*(1992)**studied heat treatment of cuttings and shoot tips of virus infected hops(*Humulus lupulus* L.), after treatment hot water young hop plants were produced RMV (*Rose mosaic virus*), PNRSV (*Prunus necrotic ringspot virus*), AMV (*Arabis mosaic virus*) and *hop mosaic virus* were elimated in 34.0, 27.3, 16.7 and 24.3% of plants, respectively. **Gooheen *et al.* (1973)** found that grapevines were freed of the causal agent of viruses disease by immersion of the cutting in water at 45°C for 180 min,50°C for 20 min, or 55°Cfor 10 min.

Table (1): Symptomatology of host plants inoculated mechanically or grafting by PNRSV.

Tested hosts	Symptoms	ELISA test
Family: Amranthaceae		
Gompherena globosa L.	0	-
Family: Apocynaceae		
Vinca rosa L.	0	-
Family:Chenopodiaceae		
Chenopodium amaranticolor Coste & Reyn.	C. L.L.	+
Chenopodium quinoa Wild	C. L.L.	+
Family:Compositeae		
Zinnia elegans L.	Cl.	+
Family:Cucurbitaceae		
Cucumis sativas L. cv. Beit Apha	M&TN	+
Cucumis pepo L. cv.Carina	0	-
Family: Fabacceae		
Phaseolus vulgaris L. cv.Giza 3	0	-
Pisum sativum L. cv. Linkolin	M	+
Vigna unguiculata L.Black eye	N.L.L	+
Family:Rosacae		
*Prunas domestica L.*cv.Calymax*	M&C.L.L.	+
Prunus persica L. cvs: Earli Gold*	C.L.S.	+
GF305*	L.P.	+
Rosa multiflora Thumb cvs.		
Charlesdemills*	M	+
Baronne prevast*	L.P.	+
Rosa setigera Michx cv.Queen Bee*	Mo.	+
Family: Solanneeae		
Datura metal L.	0	-
Datura stramonium L.	0	-
Nicotiana clevelandii L.	0	-
Nicotiana glutinosa L.	0	-
*Nicotiana rustica*L.	0	-
Nicotiana tabacum L. var. *White Burly*	0	-
*Nicotiana tabacum*L.var. Xanthi	0	-
Petunia hybrida Vilm cv. Rosa of Heaven	0	-
Physalis floridana L.	0	-

(+ Positive, - Negative) in ELISA test.

L.L. = Local Lesion. C.L.L. = Chlorotic Local Lesion. * = grafting inoculated
C.L.S.= Chlorotic Local Spots N.L.L.=Necrotic LL
Cl.= Chlorosis; M= Mosaic ; L.P.=Line pattern; Mo.= Mottle; mMo=mild mottle
mM = mild Mosaic ; 0 = no symptoms, N=Necrosis, TN=Top Necrosis

Table (2): Thermal therapy of roses, peach and plum inoculated with PNRSV as determined by ELISA.

Period of immersion cutting on hot water												
min / °C	30			60			120			180		
	I	H	D	I	H	D	I	H	D	I	H	D
45	25	0	0	13	10	2	12	10	3	9	12	4
50	25	0	0	14	7	2	10	9	8	6	9	10
55	17	8	0	10	12	3	8	6	11	3	8	15
60	15	10	0	10	12	3	7	6	12	2	5	18
65	13	12	0	8	8	9	5	2	18	0	2	22
		30			49			33			36	

I= (Infected) Cutting survived but were still diseased.
H= (Healthy) Cutting that survived and were free of PNRSV.
D = (Died) Cutting that were killed by immersion in hot water.

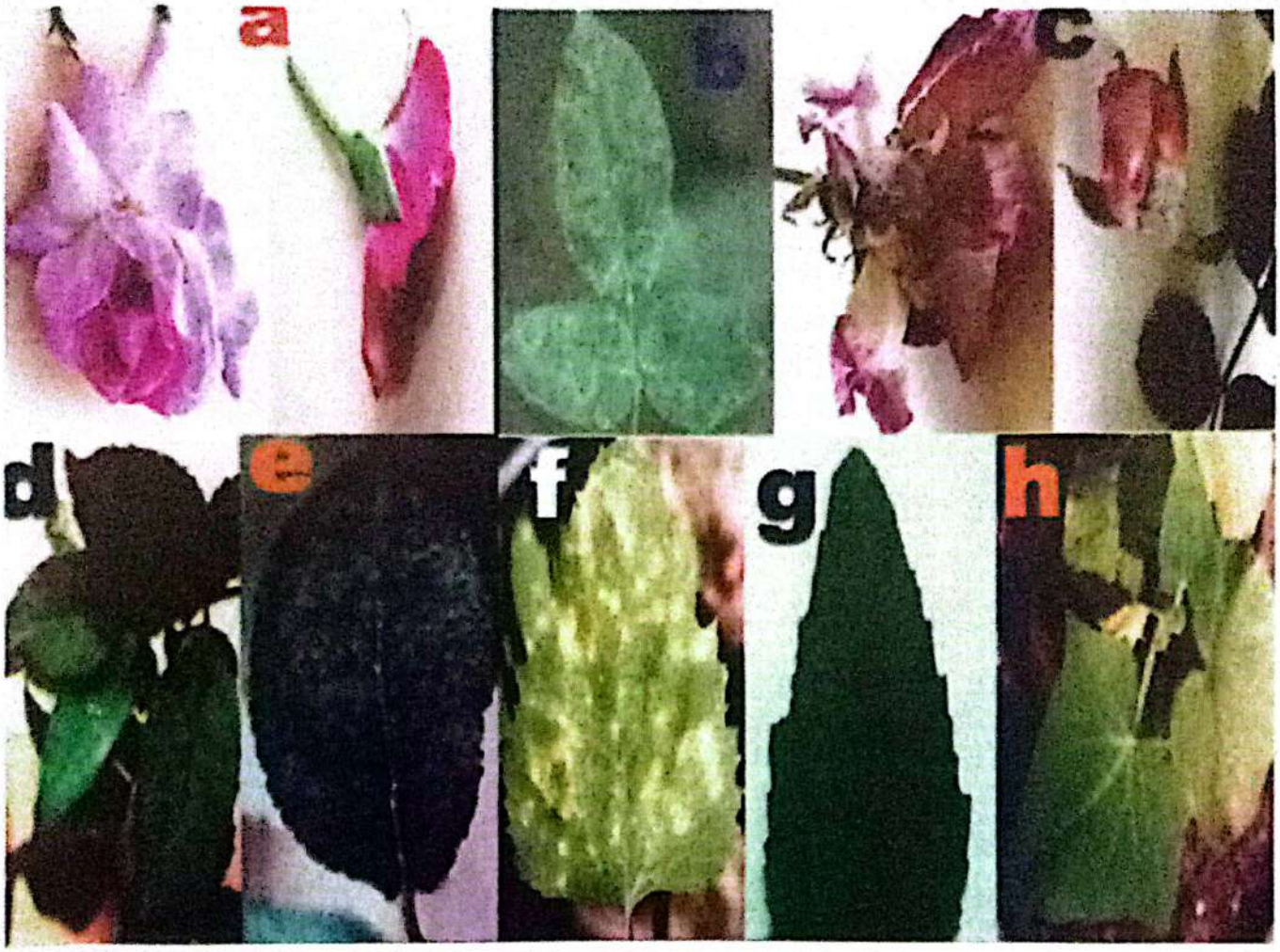

Fig. (1) : Symptoms of PNRSV on naturally and artificially infected host plants:

a: Mosaic and chlorotic spots appeared on naturally infected *rose* flower.

b: Chlorotic rings appeared on naturally infected *rose* leaves

c: Symptoms on infected rose flower *(Rosa hybrida)* showing mosaic and epinasty

d: Naturally infected rose leaves showing mosaic symptoms.

e: Mosaic and chlorotic spots **PNRSV** symptoms on artificially infected leaves of plum cv. Calymax

f: Chlorotic spots on *ch. amaranticolor* infected with **PNRSV**

g: Necrotic ring spots in *Ch quinoa* **PNRSV** infected leaf.

h: *Cucumis sativus* cv Beit Alpha infected with **PNRSV** showing top necrosis and mosaic symptoms.

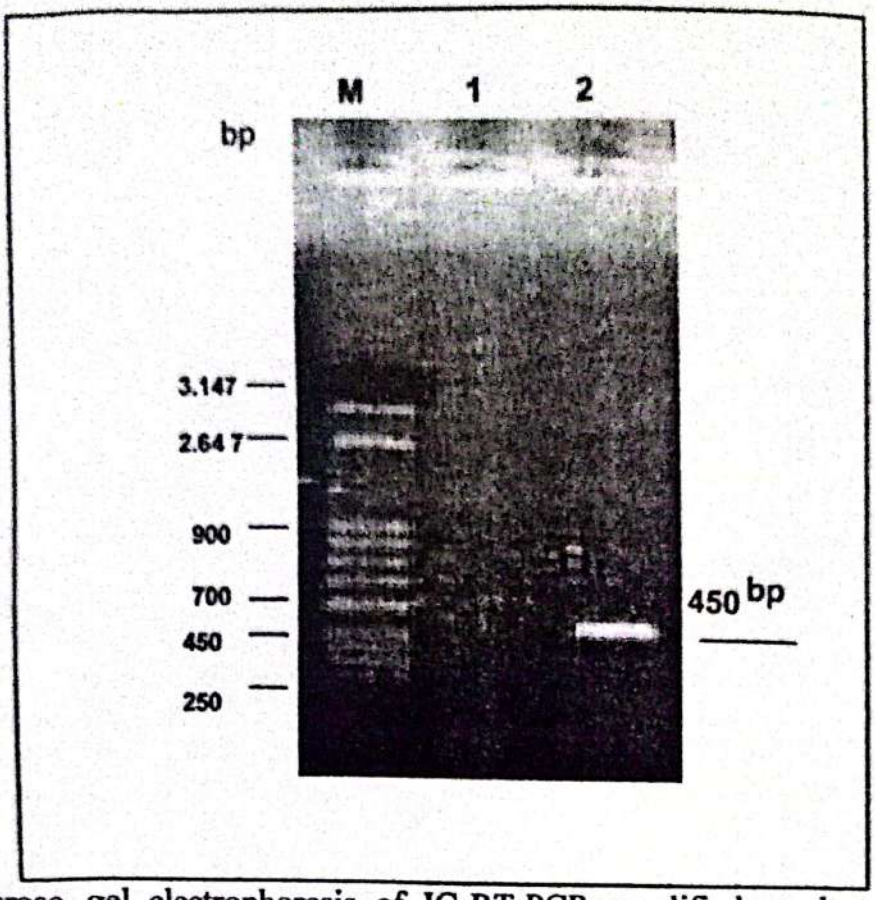

Fig. (2): Agarose gel electrophoresis of IC-RT-PCR amplified product obtained from *Prunus necrotic ring spot virus* (PNRSV) of the 3' end of the coat protein gene (RNA-3). cDNA from immunocapture-reverse transcription- polymerase chain reaction (IC-RT-PCR) using primers Mac1 and Mac2 **Lane M: markers 50bp ladder size (3.147, 2.647, 900,700,450 and 250 bp, Sigma); lanes 2 PNRSV-infected** rose(*Rosa indica L*)leaves; lanes 1, healthy rose leaves. The arrow shows the position of the amplified fragment (450 bp).

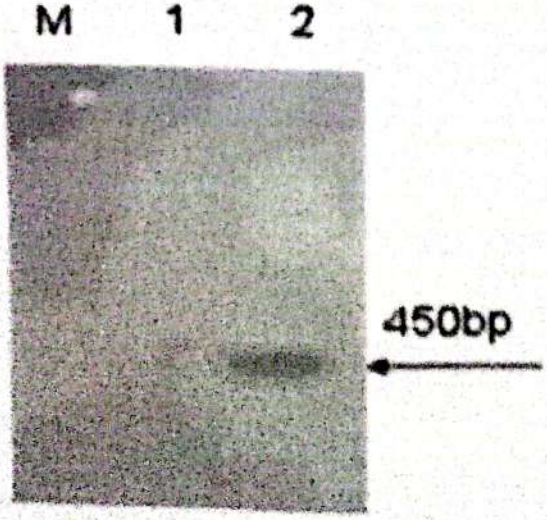

Fig.(3) Southern blot hybridization analysis using Dig- labeled probe of the same samples as for the agarose gel in **Fig. (2).** Positive reaction is shown in lane (2). (M=Marker) Lane 1= samples of healthy rose leaves.

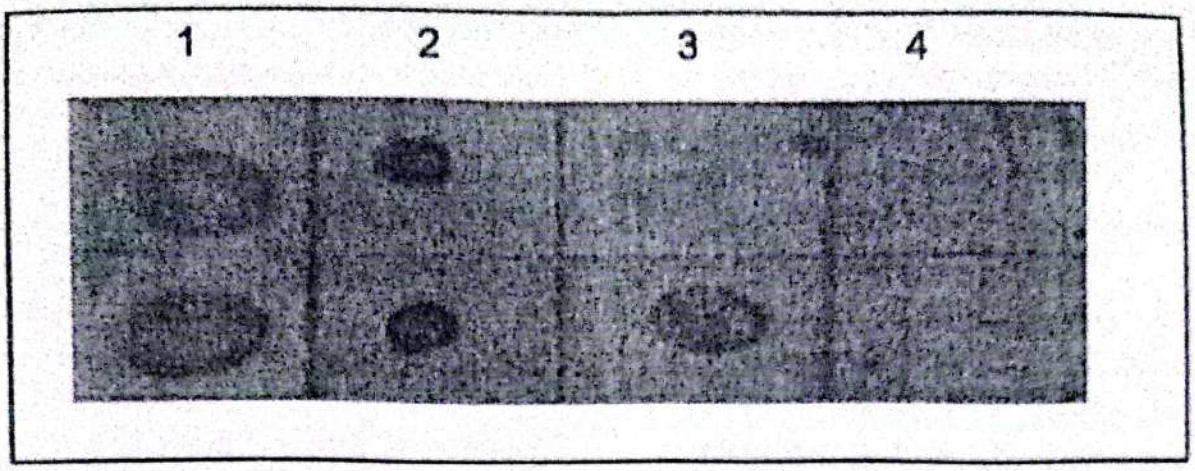

Fig. (4): Dot blot hybridization assay using dig-labeled DNA probe specific for PNRSV .Lanes: (2, and 3) strong signal with infected leaves of rose (*Rosa indica L.)* and *Chenopodium quinoa* respectively. Lane (4) no reaction with healthy sample (negative control). Lane (1) PCR product served as positive control.

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الملخص العربى

التعريف والمعاملة لعلاج النباتات المصابة بفيروس البقع الحلقية الميتة فى الورد

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تم التعرف على فيروس البقع الحلقية الميتة فى شجيرات الورد المصابة طبيعيا والمنزرعة فى حدائق نباتات الزينة بحديقة الأورمان – محافظة الجيزة. ويسبب هذا الفيروس مظهر الحلقات المميز للمجموعة الفيروسية التى يتبعها. وللتعرف على الفيروس المسبب للأصابة تم إستخدام العديد من الأجسام المضادة للفيروسات التى تسبب مظهر متشابة من مظهر الأصابة المسبب للمرض، وبالتالى تم التأكد من المسبب المرضى.وبينت دراسات العدوى أن الفيروس يصيب 12 نوع وصنف من 19 تمت عدواها بالتطعيم أو الأنتقال الميكانيكى وبدراسة الخواص الطبيعية وجد أن درجة التخفيف النهائية للفيروس 10^{-3} كما وجد أن درجة الحرارة المثبطة للفيروس 54°م فى حين أن فترة التعمير فى الأنابيب هى من 10–14 ساعة. ولقد تم التعرف على الفيروس بأستخدام المصل المضاد المتخصص فى الأختبار السيرولوجى (ELISA) وكذلك تم استخدام هذا الأختبار فى تأكيد التجارب المختلفة.

استخدمت تقنية حديثة للكشف عن فيروس البقع الحلقية الميتة فى الورد وهو تفاعل البلمرة المتسلسل المعتمد على اتحاد الفيروس المختبربالأجسام المضادة المناعية المدمصة (Immunocapture-PCR)على سطح صلب و قد أمكن في هذة التقنية الجزيئية استخدام بادئات متخصصةMac1, Mac2 للمسبب مصممة طبقا للتتابع النيوكليوتيدى لعزلة PE5 و استخدم البادىء المتخصص لعزل جين الغلاف البروتينى الموجود فى منطقة RNA-3 وتم الحصول على ناتج 450 زوج من القواعد عند استخدام البادئ .وتم استخدام طريقة تهجين الحامض النووي بنجاح فى الكشف عن فيروس البقع الحلقية الميتة فى الورد وفى النباتات العشبية باستخدام المجس المعلم الخاص بجين الغلاف البروتينى للفيروس بطريقة لونية.

ولقد تم بنجاح أستخدام العلاج الحراري للأجزاء النباتية المأخوذة من الشجيرات المصابة باستخدام الماء الساخن بدرجات حرارة مختلفة وبمدة مختلفة ولقدكانت نتيجة المعاملات المختلفة الحصول على نباتات سليمة بنسبة 29.6 %.