Expression of the coat protein gene of potato leafroll virus in *Escherichia coli* and development of polyclonal antibodies against recombinant coat protein

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A. K. El-Attar*; B. Y. Riad; A. Saad**; A. M. Soliman*; and H. M. Mazyad***
* Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
** Chemistry Dept., Faculty of Science, Cairo University, Giza, Egypt.

ABSTRACT

The coat protein (CP) gene of potato leafroll virus (PLRV) was cloned into the pBAD-TOPO expression vector upon induction; the viral protein was expressed as 6XHis-tagged PLRV fusion protein in Escherichia coli (E. coli) BL21 cells. The fusion protein was confirmed by western blot analysis using antibodies specific for the PLRV. The predicted length of the coat protein gene was 627 nucleotides, corresponding to a 209 amino acid coat protein of Mr 23 KDa. The nucleotide sequence of the coat protein gene of PLRV (PLRV-CP) was compared to the sequences of the coat protein genes of other PLRV isolates. The PLRV-CP gene of the Egyptian isolate (PLRV-CP-Eg) was found to be 95% homologous to those of other luteoviruses at the nucleotide level. Antiserum obtained from rabbit after injection with 6XHis-tagged PLRV fusion protein was immuno-reactive towards the PLRV in indirect ELISA.

Key words: *PLRV, E. coli., 6XHis-tagged fusion protein, ELISA.*

INTRODUCTION

PLRV is the type member of the genus *Polerovirus* of the family *Luteoviridae* (Pringle, 1998). PLRV has a monopartite, single stranded RNA genome, transmitted by aphids in a circulative non-propagative manner and is mainly restricted to phloem tissues of infected plants (Mayo and Ziegler-Graff, 1996). Although virus circulation within the aphid leading to transmission has been well characterized, the mechanisms involved in virus recognition at aphid membranes are still poorly understood (Rouzé-Jouan *et al.*, 2001). PLRV is a major menace for the potato production all over the world (Ehrenfeld *et al.*, 2004). PLRV has limited host range; about 20 largely *solanaceous* species have been infected experimentally. The symptoms, especially from infected seed potato stocks, causes leaf rolling and stunting, the extent depending on the potato cultivar (Taliansky *et al.*, 2003). PLRV virions play key roles in phloem-limited virus movement in plant hosts as well as in transport and persistence in the aphid vectors. These results identified amino acid residues in a surface oriented loop of the coat protein that are critical for virus assembly and stability, systemic infection of plants, and movement of virus through aphid vectors (Lee *et al.*, 2005).

PLRV forms 25 to 30 nm diameter isometric particles that encapsidate genomic RNA of about 5.9 kb that contains six large open reading frames (ORF). A 5´-located gene cluster contains three ORFs that code for a polypeptide of 28 kDa with unknown functions (ORF0) and two proteins of 70 kDa (ORF1) and 108 kDa protein (ORF1/2) that

appear to be replication-associated proteins (Mayo and Ziegler-Graff, 1996). Within the 3´-located gene cluster, ORF3 encodes the 23 kDa coat protein (CP). ORF4, which encodes a 17 kDa product (P4), is contained within the CP gene, but in a different reading frame. P4 is thought to be a movement protein. PLRV ORF5 is separated from the upstream CP gene by a single amber termination codon. During infection, ORF5 is expressed by occasional translational readthrough of this codon, presumably by a plant-encoded suppressor tRNA, to give a `readthrough' product of 79 kDa (P5). Two other ORFs (ORF6 and ORF7) were detected recently near the 3´-end of the genome (Ashoub *et al.*, 1998) but their functions are not understood. PLRV and the other viruses in the family *Luteoviridae* show remarkably diverse mechanisms of gene expression, including overlapping reading frames, subgenomic RNA synthesis, ribosomal frame shifting, stop codon read-through and protein self-processing (Miller *et al.*, 1997). Proteins encoded by the first cluster (ORF0, ORF1, ORF2) are translated directly from the genomic RNA. Two types of N-terminally His-tagged coat protein constructs were used for the expression in insect cells: one, encoding a 23 kDa protein with the C-terminal amino-acid sequence corresponding to the wild type coat protein and the second with additional clathrin binding domain at the C-terminus. The expression of these two proteins by a recombinant baculovirus was characterized by western immunoblotting with antibodies directed against potato leafroll virus (Sutuja *et al.*, 2005).

Recombinant DNA technology may help circumvent purification problems encountered with several viruses (Ling *et al.*, 2000; Hourani and Abou Jawdah, 2003). Expression of different viral genes such as those coding for CP, non-structural proteins, antisense RNAs and ribozymes, among other variants, has resulted in significant protection against infection by the corresponding viruses (Doreste *et al.*, 2002). Expression of CP genes in bacteria or in plants can give rise to virus-like particles (VLP), as shown for viruses as different as alfalfa mosaic virus (Yusibov *et al.*, 1996), cowpea mosaic virus (Wellink *et al.*, 1996). Coat protein gene expression strategy followed by immunization of animals (rabbit) with the fusion protein has many advantages over the conventional immunization. The most important advantage is the elimination of the time consuming and technically demanding steps of protein isolation and purification from plants (Soliman *et al.* 2006). The purification of protein can result in changes in protein confirmation and the loss of epitopes. This problem is probably not encountered during *in vivo* expression of the antigen after coat protein gene expression (Hinrichs *et al.*, 1997). In addition, this method causes less distress for the animal; because the administration of the purified protein does not induce any local inflammations (Davis *et al.*, 1996; Abou-Jawdah *et al.*, 2004). The overall objectives of this study are to characterize the sequence of the PLRV-CP gene of the Egyptian isolate to clone and express this gene in *E. coli*, and to produce polyclonal antiserum against the expressed protein for the detection of the PLRV in infected plants.

MATERIALS AND METHODS

Virus source

Potato (*Solanum tuberosum*, L. cv. Nicola) plants showing symptoms typical of PLRV infection were collected from Kalyoubia Governorate in Egypt and used as a source of the virus. The symptoms mainly appeared in the young leaves, which usually stand upright, roll and color slightly pale. Some cultivars showed pink or reddish discoloration. In a later stage of infection the lower leaves also may start rolling.

Extraction of total RNA from plant tissues

Total RNA was isolated from the infected potato plants using RNeasy® Plant Mini Kit obtained from QIAGEN according to manufacturer's instructions.

Design and synthesis of oligonucleotide primers

Pair of primers specific for PLRV-cp gene were designed for the full length of the cp gene according to available sequences from the GenBank. The forward (sense) primer (PLRVCPv) sequence was: 5`atgagtacggtcgtggttaragg-3`, and the complementary (antisense) primer (PLRVCPc*Nco*I) sequence was: 5`-aaaaccatggctatytggggttytgcaragcta C -3`, (Shalaby *et al*., 2002) with *Nco*I restriction enzyme site (underlined) at the 5` end.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA extracted from infected potato plants was used as template for each RT-PCR amplification reactions using QIAGEN OneStep RT-PCR Kit. Reverse transcription reaction started with incubation at 50°C for 30 min, followed by denaturation at 95°C for 15 min. PCR amplification was performed by 30 cycles in a thermal cycler starting with denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 1 min with final extension at 72°C for 10 min. Five microliters aliquots of RT-PCR products were analyzed on 1% agarose gels in 0.5X TBE buffer. DNA ladder (PCR markers, Promega) was used to determine the size of RT-PCR products. Gels were stained with ethidium bromide and visualized by UV illumination using Gel Documentation System (Gel Doc 2000, Bio-Rad, USA). The expected size of the PCR product was ~ 650 bp.

Cloning and sequencing of PLRV-CP gene

RT-PCR product was ligated directly into pBAD-TOPO® vector (pBAD-TOPO® TA Expression Kit) obtained from Invitrogen, Carlsbad, CA. The recombinant plasmids were introduced into *E. coli* strain BL21 as described by manufacturer's instructions. DNA was prepared from selected white colonies, digested with *Nco*I and fractionated on agarose gels. Automated DNA sequencing reactions were performed using dRhodamine terminator cycle sequencing ready reaction kit from PE-Applied Biosystems in conjunction with the ABI PRISMTM 310 Genetic Analyzer from PERKIN-ELMER (Applied Biosystems Division, Foster City 6, CA, USA). The forward and the reverse primers (pBAD-F and pBAD-R) were supplied with the pBAD TOPO TA® Expression System and used for DNA sequencing. The nucleotide sequence was determined automatically by the electrophoresis of the cycle sequencing reaction product on the ABI PRISMTM 310 Genetic Analyzer. The data were provided as fluorimetric scans from which the sequence was assembled using the Sequence Navigator software. The nucleotide sequence was analyzed using DNAMAN Sequence Analysis Software (Lynnon BioSoft. Quebec, Canada) and compared with those of PLRV isolates available in GenBank.

Expression of the PLRV-CP gene in *E. coli*

To express PLRV-CP gene in *E. coli* BL21, LB liquid medium containing 60 µg/ml ampicillin was inoculated with a single recombinant *E. coli* colony and incubated overnight at 37°C with shaking (250 rpm) to obtain a saturated culture. 50 ml of LB medium containing ampicillin was inoculated with 1 ml of saturated culture and incubated at 37°C with vigorous shaking to reach an OD_{600} = ~ 0.5. One ml of the uninduced culture was centrifuged at 12,000 rpm for 1 min at room

temperature; the cell pellet was stored at -20°C. The remaining culture was induced adding the optimal amount of the inducer (L-arabinose). To optimize the inducer concentration and the time of induction; 5 different concentrations of L-arabinose were used at final concentrations of: 0.00002%, 0.0002%, 0.002%, 0.02%, and 0.2%. One ml aliquots of the induced cultures was removed at 2, 3, and 4 hr after induction and immediately centrifuged at 12,000 rpm for 1 min at room temperature and the cell pellets were stored at -20°C. The remaining induced cultures were centrifuged at 4,000 rpm for 10 min at 4°C and the bacterial pellets were stored at -80°C for further use. The pellets (from-20°C) were resuspended in 100 µl of 1X SDS gel loading buffer, and heated to 100°C for 3 min, then the expressed protein was separated from other bacterial proteins on a 12% SDS-PAGE and stained with coomassie blue (Sambrook *et al.*, 1989).

Purification of 6XHis-tagged PLRV fusion protein

The rapid purification of 6XHis-tagged PLRV fusion proteins from bacteria (the pellets from -80°C) was done using the B-PER™ 6XHis Spin Purification Kit (Pierce). The eluted 6XHis fusion protein was assayed by 12% SDS-PAGE.

Western blot analysis

Polypeptides resolved on SDS-PAGE were transferred onto nylon membranes in transfer buffer using Mini Trans-Blot [Electrophoretic Transfer Cell (Bio-Rad)] as described by Towbin *et al.* (1979). The nonspecific sites on membranes were blocked in PBS containing 5% (w/v) non-fat milk for 1 h at room temperature. Blots were incubated with polyclonal antibody (Anti-PLRV) (Agdia) raised against the PLRV as a primary antibody diluted 1:100 for 1 h at room temperature with shaking. All antibody dilutions were done in PBS containing 5% (w/v) non-fat milk (blocking buffer). Membranes were washed 6 times with PBS containing 0.05% (v/v) Tween-20 with shaking. Blots were incubated with the Peroxidase-conjugated anti-rabbit antibody (Amersham) diluted 1:10,000 as a secondary antibody for 1 hr with shaking. The unbound HRP-conjugated secondary antibodies were removed by washing the membranes 6 times. Membranes were incubated with substrate working solution for 5 min with shaking. SuperSignal (West Pico substrate consists of equal volumes of the Luminol/Enhancer solution and the stable peroxide solution) was used as the substrate working solution for peroxidase. Blots were placed with the protein side facing up against X-Ray film obtained from Pierce for 15 min exposure time in dark room. The film was developed using Autex [X-Ray fixer] and X-Ray developer obtained from BALDWINsm, Cleveland, MD, USA.

Immunization of rabbit

One New Zealand white rabbit, weight ~ 4 kg, was immunized using 6 injections at weekly intervals. At the first week, the rabbit was injected intravenously with ~ 500 µl of the purified protein (1 mg/ml). The next 4 injections were subcutaneous using One ml of the purified protein (1 mg/ml). The Freund's complete adjuvant (Sigma) was used in 1:1 volume at the first subcutaneous injection. The Freund's incomplete adjuvant (Sigma) was used in 1:1 volume with the next three subcutaneous injections. One poster injection with 500 µg of the purified protein was administered intravenously two weeks after the last subcutaneous injection.

Blood collection and serum processing

Blood sample was collected from the rabbit one week before the poster injection and another blood sample was collected one week after it. Blood was obtained from the rabbit by

veinal pathway through the marginal ear vein. Once the antiserum reacted positively with PLRV infected tissues in ELISA test, the whole blood was collected from the rabbit. The whole blood was kept for one hour at room temperature for clotting then the clot was released and the blood was heated at 37°C for 30 min then stored at 4°C overnight. The serum was decanted from the clot and centrifuged at 2,000 rpm to remove cell debris. The serum was filtered through a 0.2 µm Millipore filter (Corning), and brought to 0.025% sodium azide. Aliquots of serum, mixed with equal volumes of glycerol, were stored at -20°C.

Purification of immunoglobulin G (IgG)

The IgG fraction was partially purified by selective precipitation with high concentrations of salts (ammonium sulfate, final concentration 40%, pH 7.0) and dialysis three times against 500 ml of the 1X phosphate buffered saline (PBS) (Ball *et al.*, 1993).

Indirect enzyme-linked immunosorbent assay (indirect ELISA)

One hundred mg of leaf tissues were homogenized in 2X PBS. Plates were coated with plant extracts (100 µl/well) and incubated overnight at 4°C, then blocked with 200 µl of blocking buffer (1X PBS, 0.5% of BSA) for 1 hr at room temperature. 100 µl of the anti-PLRV were added to each well then incubated at 37°C for 3 hr. 100 µl of the diluted secondary antibody alkaline phosphatase conjugated (Anti-Rabbit antibody) were added to each well and incubated for 1 hr at 37°C. All washing steps between incubations were performed with 1X PBS-T buffer. Freshly prepared pNPP substrate was added to the wells. The plate was incubated for 30 min at room temperature away of direct light. The reaction was stopped by addition of 50 µl of 3M H_2SO_4 to each well and the absorbance was measured at 405 nm.

RESULTS AND DISCUSSION

Amplification of the PLRV-CP gene

PCR primers specific for the coat protein gene within the 3′-located gene cluster and ORF3 which encodes the 23 kDa PLRV coat protein were used and illustrated in Fig.(1).

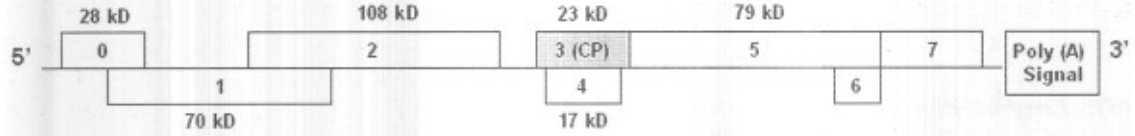

Fig. (1): Schematic representation of the PLRV genome. Non-coding regions are shown as a single line. Coding regions are shown by white boxes and indicated by the molecular mass (KD) of their products and as coat protein (CP).

RT-PCR amplification of viral RNA was carried out on the total RNA isolated from infected plants using specific primers designed to amplify the coat protein gene of PLRV. Electrophoresis analysis of RT-PCR product showed a single amplified fragment of ~ 650 bp and no fragments were amplified from the RNA extracted from symptomless or healthy plants (Fig. 2).

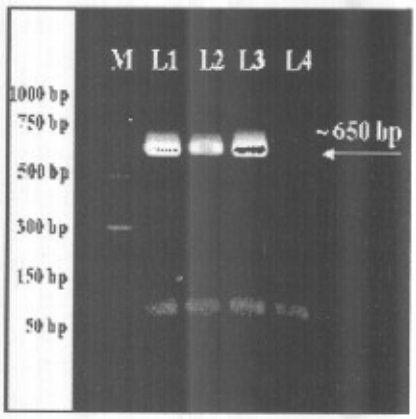

Fig. (2): Agarose gel electrophoresis analysis of RT-PCR amplified products. M: DNA ladder (PCR markers, Promega); L1, L2 and L3: different plant samples infected with PLRV; L4: healthy plant sample as a negative control

Cloning of PLRV-CP gene into pBAD-TOPO® vector

pBAD TOPO® TA Expression Kit provides a highly efficient one step cloning strategy for the direct insertion of *Taq* polymerase-amplified PCR products into a plasmid vector for regulated expression in *E. coli*. Expression in *E. coli* is driven by the *ara*BAD promoter (P_{BAD}). The AraC gene product encoded on the pBAD-TOPO® plasmid positively regulates this promoter. *Taq* Polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products. The linearized vector has single, overhanging 3' deoxythymidine (T) residues. This allows PCR inserts to ligate efficiently with the vector.

Isolation of recombinant plasmids

Several white colonies resistant to ampicillin were selected to test for recombinant plasmids containing the PLRV-CP gene. Restriction enzyme digestion with *Nco*I will release the cloned gene only if inserted in the right direction due to the presence of *Nco*I site at 5' end in the pBAD-TOPO vector and the other *Nco*I site is located in the complementary primer (Fig. 3).

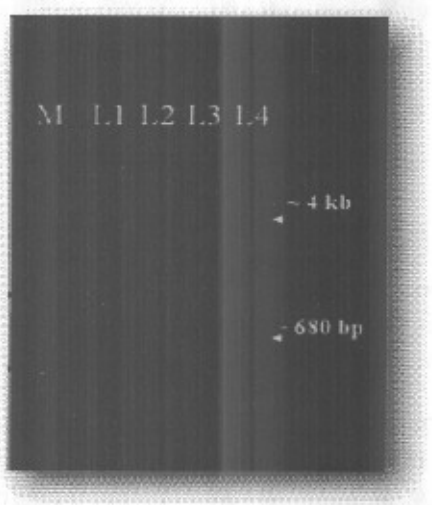

Fig. (3): Enzyme digestion of the recombinant plasmids from the cloning of the RT-PCR amplified product. M: 1 Kb DNA ladder; L1, L2, L3 and L4: DNA minipreps digested with NcoI.

Sequence analysis

Nucleotide sequencing (Fig. 4) of the RT-PCR amplified fragment in the recombinant plasmid for the PLRV-cp was completed and compared with sequences of other isolates of potato leafroll virus available in GenBank. This result was submitted to GenBank at NCBI data base as the sequence of coat protein gene of the PLRV (Egyptian isolate) with accession number: GQ376029.

The predicted PLRV-CP gene is 627 nt in size, starting from ATG start codon (methionine), as obtained by comparison with other PLRV sequences, and ending with a TAG stop codon from which the 3' NCR (non coding region) ends. The CP gene codes for a 209 amino acid protein giving a molecular weight of 23 KDa (kilo Dalton). Multiple sequence alignment of the nucleotide sequence of the coat protein gene of PLRV- Egyptian isolate (PLRV-EG) with the corresponding sequence of nine different PLRV isolates available in GenBank [Jordan (EU073861); France (AF453388); India (AF539791); Pakistan (AY307123); Iran (DQ269981); China (DQ309064); Czech Rep. (EU717545); USA (NC_001747) and Cuba (S77421)] were analyzed using DNAMAN software. Sequence comparisons showed the percentage of similarity 95% of the nine reported isolates of PLRV with the Egyptian isolate (Fig. 5). The similarity of the nucleotide sequences suggested that the architecture of the luteoviruses is highly conserved.

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1   ATGTCTACGG TCGTGGTTAA AGGAAATGTC AATGGTGGTG TACAACAACC ACGACGGAGA
61  CGAAGGCAAT CCCTTCGCAG GCGCGCTAAC AGAGTTCAGC CGGTGGTTAT GGTCACGGCC
121 CCTGGGCAAC CCCGACGACG TAGACGCAGA AGAGGAGGCA ATCGCCGCTC AAGAAGAACT
181 GGAGTTCCCC GAGGACGAGG CTCAAGCGAG ACATTCGTGT TTACAAAGGA CAACCTCATG
241 GGCAACAGCC AAGGAAGTTT CACCTTCGGG CCGAGTCTAT CAGACTGTCC GGCATTCAAG
301 GATGGAATAT TCAAGGCCTA CCATGAGTAT AAGATCACAA GCATCTTACT TCAGTTCGTC
361 TCCGAGGCCT CTTCCACCTC CTCCGGTTCC ATCGCTTATG AGTTGGACCC CCATTGCAAA
421 GTTAGATCCT TCCAGTCCTA CGTCAACAAG TTCCAAATTA CGAAGGGCGG CGCCAAAACT
481 TATCAAGCGA GGATGATAAA TGGGGTAGAA TGGCACGATT CTTCTGAGGA TCAGTGCAGG
541 ATATTGTGGA AAGGAAATGG AAAATCTTCA GATACCGCAG GATCCTTCAG AGTCACCATC
601 CGGGTGGCTC TGCAAAACCC CAAATAG
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Fig. (4): Nucleotide sequencing of the RT-PCR amplified fragment of the PLRV-cp and submitted to GenBank

Expression of the PVX-CP gene in *E. coli*

Expression of PLRV-CP was induced in *E. coli* strain BL21 transformed with the recombinant plasmid by addition of L-arabinose. PLRV-CP was expressed as a fusion protein with an estimated molecular weight of about 25 kDa, which contains about 2 kDa of tagged fragment from the vector and 23 kDa from the PLRV-CP. The expression of the protein increased continuously over time and reached maximum at 3 hr after induction at 37°C. The optimal concentration of the inducer (L-arabinose) is 0.00002% (Fig. 6).

Separation and analysis of bacterial proteins by SDS-PAGE revealed a high level of expression of the 25 kDa protein only in the induced bacterial cells containing the recombinant plasmid. This protein was readily detectable as the most abundant cellular protein upon coomassie staining of the PAGE gels. Cell fractionation experiments showed that the majority of the fusion protein was found in soluble form inside the cell and the

protein was not expressed in non-induced cells with the same plasmid. Since the expression of the protein was specifically induced and its size corresponded to the expected size, it was concluded that this protein was the PLRV-CP (Fig. 7). The purification of the proteins from bacterial cells indicated that the expressed protein was soluble, the first elution contains the highest concentration of the purified protein and used for rabbit immunization while the third and the fourth elution did not contain any proteins. In western blotting experiment, the fusion protein reacted with rabbit polyclonal antiserum directed against PLRV (Fig. 8).

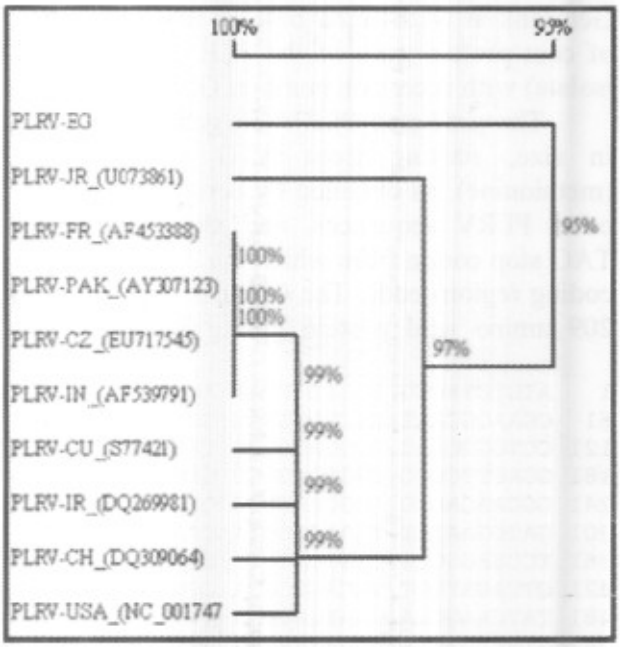

Fig. (5): A phylogenetic tree showing relationships among reported isolates of PLRV and the Egyptian isolate based on the nucleotide sequences of their CP genes. Horizontal distances indicate degree of relatedness.

Confirmation of the produced antibodies

Viruses are good antigens because of their coat protein, but their immunogenicity varies among individual viruses. This variation is probably due to differences in the complexity of their proteins, since the best immunogens are usually proteins with high molecular weight and elaborate conformation (Salazar, 1996). Antisera against plant viruses can be produced in several animal species. Rabbits are more commonly used because they can produce large volumes of serum and they respond well to most plant virus antigens. The interaction between the antigen and the specific antibody is the basis for serological tests. To testify the produced antibody raised against PLRV-CP, indirect ELISA was used for the detection of PLRV in potato plants (using the IgG prepared in this study) with the protocol described before. ELISA has been widely used for the detection of viral diseases in many plants. The indirect ELISA is used primarily to determine the strength and/or amount of antibody response in a sample, whether it is from the serum of an immunized animal or the cell supernatant from growing hybridoma clones (Salazar, 1996). Readings from the PLRV-infected leaves were compared to those of healthy leaves. Readings from healthy extracts were relatively high and attempts to reduce the back ground level by treatment of samples with DIECA were not

successful. Three independent replicates for each potato sample were made in order to minimize the variations in readings and normalize the data related to healthy potato leaves (negative control). Determination of the working dilutions of antiserum rose against PLRV-CP and IgG against the expressed CP through indirect ELISA is shown in Fig. 9. The results of the indirect ELISA revealed that both of the raised antiserum and IgG are able to recognize PLRV from infected tissues at dilutions of 1:500 and 1:200, respectively.

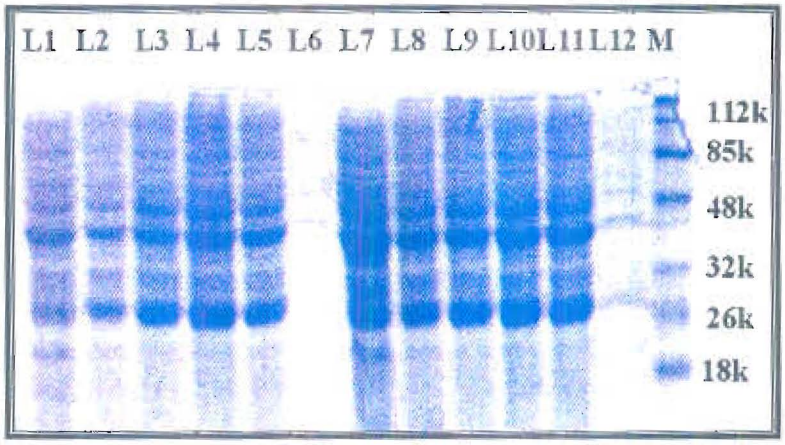

Fig. (6): SDS-PAGE for induced and uninduced bacterial cells expressing PLRV-CP. L1, 2, 3, 4, And 5: induced cultures of the same clone at five different inducer [(L) Arabinose] concentrations; L6: uninduced culture of the same clone. L7, 8, 9, 10, 11: induced cultures of another clone; L12: uninduced culture of the same clone. The inducer final concentrations are: 0.00002% in L5 and L1, 0.0002% in L4 and L10, 0.002% in L3 and L9, 0.02% in L2 and L8, and 0.2% in L1 and L7. M is the blue ranger prestained protein marker. Expected size was ~ 25 kDa.

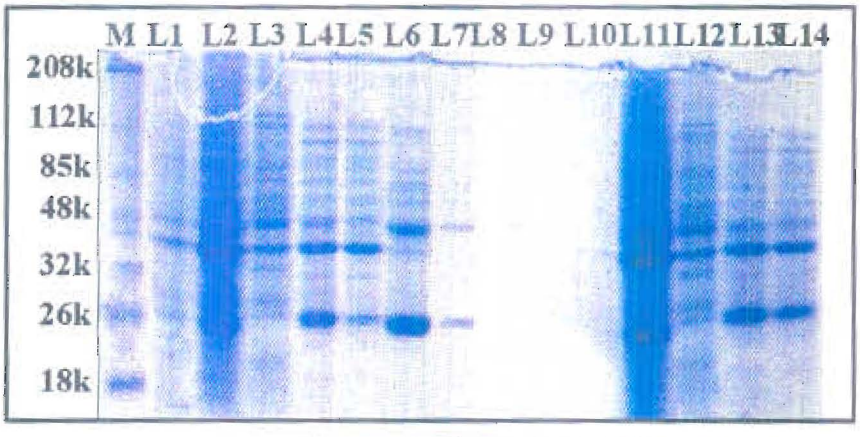

Fig. (7): SDS-PAGE for induced, uninduced bacterial cells expressing PLRV-CP and the purified PLRV-CP. M: the blue ranger prestained protein marker, L1: uninduced bacterial culture; L2: induced bacterial culture; L3: pellet collected after chemical lysis of the induced culture (insoluble proteins); L4: Bacterial cell lysate; L5: supernatant collected after resin purification; L6: purified protein (1st elution); L7: purified protein (2nd elution); L8: purified protein (3rd elution); L9: purified protein (4th elution).

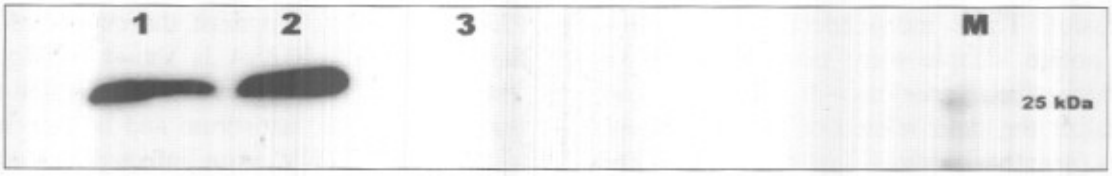

Fig. (8): Western blotting analysis. M: western blotting molecular weight marker; L1 and L2: two samples of the purified PLRV-CP while L3: Healthy plant sample used as a negative control.

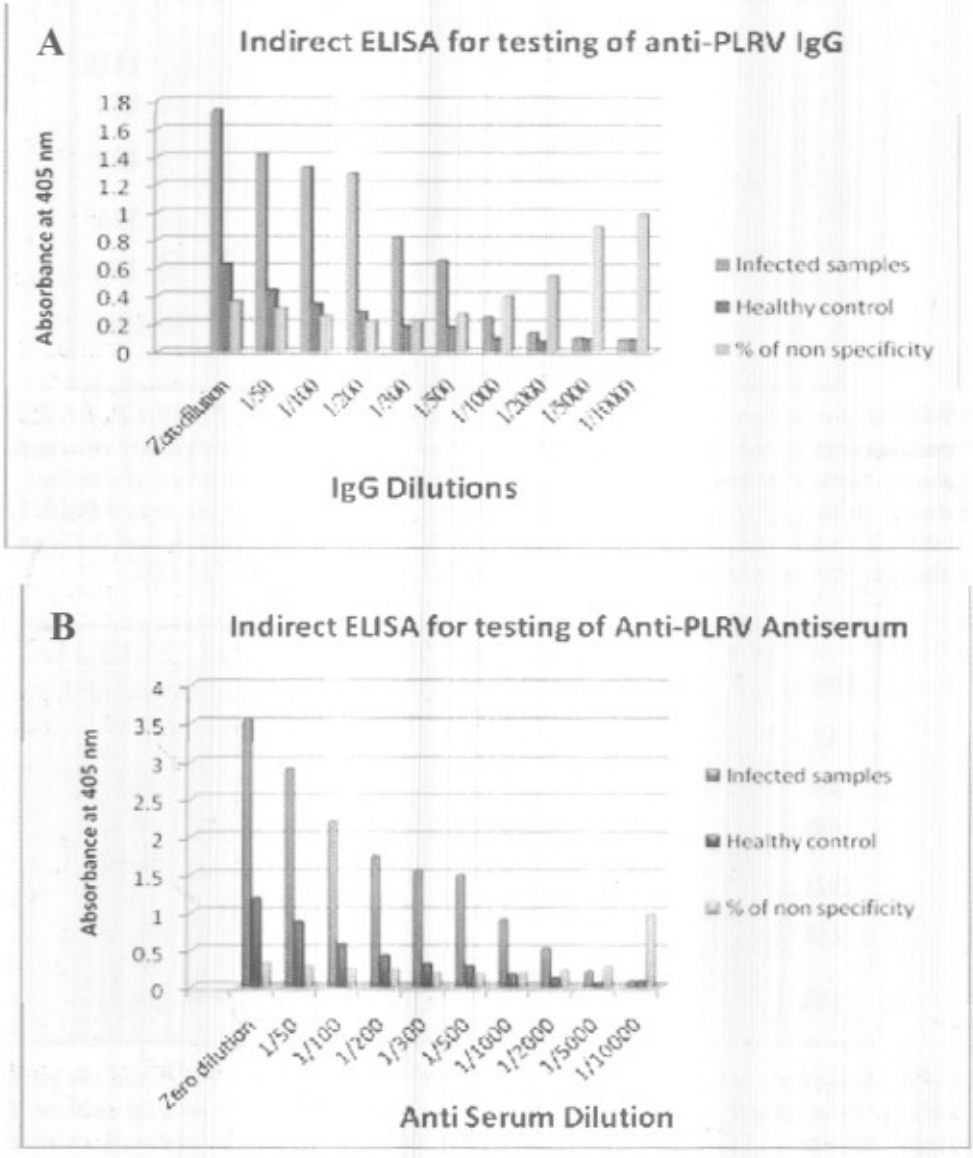

Fig. (9): Determination of serial dilutions of Anti PLRVcp- IgG (A) and antiserum (B) against the expressed CP for infected and healthy samples by indirect ELISA.

PLRV coat protein of the Egyptian isolate was successfully cloned, expressed and purified under non-denaturing conditions. Upon rabbit immunization, the produced antiserum produced was subjected to western blot analysis which showed that the antiserum was specific and gave similar results to a commercially available antibody. The suitability of produced crude antiserum and purified antibodies for use in diagnostic tests was compared to an efficient commercial DAS-ELISA kit. This study addressed the possibility of using recombinant PLRV-CP to produce PLRV specific antisera and to test their suitability for use in serological diagnostic assays for surveys or in certification programs. This investigation suggested that the recombinant virus coat proteins expressed in bacterial cells have great potential as an alternative source of antigens for raising specific antibodies to plant viruses. Such recombinant virus coat proteins can be produced in large quantities and can be manipulated or modified as needed for specific uses. A similar suggestion was reported by Soliman *et al.* (2006).

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الملخص العربي

التعبير عن جين انتاج الغلاف البروتينى لفيروس التفاف أوراق البطاطس (PLRV) للعزلة المصرية داخل بكتيريا القولون وانتاج أجسام مضادة متخصصة له

أحمد كمال العطار* ، بهية رياض ، عمرو سعد** ، أحمد محمد سليمان* ، حامد محمود مزيد***

* معهد بحوث أمراض النباتات-مركز البحوث الزراعية-الجيزة-مصر

** قسم الكيمياء-كلية العلوم-جامعة القاهرة-الجيزة-مصر

تم تحديد و دراسة التتابع النيوكليوتيدى للجين المسئول عن انتاج الغلاف البروتينى لفيروس التفاف أوراق البطاطس (العزلة المصرية) وذلك باستخدام تقنية DNA معاد الأتحاد. حيث تم كلونة هذ الجين واستنساخه داخل بكتيريا القولون من السلالة BL21 والتى تم تحفيزها للقيام بعملية الترجمة الجينية لانتاج الغلاف البروتينى للفيروس مرتبطا بعدد ستة جزيئات من الحمض الأمينى الهيستيدين. تم التأكد من حدوث عملية التعبير الجينى داخل البكتيريا باستخدام اختبار الـ western blotting واستخدام أجسام مضادة متخصصة لهذا الفيروس حيث تأكدت عملية التعبير الجينى التى أنتجت بروتين مكون من 209 حمض أمينى وزنه الجزيئى 23 ك دالتون وذلك ناتج من الترجمة الجينية لعدد 627 نيوكليوتيدة. تمت مقارنة التتابع النيوكليوتيدى الناتج من هذه الدراسة بتتابعات أخرى مكافئة ومسئولة عن انتاج الغلاف البروتينى لفيروسات الـ luteoviruses group مسجلة فى بنك الجينات الدولى حيث اوضحت النتائج وجود تشابه بنسبة 95 % بين هذه العزلة والعزلات الأخرى على المستوى الجزيئى. تم حقن ارانب تجارب بالبروتين الناتج من عملية التعبير الجينى بعد تنقيته وتم فصل الأجسام المضادة من دم الأرانب ووجد انها نشطة مناعيا تجاه فيروس التفاف أوراق البطاطس وذلك بعد اختبارها بطريقة الـ Indirect ELISA.