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Biological, serological and molecular characterization of Potato virus S isolated from potato (Solanum tuberosum L.)

التشخيص الجزيئي، السيرولوجي و البايلوجي لفايروس البطاطا S (Potato virus S)المعزول من البطاطا (Solanum tuberosum L.)

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Abstract

This study was conducted to serologically, biologically and molecularly characterize the ordinary strain of *Potato virus S* (PVS^O) isolated from different potato fields located at the James Hutton Institute (JHI), Dundee City, Scotland, U.K. Fifteen potato leaf samples with one or more disease symptoms of leaf mosaic, distortion, mottling and yellowing were collected during the 2010-2011 growing season and serologically tested by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for six plant viruses, including *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus V (*PVV), *Potato virus X (*PVX), *Potato leafroll virus* (PLRV) and *Potato virus S* (PVS). ELISA results showed that 10 samples among the 15 collected samples were found to be infected with PVS.

Mechanical inoculation of *Chenopodium amaranticolor* plants with PVS isolates induced typical symptoms of chlorotic local lesions which later developed to necrotic lesions. No obvious symptoms were observed on the non-inoculated upper leaves of *C. amaranticolor*. However, the presence of the virus was confirmed, by DAS-ELISA and polymerase chain reaction (PCR), in the inoculated leaves of *C. amaranticolor* and no virus was detected in the non-inoculated leaves of the same plants.

Examination of the virus particles by electron microscopy (EM) showed the presence of only straight filamentous particles with a length of 650 nm and a width of 12 nm, which were similar to the reported dimensions of PVS particles. However, no other virus particles related with any other plant virus with the exception of PVS particles were observed, in the examined test samples. The results also showed that all PVS isolates obtained in this study was not transmissible by *Myzus persicae* and *Aphis nasturtii* aphids.

PCR amplification, cloning and sequencing of the coat protein (CP) and 11KDa genes of PVS isolates showed 100% pairwise nucleotide identity. Based on maximum nucleotide identity, results proposed that all of these isolates were found to be belonged to the ordinary strain of PVS (PVS^O).

Key words: *Potato virus S*, PCR-based characterization

خلاصة

نفذت هذه الدراسة بهدف التشخيص البايولوجي، السيرولوجي و الجزيئي لسلالة من فايروس البطاطا (*Potato virus* S (*S* المعزولة من حقول بطاطا مختلفة في معهد البحوث جيمز هاتن، مدينة دندي، اسكتلندا، المملكة المتحدة. خمس عشر عينة جمعت من نباتات بطاطا، ظهرت عليها اعراض امراض فايروسية مختلفة مثل التبرقش، التشوة و الاصفرار، خلال الموسم الزراعي 2010-2011. تم اختبار جميع العينات بواسطة اختبار اليزا (ELISA) و لستة فيروسات متمثلة بـ *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus V* (PVV)، *Potato leafroll virus* (PLRV) و *Potato virus S* (PVS). اثبتت نتائج اختبار اليزا بأن عشرة عينات من بين تلك العينات كانت مصابة بفيروس البطاطا S.

اوضحت نتائج التلقيح الميكانيكي لنباتات الزريج (*Chenopodium amaranticolor* L.) بالعزلات المختلفة من فيروس البطاطا S ظهور اعراض مرضية متمثلة ببقع مصفرة و التي تطورت فيما بعد الى موت موضعي في الاوراق الملقحة مع عدم ظهور اي اعراض مرضية على الاوراق الحديثة وغير الملقحة بالفيروس. كما تم الكشف عن وجود الفيروس بواسطة اختبار اليزا و تفاعل البلمرة المتسلسل (PCR) في الاوراق الملقحة فقط عند اجراء تلك الاختبار على الاوراق الملقحة و غير

الملقحة بالفايروس المزعوم.
كما وجد من خلال الفحص بالمجهر الالكتروني (EM) بأن للفايروس جسيمات خيطية مستقيمة ذات طول 650 نانوميتر و عرض 12 نانوميتر و التي كانت مماثلة لأبعاد فايروس البطاطا S اضافة الى عدم ملاحظة اي جسيمات اخرى تعود الى فايروس اخر في نفس العينات المأخوذة للفحص تحت المجهر الالكتروني. اظهرت النتائج ايضا بأن جميع عزلات فايروس البطاطا S ليس لها القابلية للانتقال بواسطة حشرات المن (*Myzus persicae* و *Aphisi nasturtii*). كما اثبت تفاعل البلمرة المتسلسل و كلونة جينين (Coat protein و 11KDa) مختلفين من جميع عزلات فايروس البطاطا S و معرفة تتابع القواعد الهيدروجينية بأن جميع عزلات فايروس البطاطا S تعود الى PVS^O.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world (1), and is susceptible to many plant viruses including *Potato virus S* (PVS). PVS belongs to the genus *Carlavirus* in the family *Betaflexiviridae* (2; 3). PVS virions are straight to slightly curved filamentous particles with a length of 650-710 nm, and 10-15 nm in width (4; 5; 6).

The positive-stranded RNA genome of PVS includes a poly-A tail at the 3'-terminus, a 5'-cap structure and six open reading frames (ORFs) (2; 3; 7). ORF1 is the biggest protein of PVS (231 kDa) and encodes the viral replicase. The ORFs 2, 3 and 4 form the triple gene block (TGB) that modulates cell-to-cell movement of PVS (8). ORF-5 encodes the 34 kDa coat protein (CP) and overlaps with ORF-6 that encodes a cysteine-rich nucleic-acid-binding protein (NABP) that is required in aphid transmission, host gene transcription and viral replication (9; 10).

PVS is widespread in potato production systems, decreasing potato yield production by 10-20%, depending on the susceptibility of the potato cultivars (11). However, disease symptoms often become severe when the virus occurs in synergistic infections, and yield losses can reach up to 80% (12) when present in a mixed infection with PVX or PLRV (13; 14; 15; 16).

There are two major strains of PVS, the Andean strain (PVS^A) and the ordinary strain (PVS^O), recognized by their ability to produce systemic and non-systemic infection in *Chenopodium* spp. (17; 5; 18; 19). The PVS^A induces systemic symptoms on *Chenopodium* spp., whereas the PVS^O strain does not (6).

Standard methods of virus detection including mechanical inoculation to indicator plants, electron microscopy (EM), enzyme-linked immunosorbent assay (ELISA) and visual inspection might not be practical to reliably detect some potato viruses (20). Increased sensitivity and specificity of detection offered by reverse transcription-polymerase chain reaction (RT-PCR) that is an effective and sensitive method in detection of viruses.

In this paper, seven PVS isolates collected from infected potato plants were serologically, biologically and molecularly characterized on the basis of their responses on *C. amaranticolor* plant species, a serological test, EM and their ability for transmission by aphids.

Materials and Methods

Collection and propagation of virus isolates

A total of 15 leaf samples of potato plants showing virus-induced symptoms, severe mosaic, mottling, leaf malformation and yellowing, were collected from different fields at the James Hutton Institute (JHI) -Dundee City-UK during the 2010-2011 growing season. Laboratory and glasshouse experiments were carried out at the Plant Virology Laboratory and glasshouses at the JHI-Dundee City-UK. Moreover, the virus isolates were propagated on young *Nicitiana debenyi* plants, and maintained every 2-3 weeks by manual inoculation. All plants of *N. debenyi* were maintained in an insect-free glasshouse supplemented with artificial lights to maintain a 16 h photoperiod under a temperature of 18 ºC during the light period and 14 ºC during darkness.

ELISA detection

Leaf extracts from each fresh sample were tested using antisera (Neogen Europe Ltd, Cat No: 1043-05) against the viruses PVY (Scottish Agricultural Science Agency, SASA), PVX (Neogen Europe Ltd, Cat No: 1047-5, 1047-6), PVA (Neogen Europe Ltd, Cat No: 1041-05), PVV (Neogen Europe Ltd, Cat No: 1045-05) by double antibody sandwich enzyme-linked immunosorbent assay

(DAS-ELISA) following the manufacturer´s instructions. Each leaf sample was assayed in four duplicate wells of an ELISA plate. In addition, plants infected with known viruses, representing PVY, PVA, PVV, PVS and PVX, were also included in this experiment as positive controls for ELISA test. Leaf samples of potato plants mock-inoculated with phosphate buffer were included in the experiment as a negative control for successful ELISA test

Samples were considered positive if their mean A_{405nm} values were greater than twice the mean A_{405nm} values recorded from the mock-inoculated plants (21; 22; 23). For maintaining only PVS isolates, seven PVS-infected samples with no synergistic infection with other viruses were used to prepare sap extracts for mechanical inoculation of *N. debenyii*. All the inoculated plants were maintained in a free-virus glasshouse supplemented with artificial lights to maintain a 16 h photoperiod under a temperature of 18 ºC during the light period and 14 ºC during darkness.

Mechanical inoculation of C. amaranticolor plants

N. debneyi leaf saps infected with PVS isolates were used to mechanically inoculate on C. amaranticolor to determine the ability of these isolates to produce either local or systemic infection. Sap extracts of PVS-infected plants showing vein-clearing symptoms were prepared by grinding 5 g of infected leaf tissues in 15 ml 0.07 M Sörenson phosphate buffer.

Before performing inoculation experiment, seven plants of C. amaranticolor at the 4-5 leaf stage for each virus isolate were covered with newspapers overnight to make the plants more susceptible for virus inoculation. Two small holes were made at the tip of leaves that were going to be inoculated with the virus under diagnosis. These leaves were then given a light dusting with corundum powder and gently rubbed by a gloved forefinger dipped in the extracted leaf sap, prepared as previously described. The inoculated leaves were rinsed with water 30 minutes after inoculation and covered again overnight with newspapers to prevent wilting. Two plants of N. debneyi were also sap-inoculated with the same virus as positive controls for successful virus inoculation. C. amaranticolor plants were also mock-inoculated with 0.07 M Sörenson phosphate buffer as negative controls.

Thereafter, the inoculated plants were maintained in a free-virus glasshouse under the same condition mentioned previously and monitored daily for virus-induced symptom development for a period of 27 days post inoculation (dpi). Three weeks post-inoculation; inoculated and non-inoculated upper leaves of each plant were individually sampled and tested by DAS-ELISA.

Virus transmission by aphids

In order to investigate the ability of PVS transmission by aphids, two aphid species, *Myzus persicae* and *Aphis nasturtii*, provided by Dr. Brian Fenton (JHI), were reared on fresh plants of *N. tabacum* cv. White Burley placed in a large cage in a separate room at 18 ºC. The aphids were transferred weekly to fresh plants.

Apterous aphids of each species were chosen from a fresh culture and transferred to 1.5 ml plastic Eppendorf tubes in batches of 20. The tubes containing aphids were kept for 3 h for fasting at room temperature (22 ºC) before performing the virus transmission experiment. A total of 10 aphids of each species were given an acquisition feeding period of 5 min on freshly detached PVS-infected leaves of *N. debneyi*. Thereafter, aphids of each species were transferred to PVS-free *N. debneyi* plants, at the 2-3 leaf stage. Plants were then kept overnight in an insect-free glasshouse supplemented with artificial lights to maintain a 16 h photoperiod under a temperature of 18 ºC during the light period and 14 ºC during darkness. All plants at 21 dpi were individually tested by DAS-ELISA as described by Clark and Adams (24). This experiment was repeated three times.

Electron microscopy (EM)

EM study was carried out to determine the morphology of virus particles. Leaf extracts were prepared by macerating a leaf sample, taken from *N. debneyi* plants exhibiting vein-clearing symptoms, in a small amount of distilled water. A drop of the extract was placed on a carbon-coated grid, left for a few seconds and then drained using a filter paper. One drop of the phosphotungstic

acid dye was added and left for approximately 15 sec. The grid was then drained using a filter paper, washed with distilled water, allowed to air dry and then examined under the EM.

RNA extraction, oligonucleotide primers, cDNA synthesis and PCR amplification

Total plant RNA was extracted from a two week old *N. debenyi* plant infected with PVS using the Qiagen RNeasy Mini Kit® (Cat. No: 74904) following the manufacturer´s instructions.

For virus detection, PVS^O-specific primers {(PVS^O-F-5´- AACATTGGGG CCGTTGAAGCACC-3´) and (PVS^O-R-5´-CATATTGTCTTTATAGTTGCC-3´)}, synthesised commercially (Eurofine MWG Operon), were designed in this study to amplify a 1346bp-DNA fragment including the full CP and 11KDa genes. Specific primers, {(PVS^A-F-5´-GAATATACAGTCTCACAGCAAG-3´) and (PVS^A-R-5´- CCTGTAAACA CACAAACAGTAAC-3´), were also designed to PCR-amplify a 900bp DNA fragment, including the CP of the PVS^A strain.

The reverse transcription (RT) step was performed to copy the full CP and 11KDa genes of PVS^O and the PVS^A CP gene using the Moloney Murine Leukaemia (M-MLV) reverse transcriptase kit (Cat. No. M1701). An RT reaction mixture was set up in a 10 µl volume: containing 10 µl RNA, 1 µl each reverse primer at 10 pmol/ µl, and 14 µl free-nuclease water. The PCR reaction mixture was incubated at 70 °C for 10 min and immediately placed on ice for 2 min. Thereafter, 4 µl 5X M-MLV buffer, 1 µl of 10 mM dNTPs and 1 µl M-MLV RT were added and the mixture incubated at 42 °C for 1 h. The synthesized complementary DNA (cDNA) was used as template DNA in PCR reaction mixtures.

Duplex PCR amplification for simultaneous detection of PVS^O and PVS^A was performed using *Taq* DNA polymerase (Roche, Cat. No. 11 146 173 001) in a 20 µl PCR reaction mixture containing 4 µ1 10X PCR buffer, 2 µl each primer (10 pmol), 5 µl template DNA (30 ng/µ1), 4 µl dNTPs (2 mM), 2 unit *Taq* polymerase. Sample volume was adjusted to 40 µl with nuclease-free water. The PCR reaction parameters were: 94 °C for 1 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing temperature at 56 for 30 sec, 72 °C extension for 1 min and final extension at 72 °C. Amplified products were separated electrophoretically on a 1% agarose gel and visualised with ethidium bromide staining under UV illumination.

Molecular cloning and sequencing of the cloned PVS^O genes

Purified RT-PCR products were ligated directly into the commercial cloning vector pGEM-T Easy vector (Promega, Cat. No. A1389) following the protocol provided by the manufacturer´s instructions. Two µl of the ligation reaction mixture was electroporated, at 1.8 Kv for approximately 1 sec, into electro-competent cells of *Escherichia coli*, strain DH5α, using the electroporator (Biorad *E. coli* pulser, Life Technologies, USA). Thereafter, two independent recombinant plasmids of each isolate, were sequenced, on both strands using forward and reverse M13 primers, in-house by the Sequencing and Microarray Facility at the JHI. The identities of the cloned DNA fragments were verified by searching their nucleotide sequences on the BLAST program provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results and discussion

Virus detection by ELISA

Among the 15 potato leaf samples tested by DAS-ELISA, six samples were found to be infected with PVS, and 4 samples infected with PVY which gave mean A_{405nm} values greater than twice the A_{405} value of mock-inoculated plants. PVY, PVA, PVV, PVX antibodies gave negative ELISA readings upon testing of the same samples for these viruses (see Table 1).

Table 1: Mean absorbance values (A_{405nm}) obtained by ELISA phenotypic evaluation after testing potato leaf samples for the presence or absence of plant viruses PVY, PVA, PVV, PVX, PLRV and PVS.

Virus isolated	Potato leaf sample																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Negative control	*Positive control
PVY	+	-	-	-	-	-	+	-	-	+	-	+	-	-	+	-	+
PVA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PVV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PVX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PLRV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
PVS	+	-	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+

DAS -ELISA was used for detection of PVY, PVA, PVV, PVX, PLRV and PVS. *,Potato leaves infected with known viruses (PVY, PVX, PVA and PVV) were used as positive controls for ELISA test. A_{405nm} values were recorded following an overnight (16 h) incubation period.

PVS strain identification by Bioassay

Visible symptoms induced by seven PVS isolates were started appearing 15 dpi. Overall, all C. amaranticolor plants inoculated individually with the even PVS isolates were exhibited similar symptoms of chlorotic local lesions that later developed to necrotic local lesions (Figure 1). No visible symptoms were observed on the non-inoculated upper leaves of the same test plants. However, the presence of PVS was only confirmed, using DAS-ELISA and RT-PCR, in the inoculated leaves of C. amaranticolor plants.

Khalil & Shalla (25) reported that the PVS strains, PVS^A and PVS^O, can be differentiated by inoculating indicator plants of Chenopodium spp. PVS^O induces chlorotic local lesions 1-2 mm in diameter on C. amaranticolor, C. quinoa and C. album 20 days after mechanical inoculation. However, PVS^O does not invade the plant systemically in contrast to PVS^A, which infects all of these plants systemically (26; 27; 19).

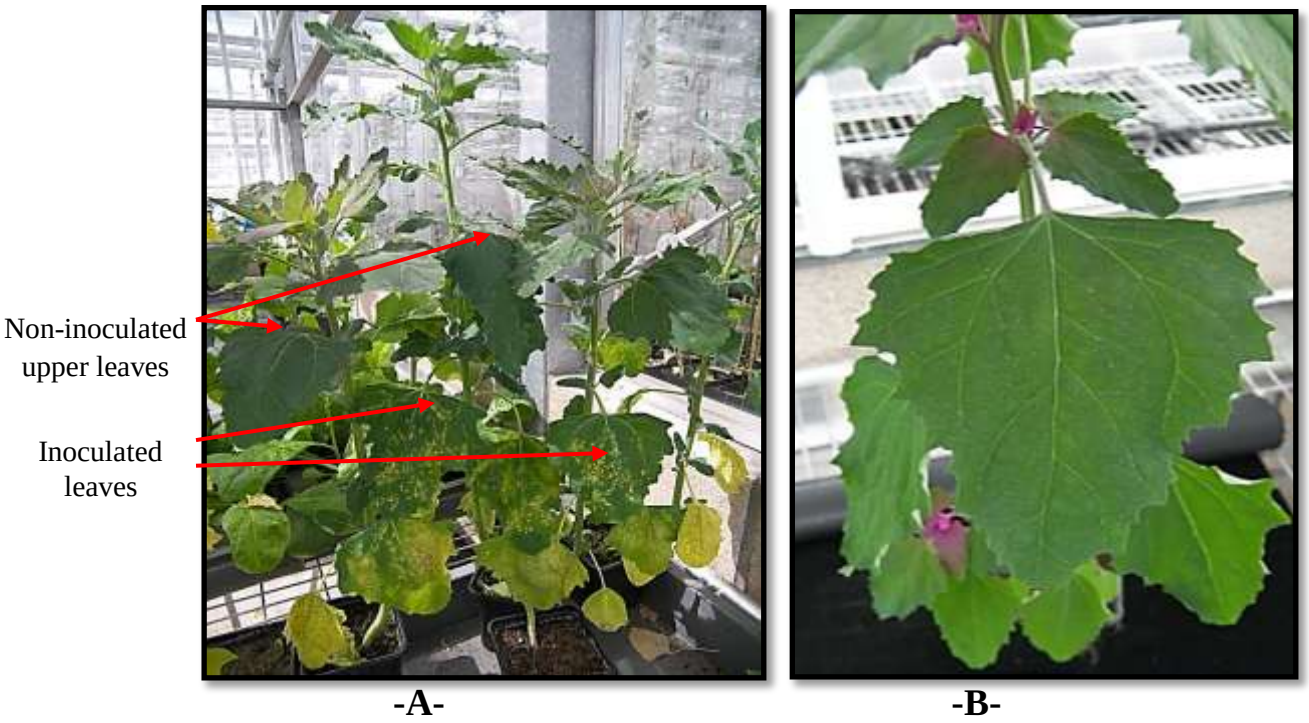

Figure 1: Plants of Chenopodium amaranticolor manually inoculated with a PVS isolate. PVS-infected plants were only induced symptoms of necrotic lesions on the inoculated leaves (A). A plant inoculated with phosphate and used as a negative control for comparative purpose (B).

Electron microscopy (EM)

EM examination showed the presence of straight filamentous particles with a length of 650 nm and a width of 12 nm, as an average of 35 particles, that were similar to the expected dimensions of PVS particles reported by De Bokx (28) and Wetter (13) (Figure 2). No other virus particle related with any other plant virus except the PVS particles were observed in the examined test sample.

Figure 2: Electron micrograph showing the morphology of PVS particles stained with phosphotungstic acid. Bar represents 100 nm.

PVS transmission by M. persicae and A. nasturtii aphids

No visible symptoms were observed in the test plants of N. debenyi and the absence of PVS was confirmed by DAS-ELISA. The mean A_{405nm} values obtained from three experiments of M. persicae and A. nasturtii transmission were 0.072 and 0.064, respectively, compared with the mean A_{405nm} value (0.071) recorded from the negative control (Table 2).

The results showed that the PVS isolate was not transmitted by M. persicae and A. nasturtii aphids. This behaviour of non-transmission of PVS is not unprecedented as it has already been reported that some isolates of PVS are not aphid-transmissible (17; 29); therefore, other factors may be responsible for PVS spread in potato fields. Wardrop et al. (30) found that none of the aphid species M. persicae, A. nasturtii, Macrosiphum euphorbiae, Aulacorthum solani and Rhopalosiphum padi were able to transmit PVS^O. As reported by Slack (17), PVS^A is transmitted by aphids in a non-persistent manner.

Cox et al. (31) showed that multiple alignment of complete PVS CP sequences revealed that most variability of amino acid sequences occurred in the N-terminal region. Such differences in the PVS strains might be responsible for the differences in symptomatology and aphid transmission (5; 19). Taken together, it is most likely that the PVS isolate used in this study is belonged to the PVS^o strain as no systemic infection was observed in plants of C. amaranticolor and C. quinoa (26; 4; 7) as well as no virus transmission by M. persicae and A. nasturtii occurred.

Table 2: Mean absorbance values (A_{405nm}) obtained by DAS-ELISA phenotypic evaluation after inoculation of N. debneyi plants with PVS using two aphid species, Myzus persicae and Aphid nasturtii

Aphid species	N. debenyi plants tested by DAS-ELISA										
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Mean	*Negative control	** Positive control
Myzus persicae	0.058*	0.069	0.077	0.053	0.078	0.092	0.095	0.058	0.072	0.071	5.593
Aphis nasturtii	0.068	0.063	0.069	0.068	0.067	0.057	0.053	0.068	0.064		

*****Mean absorbance values presented in the table were recorded at 405_{nm} following an overnight incubation period (16 h) and each represents the mean of three experiments.*, non-inoculated *N. debenyi* plants were used as negative controls for ELISA test. ****,** *N. debenyi* plants manually inoculated with PVS were used as positive controls for ELISA test.

PCR-based PVS strain identification

Testing of *C. amaranticolor* leaves, inoculated individually with different PVS isolates, using PVS^O-specific primers showed an amplification of 1346bp form the inoculated leaves, but no RT-PCR product was amplified from the non-inoculated upper leaves of the same test plants (see Figure 3). Moreover, no DNA fragment was PCR-amplified from the inoculated and non-inoculated leaves, upon using PVS^A specific-primers to test the same leaf samples (results not shown).

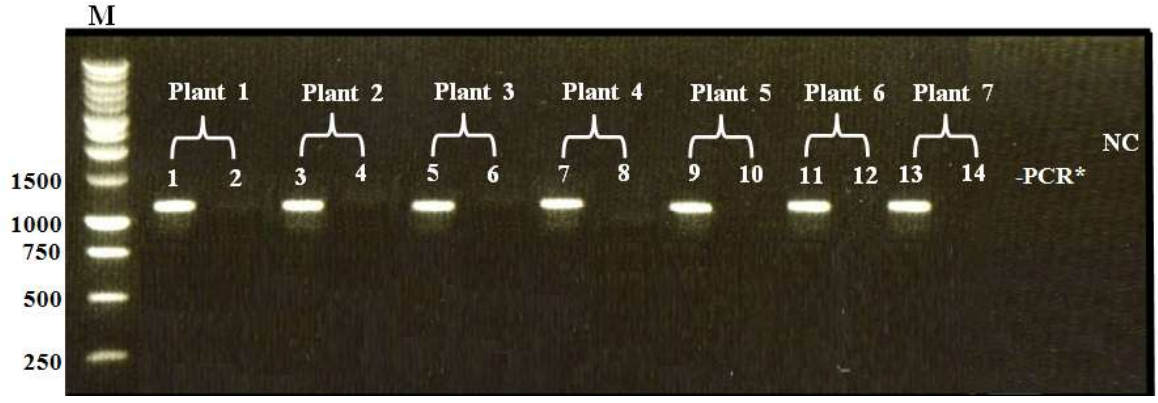

Figure 3: Agarose gel, stained by ethedium bromide, electrophoresis analysis of RT-PCR amplification of the total virus RNA extracted from the inoculated and non-inoculated upper leaves of *Chenopodium*

amaranticolor plants. RT-PCR products, representing the full CP and 11 KDa genes of PVS, were only amplified from the inoculated leaves (lanes; 1, 3, 5, 7, 9, 11 and 13), but not amplified from the non-inoculated leaves (lanes; 2, 4, 6, 8, 10, 12 and 14) of the same test plants. M, 1Kbp DNA ladder as molecular size marker given on the left in bp.

-PCR*, non-infected *C. amaranticolor* plant, inoculated only with phosphate buffer, used as a negative control; NC, negative PCR control (no template DNA added).

Molecular cloning and nucleotide sequencing

The PVS CP and 11KDa genes amplified using specific primers were ligated directly into the pGEM-T Easy vector. The recombinant pGEM-T plasmids were then transformed into *E. coli* competent cells. White ampicillin resistance colonies were selected and cultures in 5 ml LB medium supplemented with ampicillin. Thereafter, the pGEM-T plasmids were purified using the minipreperation technique and digested with the *Eco*RI restriction enzyme. The expected size (1346bp) of the full cloned PVS CP and 11KDa genes was confirmed by gel agarose electrophoresis on a 1% agarose gel (Figure 4) and the identity of the genes was verified by DNA sequencing.

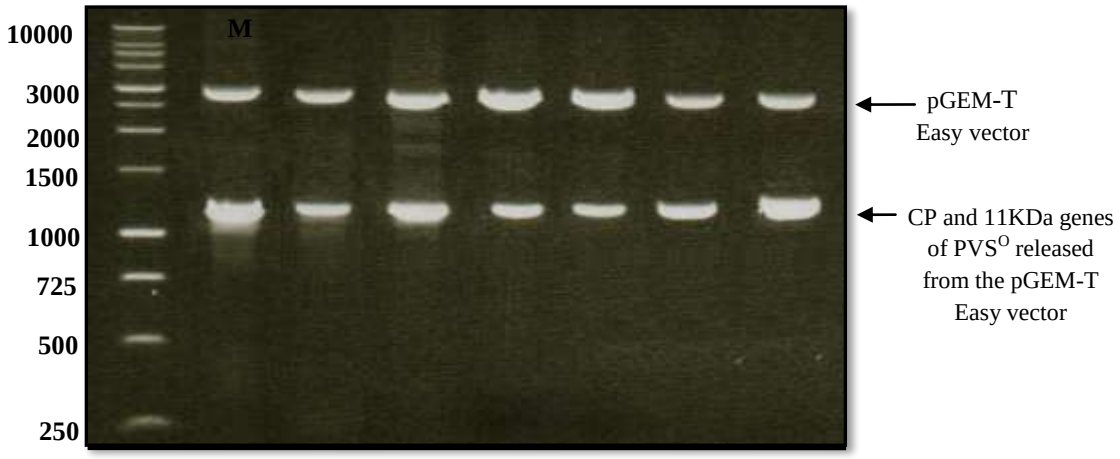

Figure 4: Restriction analysis of the PCR-amplified products of recombinant pGEM-T plasmids containing the PVS^O CP and 11KDa genes (1346bp). The top horizontal line of a DNA band is the pGEM-T Easy vector (3015bp), while the lower bands are the released PVS^O CP and 11KDa genes. M, 1Kbp DNA ladder as molecular size marker, given in bp.

BLAST searches using the deduced nucleotide sequences of the CP and 11KDa of PVS isolates were revealed highly homologous, by having 98% maximum identity, to those from PVS^{O} (GenBank Aaccession No. S45593) giving conclusive results that all of these PVS isolates are belonged to PVS^{O} (Figure 5).

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   1 AACATTGGGG CCGTTGAAGC ACCTTTAGGT TCACAGGTAA GAGTTCGAAG AAACTGTCCC
  61 ACAGAGAAAA TGCCGCCGAA ACCGGATCCG ACAAGCTCAG GAGAAACACC ACAAGCGATA
 121 CCGCTTGCGC CGCCGCCCCG GAACGTATAT TATCATAGAA TTGGCCCAAA CCAAGGGCAC
 181 GGGCAAAATG AAGAGGCTAT GCTGGAGCAG AGGCTCATCA GACTGATTGA ACTCATGGCC
 241 TCGAAAAGGC ACAATTCAAC ATTGAGCAAC ATAGCTTTTG AGATAGGTAG GCCCTCACTT
 301 GAGCCAACCC CTGAAATGCG GAGGAATCCG GAGAACCCAT ACTCGCGGTT TTCAATCGAT
 361 GAGCTGTTCA AGATGGAAAT CCGATCTGTG TCCAACAACA TGGCGAACAC TGAGCAAATG
 421 GCACAAATTA CTGCTGACAT CGCTGGACTT GGGGTCCCCA CTGAACACGT TGCAGGGGTC
 481 ATACTGAAAG TGGTGATCAT GTGTGCAAGC GTGGACAGCT CAGTTTATCT AGATCCAGCA
 541 GGAACCGTTG AGTTCCCAAC AGGCGCAGTG CCCTTGGACT CGATCATTGC AATTATGAAG
 601 AATCGCGCGG GATTGAGAAA AGTGTGCAGG CTGTATGCTC CAGTTGTGTG GAATTACATG
 661 CTAGTCCAGA ATAGACCACC TTCGGATTGG CAGGCCATGG GATTTCAATG GAATGCACGT
 721 TGCGCCGCAT TTGACACATT TGATTATGTG ACCAATGGGG CTGCAGTCCA GCCCGTAGAG
 781 GGGCTCATAC GCAGGCCCAC ACCTGAGGAA ACAATAGCTC ACAATGCCCA CAATGCCCAC
 841 AAGAGTATGG CAATTGACAA GTCGAACAGA AATGAGCGAT TGGCCAACAC TAATGTTGAG
 901 TACACTGGAG GGATGCTTGG CGCTGAGATT GTGCGCAATC ACCGTAATGC GATCAACCAA
 961 TGAAGGCAGA CCGTTTAGCT ATGTTATTAT TGTGTGTCCA TCGACTGGGA TATGTTTTGC
1021 CAGTTGAAGT TTGTGTAAAT ATAATAAGCC TAAGCGCAGG TCCAGTTTCT GGGGGTCGTT
1081 CCACTTACGC TCGTAAGCGG AGGGCCCGCA GCATTGGGCG ATGCTGGCGA TGTTATCGTG
1141 TCTATCCACC TATTTGTAAT TCTAAGTGTG ATAATAGGAC ATGCCGTCCA GGCATTAGTC
1201 AAAATTATAA AGTAGTGACT TTCATTCGGG GTTGGAGTAA CTGAGGTGAT ACCACCCATG
1261 GTGCAAAGTC AGAGTTTCGC ATAAAACTTA AATAATATAT AAGTGTGCAA CTATAAAGAA
1321 ACTATGTTTT AAAATATTTT ATCATA
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Figure 5: The nucleotide sequence of 1346 nucleotides corresponding to the full CP and 11KDa of PVS^{O}.

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