Molecular and serological studies on an egyptian isolate of plum pox potyvirus

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ABSTRACT

In this study, an Egyptian isolate of plum pox potyvirus (PPV), isolated from El-Amar apricot trees, was propagated on Nicotiana benthamiana for virus purification using a modified procedure was applied. Ultraviolet absorption spectrum of the purified virus showed a typical curve of nucleoprotein with an $A_{260/280}$ ratio ranged from 1.258 to 1.280. A yield of the purified virus ranging from 20 to 22 mg/kg virus-infected leaf tissues was obtained. The electron microscopy of the purified virus preparation, negatively stained with 2% uranyl acetate, proved the presence of flexuous filament virus particles (700-725 x 13-15 nm). The molecular weight of PPV-coat protein (CP) was about 37 KDa when estimated by 12% SDS-PAGE. ssRNA of about 10 kb was extracted from the purified PPV preparation. Polyclonal antibodies against PPV were produced and the antiserum titer of three bleedings were determined by indirect-enzyme linked immunosorbent assay (I-ELISA). The IgGs specific to the PPV virions (intact virus particles) were purified and N- and C-terminal specific IgGs were selected and successfully used to distinguish the CP subunits of PPV-El-Amar isolate via western blot analysis. From PPV-infected leaf tissues, cDNA products were amplified via immunocapture-reverse transcription-polymerase chain reaction (IC/RT-PCR) using two specific primers for 3`-terminal region of PPV-cp gene. This was followed by restriction endonuclease analysis. The results revealed that the virus isolate under investigation is a strain of PPV and belongs to either M serotype due to the absence of Rsa I restriction site.

Key words: *Plum pox potyvirus (PPV), purification, polyclonal antibodies, Western blotting, ELISA, Immunocapture/reverse transcription-polymerase chain reaction IC/RT-PCR, restriction endonuclease analysis.*

INTRODUCTION

Plum pox potyvirus (PPV), the causal agent of plum pox disease (sharka), is a member of family potyviridae, genus potyvirus (Brunt *et al.*, 1996). Sharka disease is a series disease affecting stone fruit trees (SFTs) in Europe and Mediterranean regions. Apricot, peach and plum are the most important SFTs grown in Egypt, and are susceptible to infection with several viruses. Among such viruses, PPV is causing severe

losses to the infected SFTs (Nemeth, 1986 and Roy and Smith, 1994).

The virus is transmitted by aphids, mechanical means, and grafting. The virus particles are flexuous filaments with a length 660-770 and 12.5-20 nm width (Brunt *et al.*, 1996). Infected cells contain amorphus x-bodies, pinwheel, laminated aggregates as well as nuclear inclusions (Brunt *et al.*, 1996 ; Abdel-Ghaffar *et al.*, 1998a).

The PPV genome consists of a positive sense single-stranded RNA of approximately 10 kb in length (Maiss *et al.*, 1989) with a virus-encoded protein (Vpg) covalenty linked to 5` terminus of the genome, and a poly (A) tail at 3` terminus. The complete nucleotide sequence of several PPV isolates has been determined (Lain *et al.*, 1989, Maiss *et al.*, 1989 and Teycheney *et al.*, 1989). Reverse transcription-polymerase chain reaction (RT-PCR), restriction fragment length polymorphism (RFLP), molecular hybridization and nucleotide sequencing were used by many investigators to identify and designate the PPV isolates (Varveri *et al.*, 1988, Korschineck *et al.*, 1991 ; Wetzel *et al.*, 1990, 1991b and 1992).

Serological analysis of the N-and C-terminal parts of the coat protein, as used for other potyviruses (Shukla *et al.*, 1988 ; 1989), offers another possibility to evaluate the relationships between different PPV isolates. These isolates are belonging to three serotype groups called PPV-D (Dideron isolate), PPV-M (Markus isolate) (Kerlan and Dunez, 1979, Wetzel *et al.*, 1991a ; Bousalem *et al* 1994) and PPV-C (cherry isolate) (Nemchinov and Hadidi, 1996).

In Egypt, there were a few studies on PPV (Dunez, 1988, Wetzel *et al.*, 1991b, Mazyad *et al.*, 1992 ; Abdel-Ghaffar *et al.*, 1998a). Therefore, this study aimed to develop virus purification method for increasing the purification yield, determining some molecular properties of the purified virus particles, raising polyclonal antibodies against to PPV and selection of N-and C-terminal specific IgGs. In addition, distinguishing the virus isolate by a combination of immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) and restriction endonucease analysis was also aimed.

MATERIALS AND METHODS

Source of virus isolate

The virus isolate (PPV-EL-11) was obtained from naturally infected apricot (*Prunus armeniaca* L. cv. Amar) trees cultivated in the open field of El-Amar farms, located in El-Kalubia governorate, Egypt. The isolate was identified in the Virol. Lab., Faculty of Agric., Ain Shams Univ., as a PPV isolate based on some biological properties and using I-ELISA using the polyclonal antibodies produced in 1998 by Abdel-Ghaffar *et al.* (1998a).

Virus propagation

For purification, the viral isolate under investigation was propagated on *Nicotiana benthamiana* and/or *N. clevelandii* as described by Abdel-Ghaffar *et al.* (1998a).

Virus purification

On the basis of the method given by Abdel-Ghaffar *et al.* (1998a) a procedure with some modifications was developed to improve the purity of virus preparation. PPV-infected leaves of *N. benthamiana* plants, 21 days post virus inoculation and grown in the greenhouse, were harvested and homogenized in a pre-chilled cold warring blender with two volumes ice-cold of extraction buffer. This buffer consists of 0.1 M Tris, pH 7.5 containing 10 mM sodium sulfite, 2 mM EDTA, 0.1% (v/v) 2-mercaptoethanol (2-ME) and 1% (v/v) Triton X-100. The homogenate was then

stirred at 4°C for 30 min, then filtered through double layer of cheesecloth. The resultant sap was clarified by addition of 10% (v/v) of a mixture cold chloroform:n-butanol (1:1, v/v), then centrifuged at 9,000 rpm in a Sorvall GSA rotor for 15 min at 4°C. The aqueous phase was collected and centrifuged at 25,000 rpm in a Beckman 70.1 rotor for 90 min at 4°C. The pellets were resuspended in 0.01M Tris buffer, pH 7.5 containing 0.1% 2-ME and 1% Triton X-100. The suspension was subjected to a further high speed centrifugation at 28,000 rpm for 90 min at 4°C through a 20 % (w/v) sucrose cushion prepared in 0.01 M Tris buffer, pH 7.5 (resuspension buffer, RB). The pellets were resuspended in RB and centrifuged at 9,000 rpm for 15 min at 4°C. The supernatant was layered on the top of 10 to 40% sucrose gradient, then centrifuged at 26,000 rpm in a Beckman SW 28 rotor at 4°C for 2.5 hr. Fractions were separated manually and subjected to spectrophotometry. Fractions, which showed virus-like peak, were collected and centrifuged at 50,000 rpm for 3 hr. The virus concentration was estimated spectrophotometrically using an extinction coefficient of 2.8 for a 1-mg/ml solution (Stace-Smith and Tremaine, 1970). Thc percentage of virus nucleic acid was calculated according to an equation described by Gibbs and Harison (1976).

Electron microscopy

The purified PPV preparations were negatively stained with 2% aqueous uranyl acetate (w/v) and examined with a Zeiss 10 C Transmission Electron Microscope at National Research Center, Dokki, Cairo, Egypt. The diameters of the virus particles were determined after printing the micrographs.

Electrophoresis of virus coat protein (CP)

Aliquots of purified virus preparation were dissociated by boiling for 5 min in Laemmli buffer (Laemmli, 1970). Protein samples were subjected to electrophoresis in 12% SDS-polyacrylamide slab gels using a discontinuous buffer system (Laemmli, 1970). The bands were visualized by staining the gel with Coomassi brilliant blue in the presence of a standard protein marker for estimation of the molecular weight of the viral CP.

Production of PPV-polyclonal antibodies (PAbs)

PAbs against the PPV isolate under investigation were raised according to the method described by Abdel-Ghaffar *et al.* (1998a). Two adults New Zealand white rabbits were injected subcutaneously followed by intramuscular with purified viral preparation. The rabbits were bled three times, started one, two and three weeks after the last injection. The antiserum was separated and collected, then overnight-cross absorbed at 4□C with 5% (w/v) extracted sap of healthy *N. benthamiana* plants prepared in TBS-Tween solution (10 mM Tris-HCl buffer, pH 8.0, 150 mM NaCl, 0.05% Tween 20). The mixture was then frozen for 3-4 hr, thawed and centrifuged at a low speed. The titer of the three bleedings was determined by indirect-enzyme-linked immunosorbant assay (I-ELISA) according to the methods described by Clark and Adams (1977) and Baunoch *et al.* (1992).

Isolation and purification of immunoglobulins G (IgGs)

IgGs against PPV-virion (PPV-IgGs) were isolated and purified from the crude antiserum; which gave a high titer by I-ELISA, according to the method described by Perosa *et al.* (1990) with some modifications. The antiserum was dialyzed against sodium acetate buffer, pH 4.8, then the caprylic acid was added followed by centrifugation at a low speed. The supernatant containing the IgGs

was collected and dialyzed against potassium phosphate buffer, pH 7.2, then precipitated by saturated ammonium sulfate solution and a low centrifugation. The pellets were resuspended in distilled water followed by dialyzing against phosphate buffer, pH 7.2, and stored at -20°C.

Selection of N- and C-terminal specific PPV-IgG

Removal of N- and C-terminal peptides of the coat protein from the virion (intact virus particles, IVPs) was carried out with affinity chromatography according to the method of Bousalem *et al.* (1994). To 10 mg freshly purified virus, 60 μg of trypsin (Sigma type XIII, TPCK treated) were added and the mixture was then incubated for 30 min at 25°C in a water bath. The enzyme-resistant core particles were separated from the N- and C-terminal peptides by centrifugation at 50,000 rpm for 90 min at 4°C. The pellets were resuspended in 0.02 M borate buffer, pH 8.0 and dissociated as described by Shukla *et al.* (1988). The protein preparation was coupled to 1 g CNBr sepharose 4 B (Sigma) according to the manufacturer,s instructions. Hundred μl of IgGs (1 mg/ml) were loaded onto the column in 20 ml phosphate buffer saline (PBS). The unbound IgGs were collected and stored at -20°C. The IgGs fraction isolated from the antiserum for the IVPs was termed as PPV-IgGs, and for the N-, and C-terminals was termed as PPV-N, C-IgGs.

Western blotting

Western blotting was performed using the method previously used for potyviruses (Quiot-Douine *et al.*, 1990). Proteins from 10-fold diluted sap extracts of infected and healthy apricot leaves as well as PPV, PPV (trypsin-treated), PVY and ZYMV-purified preparations (15 μg) were first separated by SDS-PAGE on discontinuous denaturing 12% polacrylamide slab mini-gel (Laemmli, 1970). The protein bands were then electro-transferred onto a nitrocellulose membrane as described by Towbin *et al.* (1979) and detected immunologically. Polyclonal IgGs either against intact PPV particles or against to N- and C-terminal peptide regions of PPV-CP were used as a primary antibody. Bound antibodies were visualized using a goat anti-rabbit IgG conjugated with alkaline phosphatase (as a secondary antibody), and nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate color development reagent as recommended by the supplier (Sigma, USA).

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

PPV-RNA was extracted from purified virions using the method described by Abdel-Ghaffar *et al.* (1998b). The IC/RT-PCR was performed according to the protocol of Wetzel *et al.* (1992). Sterile Eppendorf tubes were coated with PPV-IgG (N- and C-terminal specific PPV-IgG), and two-fold dilutions of PPV-infected apricot and *N. benthamiana* tissue extracts, as well as the purified virus preparation and healthy tissue samples were added as the antigen source. Triton X-100 solution (1%) heated to 65°C C was added to release the viral RNA; chemical denaturation, RT of viral RNA and amplification of the synthesized cDNA were done as described by Wetzel *et al.* (1991a). The region of the PPV genome selected for amplification was 243 bp cDNA fragment amplified from 3`-terminal region of the PPV *cp* gene. The nucleotide sequences of the oligonucleotide primers were designed as described by Wetzel *et al.* (1991a), (*sense primer*, 5`CCC TCA CAT CAC CAG AGC CA3`and *antisense primer*, 5`CAG ACT ACA GCC TCG CCA GA3`). Both primers were synthesized using an Applied Biosystem DNA/RNA Synthesizer at

the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt. Aliquots of 5 µl of each RT-PCR-amplified product were analyzed by electrophoresis on 1.2% agarose gel in TAE buffer at 65 V for 1.5 hr and visualized by staining with ethidium bromide (Sambrook *et al.*, 1989).

Restriction endonuclease analysis

The amplified product was digested with *Rsa* I restriction endonuclease (Gibco-BRL) according to the method of Bousalem *et al.* (1994) and Nemchinov and Hadidi (1996). *Rsa* I restriction digestion was performed using 10 units of enzyme with 10 µl aliquots of gel-purified cDNA amplified fragment (243 bp) at 37°C for 2 hr in the buffer supplied by the enzyme manufacturer. The product was analyzed through agarose gel electrophoresis as mentioned above.

RESULTS

In this work an isolate of PPV obtained from apricot (*P. armeniaca* L. cv. Amar) trees growing under field conditions in El-Amar, Kalubia governorate, Egypt was studied. These trees exhibited obvious symptoms on leaves (chlorotic blotching mosaic, veins banding, yellowish mottling, leaf roll and deformation), fruits (deformed apricot fruits) and stones (light colored ring) (Figure 1). Infected trees also showed the presence of systemic symptoms, i.e., chlorotic flecks and severe blotching on PPV-mechanically inoculated *N. benthamiana* and *N. clevelandii,* respectively.

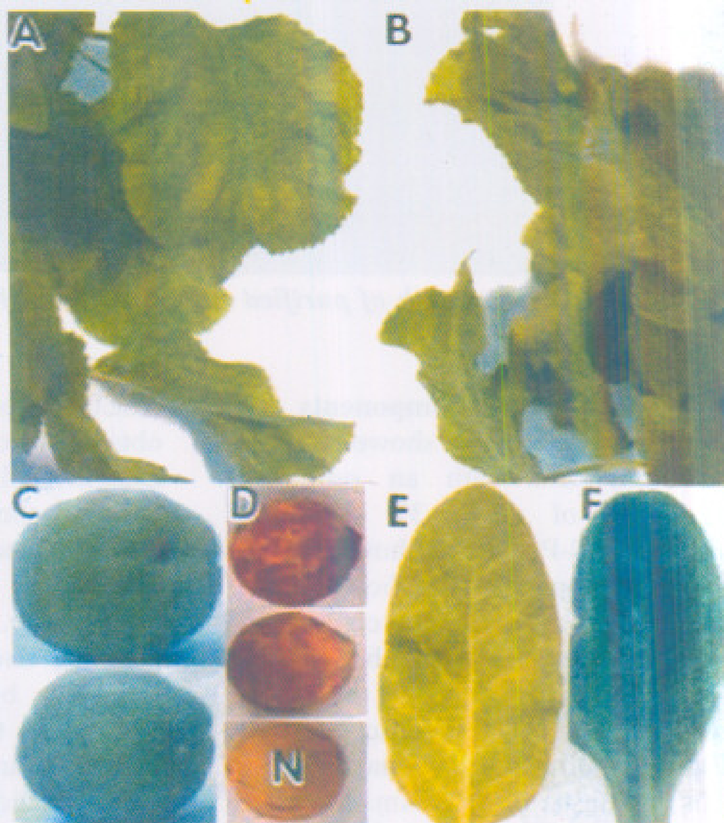

Fig. (1): El-Amar apricot (P. armeniaca L. cv. Amar) leaves exhibited PPV-like symptoms. A) Chlorotic blotching mosaics and veins banding. B) Yellowish mottling, leaf roll and deformation. C) Deformed apricot fruits. D) Light-colored rings on apricot stones. Systemic symptoms on leaves of N. benthamiana (chlorotic flecks, E) and N. clevelandii (severe blotching, F).

Virus purification

The ultraviolet absorption spectrum of PPV-purified preparations (unshown data) revealed a curve typical for nucleoprotein with a maximum ranging between 259 to 261 nm and a minimum ranging between 245 to 247 nm; no shoulder at 290 nm was observed. The value of $A_{260/280}$ was ranged from 1.258 to 1.280. The percentage of PPV-RNA was calculated to be about 7%. The average yield of the purified virus was estimated to be 20 to 22 mg/kg infected leaf tissues based on the extinction coefficient of 2.8 for a 1 mg/ml solution.

Negative staining and electron microscopy

Electron micrographs of PPV-purified preparations, negatively stained with 2% uranyl acetate, showed numerous unaggregated flexuous filamentous particles (Figure 2) ranging in length between 700 to 725 nm and in width between 13 to 15 nm.

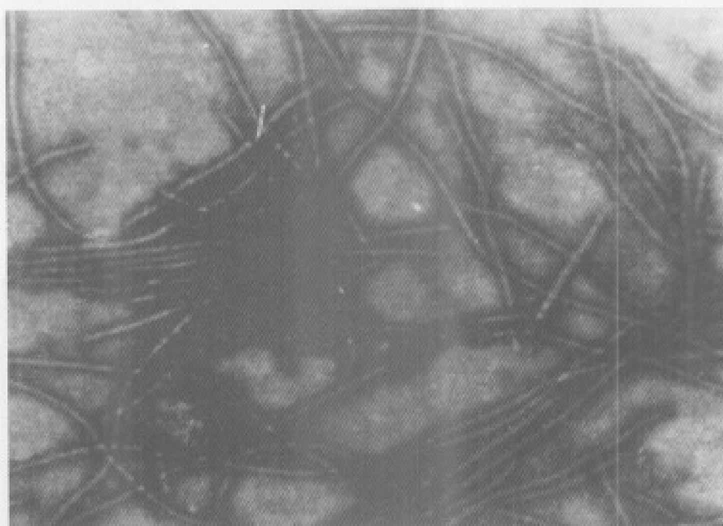

Fig. (2): An electron micrograph of purified PPV, stained with 2% aqueous uranyl acetate, (X, 85,000).

Properties of nucleoprotein components

Results in Figure (3) showed that a single CP component with an estimated molecular weight of about 37 kDa was detected in 12% SDS-PAGE. Unshown data of agarose gel electrophoresis indicated that PPV-RNA migrated as a single component with an estimated size of about 10 kb.

Production of PPV-IgGs specific to IVPs and N-, and C-terminals of PPV particles

PAbs against PPV-El-Amar isolate were raised and their titer was determined by I-ELISA technique. Three bleedings were obtained from the PPV-immunized rabbits. Data in Table (1) show that the three bleedings varied in their titers, as positive ELISA values were obtained up to dilutions 1/500, 1/1500 and 1/1000 for the 1st, 2nd and 3rd bleedings, respectively. It was also observed that the 2nd bleeding showed the highest ELISA values followed by the 3rd and 1st bleedings. Therefore, the 2nd bleeding was used for isolation and purification of IgGs using caprylic acid procedure. The concentration of

the purified IgG was adjusted with PBS, pH 7.4 to 1 mg/ml when OD at 280 nm was 1.46.

Antibodies directed to the N-, and C-termini of PPV-El-Amar CP were selected by affinity chromatography. The concentration of PPV-N, and C-IgGs was adjusted to 1-mg protein as mentioned above and used for sensitive detection of PPV-El-Amar isolate.

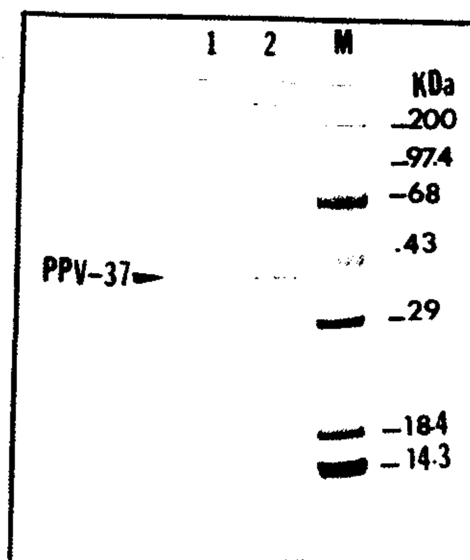

Fig. (3): SDS-PAGE (12%) electrophoresis of PPV-CP from two different purified virus preparations (lanes 1 and 2). M, protein standard marker (14.3-200 kDa, high range BRL).

Table 1: Determination of PPV antiserum titer in three bleedings via I-ELISA.

Dilutions	NS		PPV-anitserum					
			1st Bleeding		2nd Bleeding		3rd Bleeding	
	EV	R	EV	R	EV	R	EV	R
1/50	0.089	-	0.876	+	1.562	+	1.214	+
1/100	0.086	-	0.693	+	1.232	+	0.987	+
1/500	0.092	-	0.526	+	0.998	+	0.725	+
1/1000	0.088	-	0.289	-	0.682	+	0.483	+
1/1500	0.090	-	0.251	-	0.453	+	0.272	-
1/2000	0.089	-	0.211	-	0.282	-	0.221	-
1/2500	0.088	-	0.179	-	0.235	-	0.183	-
1/3000	0.091	-	0.101	-	0.126	-	0.099	-
Healthy (10^{-1})	0.112	-	0.149	-	0.156	-	0.151	-
PV (10^{-3})	0.199	-	0.321	+	0.837	+	1.573	+

EV: ELISA value at 405 nm (average of 3 replicates), 1 hr post incubation at 37°C. PV: purified virus. R: Result +: positive -: negative Healthy10^{-1}: Sap extracted form *N. benthamiana* leaves with no symptoms was used as a negative control. The ELISA values that equal two folds of healthy was considered as a positive control. NS: Normal serum.

Western blotting

Western blotting using affinity-selected IgGs directed to N- and C-termini of PPV-El-Amar

CP showed strong positive reactions with either PPV-infected apricot tissues or purified virus preparation (Figure 4). Furthermore, the serological relationship between PPV-specific IgGs and some other potyviruses, i.e., potato Y potyvirus (PVY) and zucchini yellow mosaic potyvirus (ZYMV) was studied *via* western blotting. Results also showed negative reactions, i.e., no bands were observed when the trypsin-treated intact PPV particles (core protein only) or PVY and ZYMV treated with PPV-N, or C-IgGs.

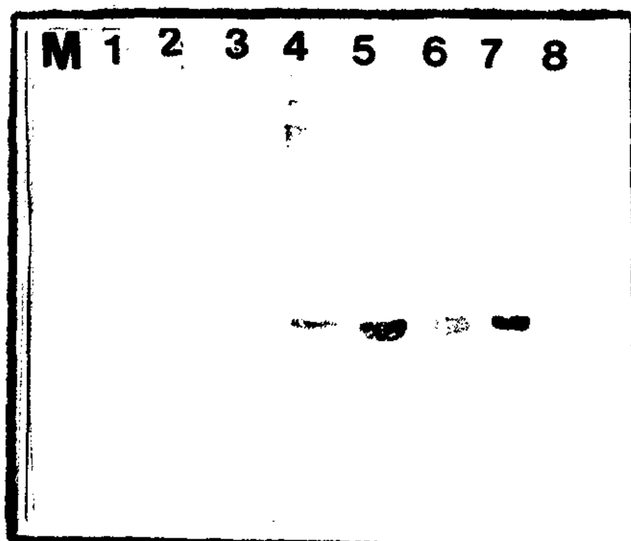

Fig. (4): Western blotting using PPV-N-, and C-IgGs against N- and C-terminal of PPV-El-Amar-CP. M, protein standard marker. Lane1, trypsin-treated PPV particles. Lane 2, purified PVY. Lane 3, purified ZYMV. Lane 4, sap extracted from PPV-infected apricot leaf tissues. Lane 5, untreated-purified PPV particles (15 µl). Lane 6, untreated-purified PPV particles (12.5 µl). Lane 7, untreated-purified PPV particles (5 µl). Lane 8, sap extract from healthy apricot leaf tissue.

IC/RT-PCR

PPV was detected in infected tissues as well as purified virus preparations using the IC/RT-PCR as a highly sensitive technique. The experimental results confirmed the specificity between the PPV-El-Amar isolate and its IgGs (PPV-N-, and C- IgGs), and also the specificity between the primers used in this study and the 3`-terminal region of the PPV *cp* gene. Results in Figure (5A) indicated that the PPV particles were immunocaptured by PPV-N-, C-IgGs. Therefore, the PPV-RNA genome could be released and used as a template in the presence of two specific primers (sense and antisense) for amplifying a 243-bp fragment from 3`-terminal region of the PPV-*cp* gene. A negative reaction of RT-PCR was obtained with healthy apricot leaf tissues and in the RT-PCR reaction without PPV template (Figure 5A).

Restriction endonuclease analysis

A gel-purified 243 bp fragment was digested with the restriction endonuclease *Rsa* I and analyzed through 1.2% agarose gel electrophoresis as mentioned before. Results in Figure (5B) showed the absence of the identical recognition site of *Rsa*I restriction enzyme, as the profile of the amplified fragments from PPV-infected apricot, *N. benthamiana* and purified virus preparation showed undigested fragment (243 bp).

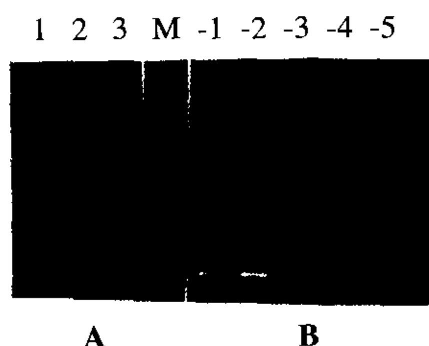

Fig. (5): A: Agarose gel (1%) in TAE buffer stained with ethidium bromide showing the IC/RT-PCR of PPV using primers specific for the 3`-terminal region of the PPV-cp gene. Lane 1, from PPV-infected apricot. Lane 2, from N. benthamiana. Lane 3, from purified PPV preparation. Lane 4, healthy control. Lane 5, reaction without any template as a negative control. M, DNA standard marker.
B: Restriction endonuclease analysis of a gel-purified 243 bp of cDNA fragments corresponding to Lane 1, 2 and 3 (Figure 7A), after treated with Rsa I restriction endonuclease and analyzed through 1.2% agarose electrophoresis. M, DNA standard marker.

DISCUSSION

In the present study, the purification method described here was found to be an efficient method for the extraction and purification of PPV-El-Amar free of cellular plant components. Using Tris-buffer containing Triton X-100, EDTA, sodium sulfite and 2-ME as an extraction buffer, improved the purification procedure. Clarification with chloroform and n-butanol, then concentrating the virus particles through 20% sucrose cushion, followed by sucrose density gradient centrifugation were found to be satisfactory without much loss of virus particles. Similar results were obtained by Abdel-Ghaffar *et al* (1998b).

The estimated yield of PPV, using an extinction coefficient 2.8, was 20 to 22 mg/kg-infected tissues. This yield seems to be good than obtained by Abdel-Ghaffar *et al* (1998a) (17-20 mg/kg-infected tissues).

A large number of flexuous filamentous particles (700 - 750 x 13-15 nm) were detected in the purified virus preparations. The molecular weight of PPV-CP and the size of PPV-RNA were estimated herein to be about 37 kDa and 10 kb, respectively. These results agree with that found by Maiss *et al.* (1989),

James *et al.* (1994) and Abdel-Ghaffar *et al.* (1998a).

In the present study, PAbs were successfully raised against IVPs and also against N- and C-termini of PPV-El-Amar isolate. Results of western blotting indicated that the PPV-N-,C-IgGs, positively strong reacted with the PPV extracts from infected tissues and no cross-reactions were observed with other potyviruses, (PVY and ZYMV), whereas using the PPV-IgG, the positive reactions as well as cross-reactions were observed (data not shown). According to Shukla *et al.* (1988), the N-terminus is the most immunodominant region of the CP of potyviruses, and the antibodies generated towards this region are highly specific and recognize only the homologous viruses and their strains. Bousalem *et al.* (1994) reported that the use of SDS-PAGE to separate proteins from crude extracts in minigels, followed by blotting into nitrocellulose sheets, is a rapid method for immunodetection and classification of PPV isolates. Also, they indicated that the unfractionated antiserum (PPV-IgGs) are suitable for the detection both M and D serotype groups, whereas directed serological differentiation is possible using IgGs directed to PPV- N- and C-terminal regions of the CP.

The sensitivity of RT-PCR as a detcetion method for PPV was evaluated by Wetzel *et al.* (1991a and 1992). They developed a sensitive RT-PCR assay using oligonucleotide primers based on consensus sequences from homologous regions of three published PPV sequences. This RT-PCR assay has been used to detected PPV isolates by amplifying a PPV-specific 243 bp fragment. IC/RT-PCR assay for 3`-terminal region of the PPV-*cp* gene has provided further evidence concerning the identity of the virus strain infecting SFTs (Nemchinov and Hadidi, 1996). Using this assay, Wetzel *et al.* (1991a) classified PPV isolates into two groups, PPV-D and PPV-M, according to RFLP analysis of amplified products with the restriction enzyme *Rsa* I. Isolates of PPV-D contain a *Rsa* I recognition sequence, whereas those of PPV-M lack this sequence. The PPV-M isolates contained the conserved *Alu* I recognition sequence (Lain *et al.* 1989; Maiss *et al.* 1989; Teycheney *et al.* 1989; Wetzel *et al.* 1991b and Cervera *et al.* 1993). PPV-SoC (sour cherry isolate of PPV) contains neither *Rsa*I nor *Alu*I restriction sites (Nemchinov and Hadidi, 1996). Data of RFLP analysis of IC/RT-PCR-amplified cDNA fragment using the two primers specific to `3-terminal region of PPV-*cp* gene, revealed that the isolate of this study is considered as a strain of PPV and could belong to M serotype group for not containing *Rsa* I restriction site.

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REFERENCES

Abdel-Ghaffar, M.H., Abo El-Nasr, M.A. and Hari, V. (1998a). Studies on an apricot strain of plum pox potyvirus isolated from El-Amar, Egypt. Acta Hort., 422: 385-391.

Abdel-Ghaffar, M.H., El-Dougdoug, K.A. and Sadik, A.S. (1998b). Serology and partial characterization of the Egyptian isolate of zucchini yellow mosaic potyvirus. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 6: 313-327.

Baunoch, D.A., Das, P., Browning, M.E. and Hari, V. (1992). R-ELISA: Repeat use of antigen-coated plates for ELISA and its

application for testing of antibodies to HIV and other pathogens, Biotechniques, 12: 412-417.

Bousalem, M., Candresse, T., Quiot-Douine, L. and Quiot, J.B. (1994). Comparison of three methods for assessing plum pox virus variability: further evidence for the existence of two major groups of isolates. J. Phytopathology ,142 : 163-172.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J. and Watson, L. (1996). Viruses of plants. C.A.B. International, Wallinggford, pp. 151-152.

Clark, M.F. and Adams, A.N. (1977). Characterization of the microplate method enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology ,34: 475-483.

Cervera, M.T., Riechmann, J.L., Martin, M.T. and Garcia, A.J. (1993). 3`-terminal sequence of the plum pox virus PS and O6 isolates: evidence for RNA recombination within the potyvirus group. J. Gen. Virol., 74: 329-334.

Dunez, J. (1988). Plum pox disease of stone fruit in Egypt. TCP/EGY/ 6759. 8 p.

Gibbs, A. and Harison, B. (1976). Plant Virology, the principles. John Wiley and Sons, Naw York, pp. 116.

James, D., Thompson, D.A. and Godkin, S.E. (1994). Cross reactions of an antiserum to plum pox virus. EPPO Bull. 24: 605 - 614.

Kerlan, C. and Dunez, J. (1979). Differniation biologique et serologique desouches du virus de la sharka. Ann. Phytopathol., 11: 241 - 250.

Korschineck, I., Himmler, G., Sagl, R., Steinkellner, H. and Katinger, W.D. (1991). A PCR membrane spot assay for the detection of plum pox virus RNA in bark of infected trees. J. Virol. Methods, 31: 139-146.

Laemmli, U.K. (1970). Cleavage of structual proteins during the assembly of the head of bacteriophage T4. Nature, London 227 : 680-685.

Lain, S., Riechmann, J.L. and Garcia, J.A. (1989). The complete nucleotide sequence of plum pox virus RNA. Virus Res., 13: 157-172.

Maiss, E., Timpe, U., Briaake, A., Jelkmann, W., Kasper, R., Himmler, G., Mattanovitch, D. and Katinger, H.W.D. (1989). The complete nucleotide sequence of plum pox virus RNA. J. Gen. Virol., 70: 513-520.

Mazayad, H.M. Nakhla, M.K., Aboul-Ata, A.E. and Hammady, M.H. (1992). Ouccurence of plum pox (sharka) virus on stone fruit trees in Egypt. Acta Hort., 309: 119-124.

Nemchinov, L. and Hadidi, A. (1996). Characterization of the sour cherry strain of plum pox virus. Phytopathology 86: 575-580.

Nemeth, M. (1986). Virus disease of stone fruit trees. In: Virus, Mycoplasma and Rickettsia Disease of Fruit Trees. Kluwer Academic Publishers Group. p. 256-533.

Perosa, F., Carbone, R., Ferrone, S. and Dammacco, F. (1990). Purification of human immunoglobulin by sequential precipitation with caprylic acid and ammonium sulphate. J. Immunol. Methods, 128: 9-16.

Quiot-Douine, L., Lecoq, H., Quiot, J.B., Pitrat, M. and Labonne, G. (1990). Serological and biological variability of virus isolates related to strains of papaya ring spot virus. Phytopathology 80: 256-263.

Roy, A.S. and Smith, I.M. (1994). Plum pox situation in Europe. *Eppo Bull. 24*: 515-523.

Sambrook, J.L., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd Ed. New York: Cold Spring Harbor Laboratory., p. 95-120.

Shukla, D.D., Jilka, J., Tosic, M. and Ford, R.E. (1989). A novel approach to the serology of potyviruses involving affinity-

purified polyclonal antibodies directed towards virus-specific N-termini of the coat proteins. J. Gen. Virol., 70: 13-23.

Shukla, D.D., Strike, P.M., Tracy, S.L., Gough, K.H. and Ward, C.W. (1988). The N and C-terminal of the coat proteins of potyviruses are surface-located and the N-terminus contains the major virus-specific epitopes. J. Gen. Virol., 69: 1497-1508.

Stace-Smith, R. and Tremaine, J.M. (1970). Purification and composition of potato virus Y. Phytopathology, 60: 1785-1789.

Teycheney, P.Y., Tavers G., Delbos, R.P., Ravelonandro, M. and Dunez, J. (1989). The complete nucleotide sequences of plum pox virus RNA (strain D). Nucleic Acids Res. 17: 10115-10116.

Towbin, H., Staehelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Nat. Acad. Sci. USA, 76: 4350-4354.

Varveri, C., Candresse, T., Cugus, M., Ravelonandro, M. and Dunez, J. (1988). Use of ^{32}P-labelled trascribed RNA probe for dot hybridization detection of plum pox virus. Phytopathology ,78: 1280-1283.

Wetzel, T., Candresse, T., Macquaire, G., Ravelonandr, M. and Dunez, J. (1992). A highly sensitive immunocapture polymerase chain reaction method for plum pox virus detection. J. Virol. Methods, 39: 27-37.

Wetzel, T., Candresse, T., Ravelonandr, M. and Dunez, J. (1991a). A polymerase chain reaction adapted to plum pox potyvirus detection. J. Virol. Methods, 33: 355-366.

Wetzel, T., Candresse, T., Ravelonandr, M., Debos, R.P., Mazyad, H., Aboul-Ata, A.E. and Dunez, J. (1991b). Nucleotide sequence of the 3`-terminal region of the RNA of the El-Amar strain of plum pox potyvirus. J. Gen. Virol., 72: 1741-1746.

Wetzel, T., Tavers, G., Teycheny, P.Y., Ravelonandro, M., Candresse, T. and Dunez, J. (1990). Dot hybridization detection of plum pox virus using ^{32}P-labelled RNA probes representing non-structure viral protein genes. J. Virol. Methods ,30: 161-172.

الملخص العربي

دراسات جزيئية وسيرولوجية لعزلة مصرية من فيروس جدري المشمش

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**قسم الميكروبيولوجيا الزراعية (معمل الفيرولوجي) كلية الزراعة-جامعة عين شمس-ص.ب. ٦٨ حدائق شبرا ١١٢٤١- القاهرة-مصر

في هذه الدراسة تم دراسة عزلة مصرية من فيروس جدري المشمش و المتحصل عليها من أشجار المشمش بالعمار. وقد تم إكثارها علي نباتات الدخان بنتميانا لتنقية الفيروس باستخدام طريقة معدلة. وكان معدل الامتصاص للأشعة فوق البنفسجية عند طول موجي ٢٦٠ و ٢٨٠ مطابق تماما للنيوكليوبروتين حيث تراوح من ١,٢٥٨ إلى ١,٢٨٠. وقد تم الحصول علي محصول فيروسي حوالي ٢٠ - ٢٢ مجم لكل كيلوجرام أوراق نباتات مصابة بالفيروس. وقد أوضح الفحص بالميكروسكوب الإلكتروني لتحضيرات الفيروس المصبوغة سالبا بخلات اليورانيل (٢%) وجود جزيئات خيطيه الشكل مرنة بأبعاد تتراوح ما بين ٧٠٠-٧٢٥ 13-15 نانوميتر. وقد ثبت أن الغلاف البروتيني للفيروس بوزن جريئي ٣٧ كيلو دالتون عندما درس بواسطة الـ (SDS-PAGE (12%. كما اتضح أن الفيروس به حمض نووي من النوع الرنا وحيد السلسلة بوزن جريئي ١٠ كيلو قاعدة. كما تم إنتاج أجسام مضادة من النوع عديد الكلون وكان تركيز السيرم المضاد في ثلاثة ادماءات والذي قدر بواسطة تكنيك الأليزا غير المباشرة. ثم تبع ذلك تنقية الأجسام المضادة من النوع G والمتخصصة لجزيئات الفيروس الكاملة ثم اختيار النوع المتخصص الطرف C , N . وقد تم استخدام تلك الأنواع المتخصصة في تمييز تحت وحدات الفيروس لتلك العزلة وذلك بواسطة تكنيك البقع الغربي. ومن أنسجة أوراق المشمش المصابة بالفيروس تم عزل الـ cDNA ثم تكبير قطعة طولها ٢٤٣ قاعدة من الطرف ٣ في الغلاف البروتيني للفيروس وذلك بواسطة تكنيك الـ .IC/RT-PCR ثم تبع ذلك إجراء دراسة للجزء المتحصل علية بواسطة الهضم بواسطة أحد إنزيمات القطع. وقد أظهرت النتائج أن العزلة الفيروسية موضع الدراسة يمكن أن تكون سلالة من فيروس الـ PPV وتتبع سيرولوجيا المجموعة M والتي لا تحتوي علي التتابع الخاص بإنزيم القطع الـ *Rsa* I.