Biological, serological and molecular studies on *Prunus necrotic ring spot virus* infecting *Rosa hybrida* L. in Egypt

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ABSTRACT

Prunus necrotic ring spot virus (PNRSV) was isolated for the first time in Egypt from naturally infected rose plants collected from the experimental farm of the Faculty of Agriculture, Cairo University). Observed symptoms circumvented necrotic ring spots on leaves, bud failure, and color breaking of petals. The virus was transmitted mechanically. The purified virus had Amax and A min at 260 and 240 nm respectively. The 260/280 ratio was 1.56. Yield of purified virus from infected Gompherina globosa was 0.182 mg/g tissue. Electron micrograph of the purified virus showed spherical (23-nm) as well as bacilliform virus particles (42x23 nm). The induced antiserum from the purified virus was successfully used to detect PNRSV in rose plants in several locations in Egypt. The full length of the replicase gene of PNRSV was successfully amplified by reverse transcription-polymerase chain reaction (RT-PCR) using different sets of specific primers. A sensitive and specific IC-RT-PCR protocol was used for the detection of PNRSV from rose tissues. Sequence analysis of PNRSV/rep gene of the rose isolate indicated 60 % similarity to that of PNRSV-AF278534 and NC-004362.

Key words: *PNRSV, Ilarvirus, electron microscopy, DAS-ELISA, rep gene, RT-PCR, IC-RT-PCR, Rosa hybrid.*

INTRODUCTION

Rose (*Rosa hybrid* L.) is the most important crop in the floriculture industry and cut flower roses grown in Egypt. The total planted area of medicinal and aromatic plants including rose was 1215 feddans in 2006 with an income output of L.E 102.421.000 (personal communication).

Roses (*Rosa hybrida*) are grown as garden plants, for the cut-flower industry, and as a source of natural fragrances. The Netherlands, with about 8000 hectares of land under Rose cultivation, is the global leader in Rose cultivation. 5000 hectares in Ecuador are under rose cultivation. Zambia, a small nation, had 80 per cent of its cultivated land under roses (Weiss, 1997; Flament *et al.* 1993).

Many modern cut-flower rose cultivars were selected for long vase life, flower shape, and color. Intensive breeding has also generated garden cultivars that have an intense "rose" scent (Knudsen and Tollsten, 1993).

Viruses that infect rose plants are mostly belonging to the genera *Ilarvirus* and *Neepovirus*. Among ilarviruses, are *PNRSV*, *apple mosaic virus = rose mosaic virus* (Fulton, 1967), and *tobacco streak virus* (Converse and Bartlett 1979). Among nepoviruses, arabis *mosaic virus* and

strawberry latent ring spot virus, alone or in complexes with other ilarviruses are infecting garden and green house roses (Johnston *et al*, 1995).

PNRSV has been isolated in many rose growing regions worldwide (Fulton, 1970; Carol and Moran 1986; Moury *et al.*, 2001, Rakhshandehroo *et al.*, 2006). Being considered as the most common rose virus in Europe, no precise evaluation of *PNRSV* prevalence or incidence is currently available (Moury *et al.* 2001). Similarly no records so far have been set for the presence of *PNRSV* in roses in Egypt.

The symptoms of disease include color breaking, necrotic ringspots on leaves, bud failure, and streaked petals (Moran, *et al.*, 1988).

A major obstacle in detection of *PNRSV* in rose is the low virus titer and the unequal distribution of virus in infected plants (Moury *et al.*, 2001). This problem represents a great challenge in sanitation programs where the need for real healthy root stocks and scions for propagation and breeding is highly needed.

In the recent years, efforts have been geared up to enhance the productivity and quality of cut flowers especially roses as a source of hard currency. However, suspected virus etioles have hindered such efforts. This paper describes, for the first time, the identification and characterization of *PNRSV* on rose plants by symptomatology, electron microscopy, serology, and molecular techniques. Additionally, tissue culture techniques, utilizing antivirus compounds, for the production of virus-free roses are discussed.

MATERIALS AND METHODS

Source of the virus isolate

Samples from rose plants showing symptoms suspected to be due to *PNRSV* infection were collected from the experimental Farm (Faculty of Agriculture, Cairo University) during spring and early summer season. The observed symptoms included necrotic ring spots, on leaves, bud failure, color breaking and streaked petals. DAS-ELISA technique was conducted to test the collected samples using an authentic and specific antiserum for *PNRSV* (SANOFI, Sante Animale, Paris, France). The isolated virus isolate will be referred to as *PNRSV*-rose (*PNRSV*-R).

Other *PNRSV* isolates used

Other virus isolates for *PNRSV* from *Prunus* spp. (Apricot and, Peach) were supplied by Prof. Dr. Aly M. Abdel-Salam as positive controls for ELISA and IC-RT-PCR.

Isolation and propagation of *PNRSV-R*

Naturally infected rose leaf tissues, which reacted positively with DAS-ELISA, were used for virus isolation and identification. About 5 g of naturally infected leaf tissues were ground in 10 ml of buffer (0.01M phosphate buffer, pH 7.4), then inoculated onto carborandom dusted leaves of the local lesion host of *Cucumis sativus* and *Chenopodium quinoa*. The inoculated seedlings were kept in the greenhouse and were observed for symptoms development. Systemic mottled leaves developed on inoculated *C. quinoa* afterwards were used to inoculate the primary leaves of *Gompherena globosa*. The single local lesion technique (Noordam, 1973) was used for biological purification of the virus isolate. Successive leaves with ringspot symptoms reacting positively against *PNRSV* antibodies were served as source of virus inoculum in the subsequent experiments (Abdel-Salam *et al.*, 2006).

Virus purification

PNRSV-R was purified from infected gompherena according to the technique of

electro-elution (Abdel-Salam, 1999; Abdel-Salam *et al.*, 2006). An additional final step was the precipitation of virus suspension with 0.277 g/ml of $(NH_4)_2SO4$. The virus was recovered with low speed precipitation and dialyzed against 1mM phosphate buffer, pH 7.4, for 4-6 hr at 4^0C. Virus concentration was estimated using $E^{0.1\%}_{260}$ of 5.3 (Crosslin and Mink (1992).

Electron microscopic examination (EM)

The carbon-coated grids were floated on drops containing purified preparation of *PNRSV*. The grids were stained with 2% uranyl acetate for 2 min and air dried. Grids were then examined under SEO (Sumy Electron Optics) TEM-100 at the Electron microscopy unit, VACSERA, Egypt.

Serologic tests

Antiserum Production

A polyclonal antiserum specific for *PNRSV* was raised through applying six weekly consecutive injections of purified virus preparation (1 mg/ml, each) into a New Zealand rabbit (Animal Immunization Unit, Faculty of Agriculture, Cairo University). In the first injection, the purified virus was emulsified with complete Freund's adjuvant in an equal volume ratio and injected subcutaneously. In the subsequent injections, incomplete Freund's adjuvant was used and the virus was injected intramuscularly. The rabbit was bled weekly for six weeks. The blood was collected in a 50 ml Falcon tube and centrifuged at 5000 g for 10 min.

Purification of IgG

IgG fractions of *PNRSV* antiserum were separated with caprylic acid according to the technique described by Mckinney and Parkinson (1987).

DAS-ELISA

The DAS-ELISA technique described by Clark and Adams (1977) was used for testing tissue preparations infected with *PNRSV*-R during virus isolation and in surveying the incidence of *PNRSV*-R in the field. Samples were collected from three locations visualized as the Orman Garden in Giza, Governorate, The Horticulture Department at the Faculty of Agriculture, Cairo University, Giza Governorate, and from Sherbeen County, El-Dakahlya Governorate. Sixty samples were collected from each location. To overcome the problem of the unequal distribution of the virus inside infected rose plants, a compound sample technique was followed where six to eight leaves located in different directions on the plant were collected shredded and random tissues were taken for ELISA processing. The O.D. readings were recorded at 405 nm with Tecan Spectra ELISA Reader. Samples with an absorbance of at least twice that of the healthy controls were considered positive for the presence of virus. Percentage of infection was calculated by dividing the total positive samples by the total tested samples then multiplied by 100.

Molecular studies

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Oligonucleotide primers for RT-PCR were derived from the published sequences of *PNRSV* RNA-1 (Terlizzi *et al.* 2001). Total RNAs were extracted from *PNRSV*-infected rose petals and leaves as described by Manning (1991). First strand cDNAs were synthesized in a 20 µl reaction containing 2 µl of total RNA (~10 µg), 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM spermidine, 1 mM each of dNTP, 40 units of ribonuclease inhibitor (Amersham International, Cleveland), 2 units of AMV-RT enzyme (Roche), and 1 µg of each antisense primer separately.

To perform specific PCR amplification for *rep* gene, the primer set Rep 5 ter (+) sense primer (GTT TTT AGT TGT GGT TGA ACT AT) at nucleotide position (1-23) and Rep 5 ter (-) antisense primer (GAA CTT CAA CGT AGT TGG GAG A) at nucleotide position (720-697) complementary to the conserved ultimate 5' terminal region was used to generate 720 bp fragments from *rep* gene (RNA-1). The 3'-end of the *rep* gene was also amplified using Rep (+) sense primer (AGT GAG ATG AAC GCA CTC GAT TCC CAG) at nucleotide position (3167-3194) and Rep (-) antisense primer (GCT TCC CTA ACG GGG CAT CTA CAA CT) at nucleotide position (3306-3332) to amplify 170 bp DNA fragments. The full length *rep* gene was amplified using the primer set Rep 5 ter (+) and Rep (-) to amplify 3332 bp DNA fragments.

The RT reactions were carried out with AMV reverse transcriptase (Roche) and subsequent PCR amplification was done by using *Taq* DNA polymerase (Roche). PCR cocktail included: 2.5 µl of the reverse transcription products, 2.5 µl of 10× buffer (166 mM $(NH4)_2SO_4$, 670 mM Tris-HCl at pH 8.8, and 0.1% Tween 20), 2 mM $MgCl_2$, 0.2 mM each of dNTP, and 25 pmol of each primer in a total volume of 25 µl. The cDNAs were amplified for 35 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. All PCR reactions have been optimized using a technique developed by Cobb and Clarkson (1994) using different concentrations of, cDNAs, $MgCl_2$, dNTPs and primers in order to obtain the best amplification of PCR products. The RT-PCR fragments were analyzed on 1 % agarose at 60 V in TAE (40 mM Tris/acetate, 1 mM EDTA, pH 8.0) and stained with ethidium bromide.

IC-RT-PCR

The PCR primers used in this technique were the Rep 5 ter (+) sense primer and the Rep 5 ter (-) antisense primer The IC-RT-PCR was performed in a sterile micro Amp PCR tubes (0.5 ml) coated with anti-*PNRSV* IgG (150 µl) as for ELISA and at the same dilution for 3 hr at 37°C. The tubes were then washed once with PBS-T. A 150-µl aliquot of plant extract supernatants obtained as for ELISA was added, and the tubes were incubated either overnight at 4°C or for 3 hr at 37°C. The micro tubes were washed one to three times with PBS-T, briefly centrifuged, and the remaining droplets were removed. Micro tubes were reheated for 15 min at 65°C before adding 50 µl of the RT-PCR mix: 10 mM Tris-HCl (pH 9), 1.5 mM $MgCl_2$, 50 mM KCl, 0.3% (vol/vol) Triton X-100, 250 µM each dNTP, 1 µM each of the two primers, 2 U of AMV reverse transcriptase (Roche), and 1 U of *Taq* DNA polymerase (Roche). After vortexing and spinning down the droplets, the reaction mix was placed in the thermal cycler. The cycling parameters used, performed on a UNOII cycler (Biometra), was 1 hr at 42°C (reverse transcription); 2 min at 94°C; followed by 40 cycles of amplification of 1 min at 92°C, 1 min at 52°C, and 90 sec at 72°C. At the end of the program, the micro tubes were held for 10 min at 72°C. Ten-microliter aliquots of the amplification reactions were analyzed by 1 % agarose gel electrophoresis.

Molecular cloning

The DNA fragment (720 bp) encoding partial sequence of the *rep* gene of *PNRSV* (rose isolate) was purified from the agarose gel using GFX Agarose Gel Extraction Kit (Amersham, Pharmacia, Uppsala.) and ligated into the pGEM-T Easy plasmid vector system supported by Promega (Madison, Wisconsin, USA) according to Maniatis *et al.* (1987) and

Spiegel *et al.* (1999). The protocol of Hanahan (Hanahan and Meselson, 1983) was used for bacterial transformation in *E. coli* (JM109) cells with the ligation reactions. The recombinant DNA plasmids containing the *rep* gene of *PNRSV* were purified from the transformed cells using the High Pure plasmid preparation kit (Roche). Successful clones were validated by PCR using Rep 5 ter (+) / Rep 5 ter (-) primer pairs.

Automated DNA sequencing

DNA sequencing of the partial sequence of the rep gene (~720 bp) was performed using ABI prism 310, an automated DNA sequencer at Gene Analysis Unit, VACSERA, and Cairo, Egypt. The resulting sequence for *rep* gene was then compared to published sequences of *PNRSV* isolates using the Clustal method (Thompson *et al.*, 1994). A phylogenetic tree of PNRSV/*rep* was also performed on all of the sequences generated in this study as described by Olsen *et al.* (1994).

RESULTS AND DISCUSSION

Sources of virus isolate

The virus under investigation was isolated from naturally infected rose plants grown in the Faculty of Agriculture, Cairo University. The virus was identified on the basis of symptomatology, mechanical transmission, and morphology of virus particles, serology, and molecular testing. All data obtained confirmed that the virus under study was *prunus necrotic ringspot virus* (*PNRSV*).

Symptomology

Infected rose plants with *PNRSV*-R showed symptoms of ringspots, color breaking, and shoot holes on the petals and streaked necrosis on the stems (Fig. 1-A,B and D). In some rose plants, flowers remained undeveloped and malformed (Fig.1-C). These symptoms are similar to those described by Moury *et al.* (2001) upon infection of roses with *PNRSV*. Carol *et al.* (1986) showed the effect of *PNRSV* infection on the induction of malformed flowers. Leaves in infected plants develop ringspots which later are changed to shoot holes afterwards (Fig. 1-E). Bud failure on *PNRSV*-infected roses (Fig.1-F) seems to be a common denominator between *PNRSV* isolates infecting peach and apricot (Abdel-Salam *et al.*, 2007 in press). Therefore the bud failure syndrome should be considered as a prominent indication for the presence of *PNRSV*.

Virus purification

Purified virus had A_{max} and A_{min} at 260 and 240 nm, respectively. The 260/280 ratio was 1.56 (Fig. 2). Yield of purified virus was 0.182 mg/g tissue. The previously measured physical properties of the purified *PNRSV*-R indicate its nature as a nucleoprotein. Further, the 260/280 ratio of 1.56 is typical to what have been reported for several isolates of *PNRSV* by Fulton (1981) and Abdel-Salam *et al.* (2006).

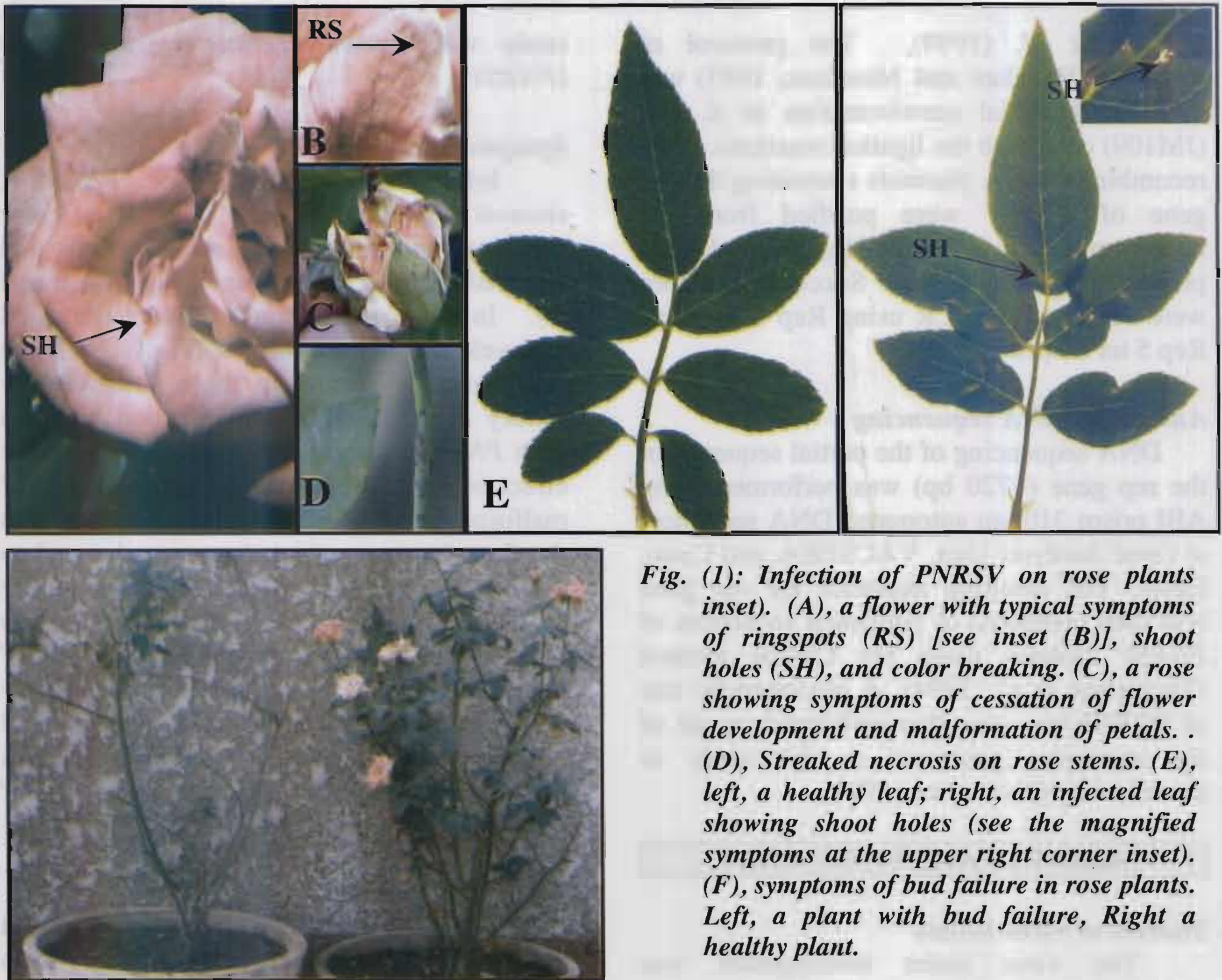
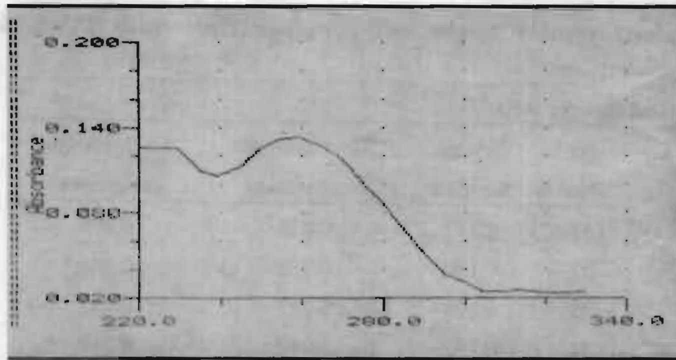


Fig. (1): Infection of PNRSV on rose plants inset). (A), a flower with typical symptoms of ringspots (RS) [see inset (B)], shoot holes (SH), and color breaking. (C), a rose showing symptoms of cessation of flower development and malformation of petals. . (D), Streaked necrosis on rose stems. (E), left, a healthy leaf; right, an infected leaf showing shoot holes (see the magnified symptoms at the upper right corner inset). (F), symptoms of bud failure in rose plants. Left, a plant with bud failure, Right a healthy plant.

Shape and size of virus particles

Electron microscopic examination (Fig. 3) reveled that *PNRSV*-R had isometric (*ca* 23 nm, 25 nm and/or bacilliform particles (42 x 23 nm). Fulton (1970, 1981) showed that *PNRSV* virions were spherical with mostly 23 nm in diameter. Other particles, however, were larger in size. Fulton (1981) also reported the presence of bacilliform particles. These results are in agreement with the obtained result in the present study.



Wave Lengths

Fig. (2): Ultra-virolet spectrum of purified PNRSV-R purifed with the electro-elution technique.

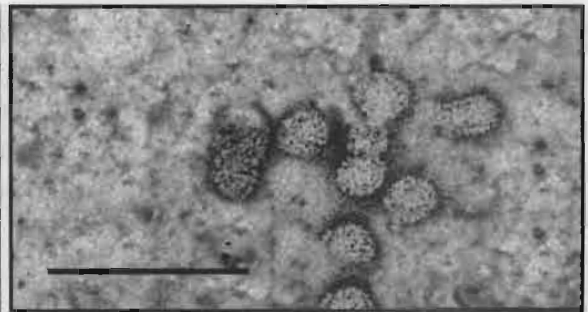


Fig. (3): An electron micrograph of purified PNRSV-R preparation stained with 2% Uranyl acetate. Bar = 100 nm

Serologic studies

Separation of the IgG fraction

The IgG fraction of the prepared antiserum was successfully separated using caprylic acid. It had an A_{max} and A_{min} at 280 and 250 nm, respectively (Fig. 4). The obtained UV spectrum Indicate the IgG nature as a protein.

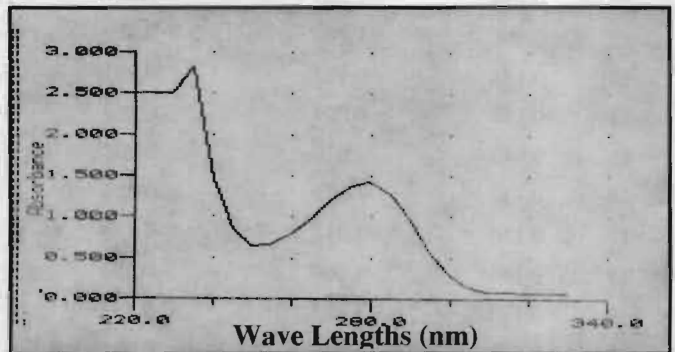


Fig. (4): Ultra-violet spectrum of the IgG fraction (1 mg/ml=1.4 O.D) of PNRSV-R antiserum after separation with caprylic acid.

Field survey for PNRSV-R in rose plants

Primary field survey for *PNRSV*-R using DAS-ELISA in three locations (Table 1) indicated a high-virus incidence in samples collected from the Sherbeen, Dakahlya (65%) and The Horticulture Department, Cairo University (80%). The least infection was in the Orman Garden being 13.3%. These results, though indicate the presence of *PNRSV* in rose plants, do not imply the presence of an epidemic situation, because only a total of 180 samples were collected from the three locations which are not enough to draw a wide conclusion. Also the infection in Orman Garden was low comparing to the Horticulture Dept., Cairo University, where both locations are closely adjacent. Since *PNRSV* is known to be transmitted in roses by root stock cuttings and scions, selection for healthy cuttings should be of great importance in disease reduction.

Table (1): Field survey of rose (Rosa hybrida) plants suspected for infection with PNRSV different location in Egypt.

ID No	Optical Density (OD)*			ID No			
	Orman Garden	Horticulture Department	El-Dakahlya (Sherbeen)		Orman Garden	Horticulture Department	El-Dakhalya (Sherbeen)
1	0.166	1.031	0.137	31	0.112	0.825	0.396
2	0.154	1.191	0.365	32	0.179	0.952	0.344
3	0.154	1.387	0.338	33	0.194	1.137	0.135
4	0.173	1.004	0.347	34	0.198	1.010	0.240
5	0.155	1.119	0.124	35	0.174	0.757	0.635
6	0.108	0.995	0.425	36	0.174	0.759	0.552
7	0.154	1.440	0.360	37	0.171	0.995	0.133
8	0.107	1.110	0.321	38	0.162	0.677	0.303
9	0.158	0.793	0.133	39	0.158	0.126	0.260
10	0.156	1.261	0.339	40	0.166	0.109	0.245
11	0.134	1.240	0.337	41	0.255	0.165	0.111
12	0.160	1.240	0.419	42	0.176	0.517	0.166
13	0.154	0.912	0.101	43	0.160	0.217	0.478
14	0.159	0.879	0.315	44	0.360	0.430	0.123
15	0.143	1.010	0.401	45	0.160	0.107	0.131
16	0.152	1.025	0.304	46	0.698	0.130	0.341
17	0.150	1.012	0.136	47	0.854	0.493	0.764
18	0.146	1.060	0.352	48	0.159	0.122	0.336
19	0.172	1.172	0.352	49	0.171	0.642	0.350
20	0.169	1.149	0.324	50	0.586	0.221	0.399
21	0.158	1.184	0.112	51	0.207	0.330	0.899
22	0.157	1.212	0.386	52	0.725	0.751	0.110
23	0.239	1.252	0.340	53	0.295	0.119	0.423
24	0.157	1.084	0.322	54	0.241	0.624	0.162
25	0.175	0.587	0.131	55	0.173	0.425	0.306
26	0.170	0.927	0.346	56	0.166	0.116	0.134
27	0.125	0.602	0.343	57	0.693	0.173	0.128
28	0.159	0.721	0.359	58	0.182	0.107	0.787
29	0.169	0.641	0.127	59	0.125	0.588	0.386
30	0.198	0.601	0.360	60	0.288	0.711	0.303

OD = Optical density at 405 nm for Positive control = 1.440 & Negative control = 0.138. Samples with reading more than two folds of the negative control are considered as positive and marked with bold font.

Percentage of infected samples = Number of infected samples / Total number of samples.

Orman garden =13.3% Horticulture Department =80% El-Dakhalya Gov. =65%

Molecular studies

RT-PCR detection of PNRSV-R in rose tissues

RT-PCR successfully detected *PNRS* viral RNA in rose tissues and in purified virus respectively. A full length *rep* gene DNA. fragments about 3.3 kb in size were detected from purified virus preparation or from infected rose tissues by using specific primers for PNRSV *rep* gene (Fig.5). The size of all PCR products fell within the estimated range of *rep* gene primers. No equivalent DNA product could be generated from uninfected tissues by the same procedure. Similar results were obtained by other authors using RT-PCR for the detection of *PNRSV* in roses and stone fruits (Moury *et al.*, 2001; Ulubas and Ertunc, 2004).

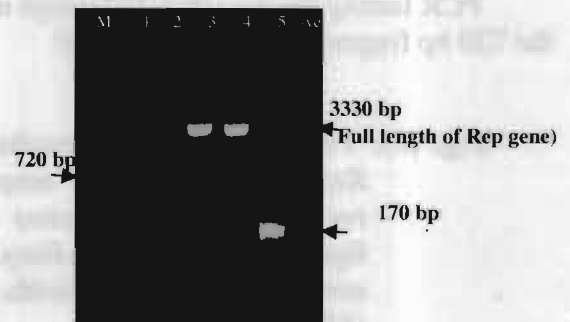


Fig. (5): A multiple RT-PCR results where the 3330 bp fragment corresponds to the full length replicase gene of PNRSV isolated from rose tissue (Lanes 3); from purified virus (Lane 4). Lanes 1 & 2 corresponds to the 5'-terminal of the rep gene amplified from rose tissues and purified virus respectively) and the 170 bp fragment corresponds to 3'-terminal of the rep gene amplified from rose tissue (Lane 5). Lane 6: negative control for PCR (No cDNA template). M: Molecular weight DNA marker (100 bp ladder).

IC-RT-PCR for PNRSV detection in rose

The efficiency of IC-RT-PCR was improved by changing the number of washings before reverse transcription, and adjusted pretreatment temperatures and reverse transcription incubation time. Positive PCR reactions lead to the amplification of the expected 720 bp DNA band. The most crucial step in the IC-RT-PCR protocol was the number of washings before reverse transcription. At a 10^{-1} dilution factor (in PBS buffer), a single washing is adequate for IC-RT-PCR amplification from cucumber cotyledon extracts (Fig.6 , Lane 1), whereas three washings were necessary for peach leaves, apricot leaves, rose leaves, and/or rose petals (Fig.6 , lanes 2, 3, 4, and 5, respectively. Such results indicate that the increment of washing removes unbound plant material associated with virions attached to specific antibodies. This, in turns, removes any taq-polymerase inhibitors and increases the exposure of the nucleic acid template to the enzyme. Preheating the tubes (65°C for 15 min) before reverse transcription increased the efficiency of IC-RT-PCR especially in tissues containing viscous materials as in roses. This can be attributed to the heat effect in loosening up and/or degrading the virus coat protein and exposes the nucleic acid target to reverse transcriptase enzyme. Similar results obtained by Rosner *et al.* (1998) and Moury *et al.* (2000) upon using IC-PCR for the detection of *PNRSV* have verified our above mentioned finding.

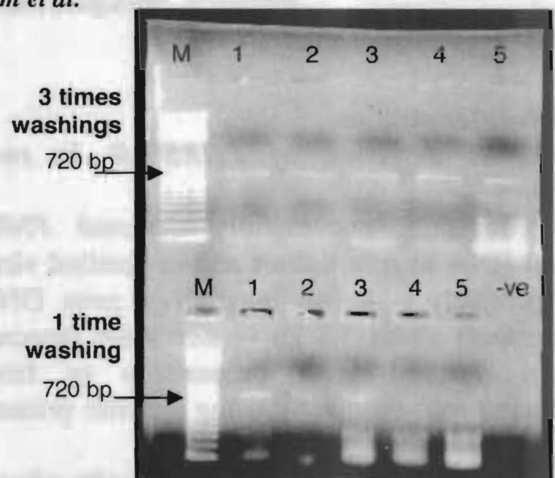


Fig.(6):Immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) showing the effect of plant extracts and the number of washings before reverse transcription on the detection of Prunus necrotic ringspot virus. 1: cucumber cotyledon; 2: peach leaves 3: apricot leaves; 4: rose leaves and 5: rose petals. All samples are diluted 10^{-1} in extraction buffer.

Molecular cloning

PCR testing for the DNA minipreps of the 720 bp fragment of the *rep* gene of *PNRSV*-R indicated the validity of the ligation and cloning process (see Fig. 7, lanes 1, 3, 5, and 5).

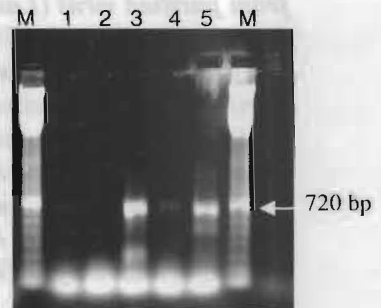


Fig. (7): 1% agarose gel electrophoresis showing the Rep-gene fragment amplified from the recombinant clones lanes 1,3,4, and 5 after ligation into pGEM-T-Easy. Lane 2 contained non-recombinant plasmids. M: 100 bp ladder (Sigma).

Sequence analysis of PNRSV/rep gene

PNRSV/rep gene under study (rose isolate), *Bromoviridae*, was found to have 60% similarity to that of *PNRSV* (AF278534) and (NC-004362) (Terlizzi *et al.*, 2001). Phylogenic data seem to suggest that though it is distantly related to these two isolates, but it belongs to the proposed *PNRSV*-rep of *Ilarvirus* genus. This low similarity might be related to difference of geographic location between the tested isolates especially if we know that *PNRSV* is mostly transmitted vegetatively in roses and most propagation process for rose in Egypt is through local rose cultivars.

In conclusion, based on its similarity in sympto-matology, serology, electron micro-scopy, UV spectral measures, and molecular characters with other isolates of *PNRSV*, we suggest that the isolated virus is an isolate of *PNRSV*. However, further verification for coat protein, movement protein sequence analysis and restriction fragment analysis are needed to study the phylogenic relatedness between the Egyptian *PNRSV*-R. and other isolates of *PNRSV* on a larger scale.

The present study has indicated for the first time, the presence of *PNRSV* in rose plants in Egypt. Effort should be geared up to control this new disease. Sanitation seems to be the key role in combating this dilemma. With the presence of the induced antiserum for *PNRSV*-R, field survey on a larger scale should be conducted in the areas of rose plantations to evaluate the disease incidence precisely. Efforts are being done by our group to induce virus-free roses through tissue culture using antiviral compounds. The out coming results are very promising.

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PNRSV-REP-ROSE   CTCCTTCTCTGATCCTCGACCATCCTTTGCTCTATATTA    40
AF278534_PNRSV   AGACTCTTCGAGTCATAGTTTTGCGGCAGCCCATAGGTTG   40
NC-004362        AGACTCTTCGAGTCATAGTTTTGCGGCAGCCCATAGGTTG   40
Consensus           ct tc    tc t g     c      c ta  tt

PNRSV-REP-ROSE   GTCTCAAAGACTGCTCTTTATAAGTGCTTCATCATAACGA   80
AF278534_PNRSV   TTAGAAACCGATTATATTTATAAGTGCTTCGGCA...CGA   77
NC-004362        TTAGAAACCGATTATATTTATAAGTGCTTCGGCA...CGA   77
Consensus         t   aa    t  t tttataagtgcttc  ca   cga

PNRSV-REP-ROSE   CTGATTTCTTCCCAATCAGCCGATTGATCTTAACCTCTCT   120
AF278534_PNRSV   CTGAAGAATCGGTGATTGATCTTGGGGG...AAATTTTGT   114
NC-004362        CTGAAGAATCGGTGATTGATCTTGGGGG...AAATTTTGT   114
Consensus        ctga    t      at    c   g     aa  t t t

PNRSV-REP-ROSE   TTCTCACATAAATCACTTACGGTCTCATGTGCTACAATCA   160
AF278534_PNRSV   TTCTCACATAAAGCAAAAGAGGTATAATGTGC.ACAGCTG   153
NC-004362        TTCTCACATAAAGCAAAAGAGGTATAATGTGC.ACAGCTG   153
Consensus        ttctcacataaa ca     ggt t atgtgc aca

PNRSV-REP-ROSE   ATAGTCCCCATACCACGATCGCGATGGTGCCAT.CTTACC   199
AF278534_PNRSV   TTGCCCACTTTTGGACGATCGCGATGGTGCCAGATTTACC   193
NC-004362        TTGCCCACTTTTGGACGATCGCGATGGTGCCAGATTTACC   193
Consensus         t   c c  t    acgatcgcgatggtgcca   ttacc

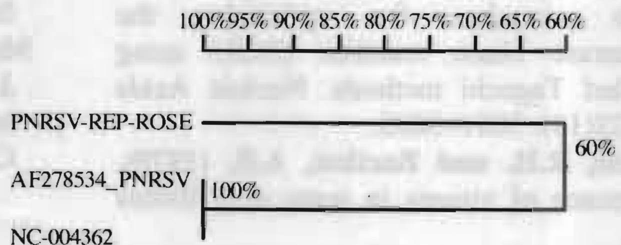
PNRSV-REP-ROSE   GTTCATTAGATCTCATTGAAGACCTACTAACGAACATACA   239
AF278534_PNRSV   GAACGTTTGATCTCATTGAAGACCTACTTGCGAACGCACA   233
NC-004362        GAACGTTTGATCTCATTGAAGACCTACTTGCGAACGCACA   233
Consensus        g  c tt gatctcattgaagacctact  cgaac  aca

PNRSV-REP-ROSE   ATGACCTCCGACATGACGCACACTCC.....ATGTTCCTT   274
AF278534_PNRSV   AGGAGGAAAGACATGAGGCAGACTATTGCGAATGCCGCTT   273
NC-004362        AGGAGGAAAGACATGAGGCAGACTATTGCGAATGCCGCTT   273
Consensus        a ga      gacatga gca act       atg   ctt

PNRSV-REP-ROSE   CGATCAGTGCCCGAGAACTCAGGGCCGTCATCAATTGACT   314
AF278534_PNRSV   CGAGGAGTGCCCGAGAA...GGGCCGATTATGTGATGGCT   310
NC-004362        CGAGGAGTGCCCGAGAA...GGGCCGATTATGTGATGGCT   310
Consensus        cga  agtgcccgagaa    gg c  t at     tg ct

PNRSV-REP-ROSE   CCA                                        317
AF278534_PNRSV   GTG                                        313
NC-004362        GTG                                        313
Consensus
```

A



B

Fig. (8):(A). Alignment of a 310 nt stretch in RNA1 of PNRSV (rose isolate) with portions of the replicase region of Prunus necrotic ring spot virus (PNRSV) (AF278534) and (NC-004362). Conserved sequences are indicated below the alignment. Nucleotide sequences common to PNRSV are boxed. (B). Phylogenetic tree based on the alignment of the RNA 1 (replicase gene) (Di Terlizzi et al.2001) of Bromoviridae and PNRSV-rose isolate using the CLUSTALX package (Thompson et al. 1997).

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الملخص العربي

دراسات بيولوجية وسيرولوجية وجزيئية على فيروس التبقع الحلقي النيكروزي الذي يصيب الورد في مصر

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تم للمرة الاولى في مصرعزل فيروس البقع الحلقية الميتة في البرقوق Prunus necrotic ring spot virus (*PNRSV*) من نباتات الورد المصا بة طبيعيا بالفيروس والمزروعة في مشاتل الزينة بالمزرعة التجريبية بكلية الزراعة جامعة القاهرة بمصر. وتمثلت مظاهر الاصابة الاساسية على تكون البقع الحلقية على الاوراق وتكسر لون البتلات وتشوهها وكذا عدم تكشف البراعم في النباتات المصابة. واظهرت الدراسة التجريبية امكانية نقل الفيروس ميكانيكيا. وبينت خواص الفيروس المنقى ان اعلى وادنى امتصاص للاشعة فوق البنفسجية كان عند ٢٦٠ و ٢٤٠ نانوميتر على التوالي. تركيز يبلغ 0.182 mg من انسجة نبات *Gompherina globosa* . وبينت دراسات المجهر الالكتروني تواجد جزيئات كروية (23-nm) واخرى ذات شكل باسيلي (42x23 nm) في تحضيرات الفيروس المنقى ولقد تمت بلمرة جين الـ replicase الخاص بالفيروس بواسطة انزيم النسخ العكسي لتفاعل تسلسل البلمرة reverse transcription-polymerase chain reaction (RT-PCR) بأستخدام بادئات متخصصة. كذلك امكن تطويع اختبار الارتباط المناعي المدمج مع تفاعل تسلسل البلمرة IC-RT-PCR في الكشف على الفيروس في التحضيرات غير المنقاة للنباتات المصابة بالفيروس. . وقد اوضحت دراسات التتابع النيوكليوتيدي والخاص بعزلة الورد المصرية لـ PNRSV وجود درجة تشابه تبلغ ٦٠% مع العزلتين PNRSV-AF278534 و NC-004362 والمسجلتين في بنك الجينات الامريكي.