Int J Pharm Bio Sci 2016 Oct; 7(4): (B) 757 - 764

Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IDENTIFICATION OF MOLECULAR MARKER LINKED TO *POTATO VIRUS Y* RESISTANCE IN POTATO CULTIVARS TREATED WITH RIBOSOME INACTIVATING PROTEINS (RIPS)

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ABSTRACT

Potato virus Y Potyviridae (PVY) is one of the most destructive viruses of the potato crop in Egypt and the world. Ribosome inactivating proteins (RIPs) isolated from *Mirabilis jalapa* and *Phytolacca* sp plants, which inhibit the infection with necrotic tuber necrosis strain of *potato virus Y* (PVY^{NTN}) by depurinating from the 28S RNA. The primary objective of this work, was the development of an easy and simple strategy for controlling PVY^{NTN} by applying of aqueous extract from *M. jalapa* and *Phytolacca* sp plants. In addition, assessment of genetic diversity among PVY^{NTN} resistance, susceptible and the control potato plants was performed by Random amplified polymorphic DNA (RAPD). Five potato cultivars ('Cara', 'Diamond', 'Nicola', 'Selan' and 'Spunta') sprayed with aqueous extracts before virus inoculation led to inhibition of PVY^{NTN} infection with 100%; these results were confirmed by Double antibody sandwich enzyme linked immuno-sorbent assay technique (DAS-ELISA). Analysis of variance displayed there were no significant differences in the number, weight and volume of tubers between extract-pretreated potato plants and the healthy control in all five potato cultivars. RAPD analysis using five arbitrary decamer primers was applied to determine DNA polymorphism among the PVY^{NTN} resistance, diseased and the control potato plants. A total of 65 reproducible fragments ranged from 100-1200 bp were recorded using five primers. Thirty-three out of 65 (50.77%) loci were polymorphic and other 32 (49.23%) were monomorphic. The number of amplicons for each primer varied from 5 to 22 bands. Among the 65 bands, 18 (27.69%) were reproducible and considered as reliable RAPD markers for further analyses. Aqueous extract treated potato cultivars which gave resistance against the PVY^{NTN} strain diversified considerably using the five primers of RAPD-PCR, whereas the 'Selan' cultivar showed to have the highest number of specific bands (seven), followed by 'Spunta'(six), 'Nicola' (five) and 'Diamond' (four), while 'Cara' scored the lowest number of unique bands (one). This information should be taken into consideration in future breeding programs. Spraying of aqueous extract from *P. americana*, *P. acinosa* and *M. jalapa* plants in different crops led to prevent or control viral infection.

KEYWORDS: ***Phytolacca sp.*, *Mirabilis jalapa,* virus, RAPD-PCR, cluster analysis.**

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INTRODUCTION

Potato virus Y Potyviridae (PVY) is one of the most destructive viruses of family *Solanaceae*, genus *Potyvirus* and it induces crop losses from 10 to 90%. PVY causes severe mosaic, crinkling, rugosity, necrosis and decrease potato yield productivity and quality.[1-3] A further strategy for inserting virus resistance in plants is applying of natural inhibitors of virus multiplication.[4-6] Different plants generate enzymes known as ribosome inactivating proteins (RIPs), or rRNA *N*-glycosidases, which inhibit ribosome by removing a specific adenine base from rRNA in a highly conserved stem-loop structure in the 28 sRNA.[7-10] RIPs have been isolated from several various plant species such a pokeweed (*Phytolacca americana*), *P. acinosa* and native peruvian (*Mirabilis jalapa*). RIPs are divided into two types depending on their subunits configuration. Type-I RIPs composed of a single polypeptide chain, whereas type-II consisted of two polypeptides, an active A-chain and a B-chain which is a galactose-binding lectin. Ribosomes depurinated are not able to link the EF-2/GTP complex and protein synthesis is inhibited at the translocation stage.[11]Three different types of RIPs: Pokeweed antiviral protein (PAP-I), PAP-II and PAP-S have been extracted and purified from pokeweed (*P. americana*) plants. These proteins are identical in molecular weights (MWs) (29, 30 and 29.5 KDa, respectively) but are expressed at various developmental stages and in different tissues of pokeweed.[12-13] PAP depurinates ribosomes of pokeweed and several of other plants such as *M. jalapa*, also mammalian, yeast and bacterial ribosomes. In addition, PAP efficaciously prevents infection by a number of various animal[14] and plant viruses,[15-18] including human immunodeficiency virus (HIV).[19] Besides, PAP suppresses growth of tumor cells.[8,20] Positive correlations were observed between RIP-catalyzed depurination of tobacco ribosomes and antiviral activity of exogenously used RIPs[21] and between the extent of virus infection prohibition by PAP and the depurination of plant ribosomes.[14] Transgenic potato plants expressing PAP give broad-spectrum resistance against various viruses' infection.[22] The aim of this study, was the development of an easy method for controlling *Potato Virus Y* (PVY^{NTN}) by spraying of aqueous extracts from *M. jalapa* and *Phytolacca* sp on potato varieties. Furthermore, assessment genetic variability among PVY^{NTN} resistance, infected and the healthy control potato plants was performed by RAPD-PCR.

MATERIALS AND METHODS

Plant Materials

P. americana, *P. acinosa* and *M. jalapa* seeds were collected at Leibniz Institute of Plant Science and Crop Plant Research (IPK), Gatersleben, Germany. Seeds were sowed in pots and kept in a greenhouse for eight weeks. Temperature in the greenhouse was kept at 21-25ºC.

Source of PVY^{NTN} strain

The necrotic tuber necrosis strain of *potato virus Y* (PVY^{NTN}) was obtained from the Virology Laboratory, Department of Agriculture Microbiology, Faculty of Agriculture, University of Ain Shams which was previously isolated and identified from systemically infected potato plants.[23] The isolate was maintained on *Datura metel* L. plants. Systemically infected leaves were used as sources of inoculum in all experiments.

Plant extract

The mixed aqueous extract of three medicinal plants was prepared by 1 g fresh leaves of each *P. americana*, *P. acinosa* and *M. jalapa* plants was blended in the pestle and mortar with a 5 ml sterilized dsH_2O. The homogenate was squeezed through two layers of cheesecloth before spray as described by Mahdy *et al*.[24]

Preventive treatment with antiviral protein (AVP) extracts

Tubers of the five potato cultivars ('Cara', 'Diamond', 'Nicola', 'Selan' and 'Spunta') were tested for free from potential infection with potato viruses [PVY, *Potato virus X* (PVX) and *Potato leaf roll virus* (PLRV)] by DAS-ELISA technique were provided by Sanofi Company Sante Animal, Paris, France and used as described by Clark *et al*.[25] Thirty healthy tubers from each cultivar were planted in the open field in winter season 2014/15 with factorial completely randomize design. Twenty rows, including ten holes each per row and intra-and inter-row distances of 20 cm and 60 cm, respectively for each cultivar.[26] Potato plants grown from thirty tubers of each cultivar were divided to three treatments:

Treatment-1: Ten antiviral extract-treated potato plants of each cultivar and subsequently inoculated with the sap from PVY^{NTN} infected plants (AVP).
Treatment-2: Ten potato plants of each cultivar inoculated with PVY^{NTN} strain only.
Treatment-3: Ten dsH_2O treated healthy potato plants of each cultivar (the healthy control).

Potato leaves and tubers of three treatments were collected after 30 and 120 days, respectively. Mean number, weight and volume of potato tubers per plant were measured.

Determination of potato volume by water displacement

This is the most accurate method for measuring the potato volume. A graduated cylinder was half filled with water. Each potato was then immersed carefully, increasing the level of water. The difference between the final and the initial water levels gives a measure of the volume of the potato tubers.[27]

enzyme linked immuno-sorbent assay (DAS-ELISA)

All the plant samples of treatments were tested for the presence of PVY^{NTN} strain by the double antibody sandwich enzyme linked immuno-sorbent assay technique (DAS-ELISA) as described by Clark *et al*.,[25] PVY ELISA kit provided from Sanofi Company Sante Animal Paris, France.

DNA isolation

0.5 g of young leaves of the three treatments from each cultivar were collected and soaked in liquid nitrogen for DNA extraction using the 2% CTAB (Cetyltrimethylammonium bromide) method modified by Doyle *et al*. and Mahfouze.[10,28]

RAPD-PCR analysis

A total of five primers were used to amplify DNA[29] (manufactured by Bioneer, New technology certification from ATS Korea) (Table 1). The total reaction mixture was 25 µl contained 10× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 *p*mol primer, 1.25 U *Taq* polymerase and about 150 ng genomic DNA. RAPD-PCR amplification was performed in a thermal cycler (Biometra Inc., Germany). The temperature profile was as follows: an initial denaturation at 94°C for 3 min; followed by 35 cycles of denature temperature 94°C for 5 min; annealing temperature 37°C for 1 min and extension temperature 72°C for 1 min, final extension at 72°C for 5 min.

Amplification product analysis

The amplified DNA (25 µl) for all samples was electrophoresed on 1% agarose containing ethidium bromide (0.5 µg/ml) in 1x TBE buffer (89 mM Tris-HCl, 89 mM Boric acid, 2.5 mM EDTA, pH 8.3) at 75 constant volt and determine with UV transilluminator. The size of each fragment was estimated with reference to a size marker of 100 bp DNA ladder (BioRoN, Germany). The gel analysis was applied by a program (UVI geltec version 12.4, 1999-2005, USA).

Data analysis

A matrix for RAPD was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the treatments. Genetic similarity coefficients were computed according to Nei *et al*.[30] The data were subsequently used to construct a dendrogram using the un-weighted pair group method of arithmetic averages (UPGMA)[31] employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1.[32] Correlation coefficients were calculated using similarity coefficients obtained from combined RAPD analysis.

Statistical analysis

All experiments were arranged in factorial completely randomize design and data were compared according to the method described by Snedecor *et al*.[33] Analysis of variance (ANOVA) for all measured variables was performed using the software new MSTAT-C (version 2.1). The level of significance was measured using Duncan's multiple range test; $P \leq 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Inhibitory activity of AVP-extracts against PVY^{NTN} strain

Antiviral-extracts (AVP) prepared from *M. jalapa* and *Phytolacca* sp plants were sprayed on potato leaves of five cultivars; Results found that the AVP extracts were inhibited PVY^{NTN} infection with 100%. On the contrary, Non-AVP-treated potato varieties (PVY^{NTN} infected) gave different viral symptoms. 'Cara' cultivar appeared systemic necrotic spots, 'Selan' produced systemic necrotic spots and yellowing, 'Nicola' showed apical necrosis. Besides, 'Diamond' and 'Spunta' displayed mild mosaic. These results were confirmed by DAS-ELISA. These results were in an agreement with Yoonkang *et al*.; Vivanco *et al*.; Picard *et al*.; Mahfouze *et al*.[10,15,18,34] found that *Phytolacca* sp and *M. jalapa* extracts, contained on a ribosome inactivating proteins (RIPs) when were examined against different plant viruses such as PLRV, PVY and PVX. Also, viroid as *Potato spindle tuber viroid* (PSTVd). Root extracts of *M. jalapa* plant sprayed on test plants 24 h before the viroid or virus inoculation suppressed infection of 100%, as confirmed by infectivity assays and the Nucleic acid spot hybridization technique (NASH)[15]. The antiviral activity of RIP extracts was showed against several of mechanically transmitted plant viruses, but not against the insect transmitted viruses. The effect of phytoantiviral extracts against infection with the virus suggests that a machinery by which RIP permeate the upper layers of the epidermis cells and locates itself in the intercellular places. While, the plants are wounded due to viral infection, RIP is capable penetrate epidermal and leaf cells, wherever it deglycosilates the 28S rRNA. This prohibits viral multiplication at an early phase, by inactivating the cell protein chemistry mechanism[9]. When viral inoculation is achieved, the virus permeates the cell along with RIP. Once within the cell, RIP and the virus contend for the active sites on the 28S rRNA. RIP depurinates the 28S rRNA and hence deactivates the protein synthesis of the cell. Our results showed that RIP arrives at the active site of the ribosomes first, prohibition viral infection at an early phase, before viral de-encapsidation. Poyet *et al*. and Picard *et al*.[18,35] who found that RIPs have not influenced on protein synthesis in healthy plant cells, which indicates that intact, healthy cells. Nevertheless, it has been proposed that RIPs entry is intermediated by alterations in the cellular membrane stimulated by the adsorption of viral particles on the plant cell surface. In this action, virus entry is not demanded. An alteration in the cell membrane integrity could lead to the ingress of RIPs and hence would allow a cellular suicide mechanism. Since this double inhibitory activity, RIP_S have become the object of a wide range of studies interesting their potential using as new therapeutic materials and as putative protective proteins applied by plants as a defense against viruses.

Number, weight and volume of tubers

Variance analysis results showed that there was a significant difference between AVP-treatment potato plants which appeared resistance against PVY^{NTN} and infected ones. Potato cultivars treated with AVP extract showed a better number of tubers compared with infected plants such as 'Selan' (10.17) and 'Spunta' (7.67) (Table 2). On the other hand, there was a significant difference in tubers number between PVY^{NTN} infected potato plants and the healthy control such 'Spunta' and 'Diamond' as shown in Table (2). A significant difference ($P \leq 0.05$) was observed between the AVP treated potato plants and PVY^{NTN} infected ones

and between the latter and the control in weight and volume of tubers, as 'Spunta' cultivar (Table 2). Moreover, there was no significant difference in number, weight and volume of tubers between AVP-treated potato plants and the control in all five potato cultivars. The findings were in an agreement with Poyet *et al*. and Picard *et al*.[18,35] who found that RIPs have not effected on protein composition in the healthy plant cells. Hemmat *et al*.[36] mentioned that the potato is propagated by tubers and annual infection with viruses into the generated tubers increases, the potato tubers number has reduced as a result of viral infection, it significantly decreases the quality and quantity values. Consequently, efforts are made to supply pathogen-free tubers. Thus, that is evaluated that the application program of the healthy potato tubers will increase crop by at least 30%.[37]

RAPD-PCR analysis

The molecular genetic variability in genomic DNA among the AVP-treated potato cultivars (PVY^{NTN} resistance), infected and the healthy control ones were detected by RAPD-PCR analysis using five random decamer primers (Fig 1 and Table 3). A total of 65 reproducible and scorable ranging from 100 (primer-2) to 1200 bp (primer-3) were scored using five primers. Thirty-three out of 65 (50.77%) bands were polymorphic loci and the remaining 32 (49.23%) were monomorphic. The number of amplicon varied from 5 (primer-4) to 22 (primer-3). The maximum polymorphism recorded in primer-5 (72.73%), while the minimum polymorphism showed in primer-3 (40.91%). Among the 65 fragments, 18 (27.69%) bands turned out to be reproducible and regarded as a reliable RAPD marker for further analysis (Table 3). The AVP-treated potato cultivars (PVY^{NTN} resistance) were diversified considerably using the five primers of RAPD-PCR, whereas the 'Selan' cultivar appeared the highest number of unique markers (seven), followed by 'Spunta' (six), 'Nicola' displayed (five), 'Diamond' (four), while 'Cara' scored the lowest number of the specific bands (one) (Table 3). These results were agree with those obtained by Shiran *et al*.[38] who found that some of the primers produced numerous markers and were capable show a high level of genetic variability, while others generated a single or few markers and discovered a little diversity. The number of RAPD alleles was observed by each primer relies on primer, sequence and the extent of variance in the potato cultivar. Mahfouze *et al*.[10] studied alterations in genomic DNA induced by AVP-treated potato cultivars by ISSR-PCR marker, using five primers compared to PVY^{NTN}-diseased and the healthy plants. The total number of 63 loci amplified DNA amplicons ranged from 90 to1105 bp. Forty-three bands were polymorphic and the other fragments were monomorphic. The five primers displayed mean polymorphic percentage of 68.25%. The maximum percent from polymorphic recorded using primer ISSR-1 (87.50%), followed by primer ISSR-C4 (72.73%), while primer ISSR-C1 scored the minimum percentage (33.33%). The AVP-treated potato cultivars were frequently differed using the five primers of ISSR-PCR profiles, while the 'Nicola' cultivar appeared the highest number of markers (nine), followed by 'Selan' cultivar (eight). Finally, 'Spunta' and 'Diamond' revealed (five) and (one), respectively. On the contrary, 'Cara' cultivar has not recorded any markers. Langridge *et al*.[39] mentioned that RAPD analysis has frequently been applied for fingerprinting ascribable efficiency, simplicity and non-requirement of sequence data. RAPD-PCR assay has been widely applied for characterization of cultivars in crop plants, for studying the genetic diversity within species and to display relationships among populations. RAPD-PCR uses 10-base pair primer to amplify the random segment of genomic DNA. It is a high throughput marker technology, which provides the analysis of individual and large number of markers in a relatively short time, as only a few primers allow the production of sufficient information to get a robust estimate of variability index and have provided the resolution of complex taxonomic relationships.[40-42] RAPD includes the use of a single arbitrary primer in a PCR reaction led to the amplification of many distinct DNA products. Each amplicon is produced from a segment of the genome that involves two short regions in an inverted orientation, on opposite fragments that are complementary to the primer and sufficiently close together for the amplification to gene.[43-44]

Cluster analysis

In the present study, cluster analysis was done to estimate the level of polymorphism between the control plants and treatments. The dendrogram resulted from the banding patterns of RAPD (Table 4 and Fig 2). A dendrogram was constructed using a distance matrix by using the UPGMA method (Fig 2). The control and treated samples were clustered in three major groups: The first group I: (ranged from 0.69 to 0.98 similarity) contained on 'Cara' and 'Selan' cultivars (the control, AVP-treated and PVY^{NTN} diseased plants). The second group II: (genetic similarity 0.62 to 0.90) composed of 'Spunta' (the control and PVY^{NTN} infected plants), 'Nicola' and 'Diamond' cultivars (the control, PVY^{NTN} diseased and AVP treated plants). The third group III: (genetic similarity 0.67 to 0.77) consisted of the AVP-treated plants of 'Spunta' cultivar. These results were agree with those obtained by Swaileh *et al*. and Raj[45-46] who mentioned that the cluster analysis method is believed among the most efficient ways in numerical analysis concerning band scoring and analysis of RAPD-PCR fingerprinting.

Table 1
The nucleotide sequences of five RAPD-PCR primers used in the study

Primer name	Sequence
Primer-1	GTTTCGCTCC
Primer-2	AACGCGCAAC
Primer-3	CCCGTCAGCA
Primer-4	GGACGGCGTT
Primer-5	AAGCCCGAGG

Table 2

Variance analysis of number, weight and volume of AVP-treated, PVY^{NTN} infected and the healthy control potato cultivars

Cultivar	Treatment	Number	Weight (g)	Volume (Cm^3)
Selan	PVY-Infected*	4.00DE	150.0CD	120.0D
	AVP+PVY**	10.17 A	283.3BCD	262.5CD
	Control***	7.00ABCD	500.0ABC	450.0 ABCD
Spunta	PVY-Infected	2.00E	50.0D	145.0D
	AVP+PVY	7.67ABCD	783.3A	768.3A
	Control	7.00 ABCD	658.3AB	580.0ABC
Cara	PVY-Infected	6.00BCD	700.0A	640.0AB
	AVP+PVY	8.16ABC	408.3ABCD	371.2BCD
	Control	9.16AB	685.0 AB	650.0AB
Diamond	PVY-Infected	5.00CDE	500.0ABC	440.0ABCD
	AVP+PVY	8.00ABC	470.0ABC	410.0ABCD
	Control	9.83AB	683.3AB	615.0ABC
Nicola	PVY-Infected	7.00ABCD	750.0 A	660.0AB
	AVP+PVY	7.66ABCD	618.3AB	655.0 AB
	Control	8.83ABC	701.7A	670.0AB

Means within the same column and treatment followed by the same letter are not significantly different according to Duncan ($P \leq 0.05$).
PVY-Infected* = Potato plants inoculated with PVY^{NTN} strain only.
AVP+PVY = Antiviral protein extract pre-treated potato plants and subsequently inoculated with the sap from PVY^{NTN} infected plants.**
Control* = dsH_2O treated healthy potato plants**

Table 3

RAPD amplified bands, polymorphic bands and unique markers for AVP-treated potato cultivars (PVY^{NTN} resistance) using five primers

Primer name	Polymorphism		No. of markers	With pb	Selan	Spunta	Cara	Diamond	Nicola
	Total	*P %							
Primer-1	19	9	5	1000	+				
				800					+
				700	+				+
				273		+			
				200		+			
	47.37%			**26.32%**	**2**	**2**	**0**	**0**	**2**
Primer-2	8	4	3	350	+				
				290	+				
				100					+
	50%			**37.50%**	**2**	**0**	**0**	**0**	**1**
Primer-3	22	9	5	1200				+	
				800	+			+	+
				550		+			
				510				+	
				130		+			
	40.91%			**22.73%**	**1**	**2**	**0**	**3**	**1**
Primer-4	5	3	2	600		+			
				505		+			
	60%			**40%**	**0**	**2**	**0**	**0**	**0**
Primer-5	11	8	3	750	+		+		
				320	+				
				110				+	+
	72.73%			**27.27%**	**2**	**0**	**1**	**1**	**1**
**Total=	**65**	**33**		**18**	**7**	**6**	**1**	**4**	**5**
Polymorphic=	**50.77%**			**27.69%**					

AVP= Antiviral protein extract.
**** P = Number of polymorphic bands with polymorphic percentages.***
***** Total = Total number of amplified fragments. + = presence of marker band.***

Table 4

Similarity index between AVP-treated, PVY^{NTN} infected and the control potato cultivars by RAPD analysis

Cultivar		Selan			Spunta			Cara			Diamond			Nicola		
		C*	PVY**	AVP***	C	PVY	AVP	C	PVY	AVP	C	PVY	AVP	C	PVY	AVP
Selan	C	1.00														
	PVY	0.96	1.00													
	AVP	0.81	0.81	1.00												
Spunta	C	0.79	0.83	0.74	1.00											
	PVY	0.69	0.69	0.64	0.78	1.00										
	AVP	0.75	0.75	0.67	0.77	0.77	1.00									
Cara	C	0.87	0.91	0.77	0.82	0.71	0.77	1.00								
	PVY	0.81	0.85	0.75	0.84	0.76	0.79	0.93	1.00							
	AVP	0.85	0.89	0.79	0.80	0.69	0.76	0.98	0.91	1.00						
Diamond	C	0.73	0.77	0.69	0.84	0.80	0.71	0.84	0.86	0.82	1.00					
	PVY	0.73	0.76	0.62	0.82	0.74	0.73	0.79	0.80	0.77	0.84	1.00				
	AVP	0.66	0.69	0.65	0.78	0.71	0.73	0.75	0.77	0.73	0.80	0.83	1.00			
Nicola	C	0.70	0.73	0.65	0.84	0.88	0.74	0.80	0.86	0.78	0.90	0.84	0.80	1.00		
	PVY	0.70	0.70	0.63	0.80	0.84	0.78	0.76	0.82	0.74	0.86	0.88	0.84	0.90	1.00	
	AVP	0.67	0.67	0.66	0.75	0.75	0.74	0.72	0.77	0.71	0.77	0.79	0.83	0.80	0.89	1.00

C* = the healthy control, PVY** = potato plants inoculated with PVY^{NTN} strain only, AVP = antiviral protein extract pre-treated potato plants and subsequently inoculated with the sap from PVY^{NTN} infected plants.***

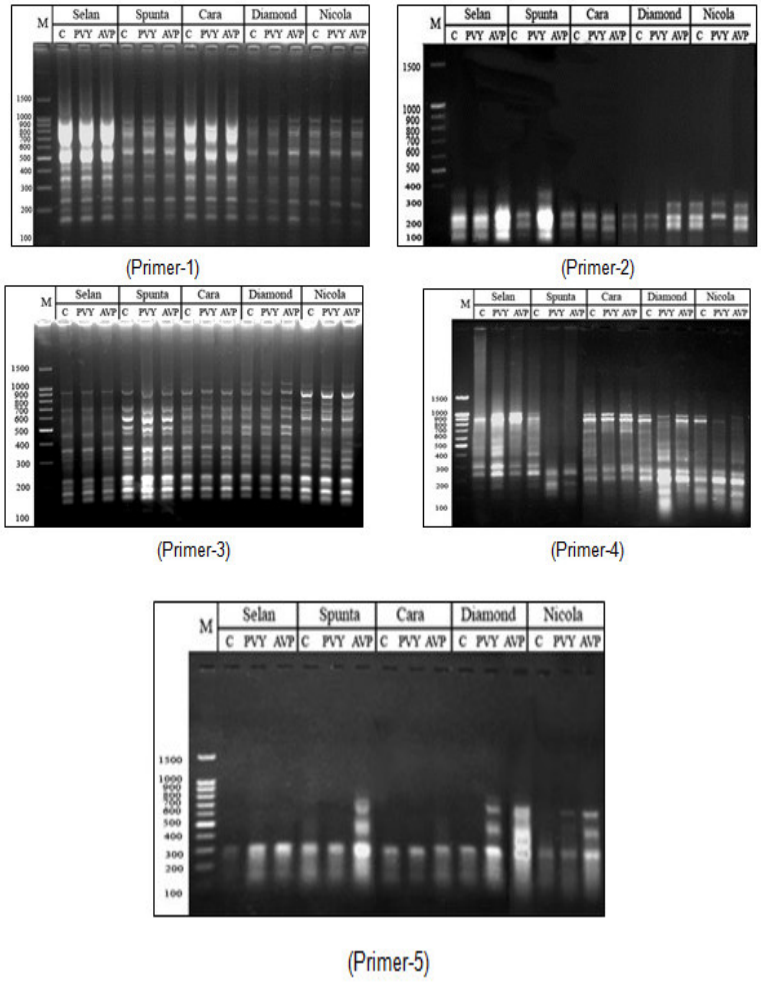
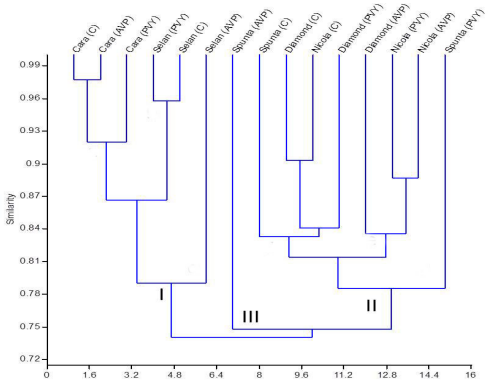


Figure 1

RAPD-PCR analysis of AVP-treated potato plants using five arbitrary primers, compared to PVY^{NTN} infected and the healthy ones of five potato cultivars. Lane M = 100 bp DNA ladder. Lane C = the healthy control, PVY= potato plants diseased with PVY^{NTN} strain only, AVP= antiviral extract treated potato plants and subsequently inoculated with the sap from PVY^{NTN} infected plants.

Figure 2
UPGMA dendrogram showing clustering of the Treated and control five potato cultivars



C = the healthy control, PVY= potato plants diseased with PVY^{NTN} strain only, AVP= antiviral extract treated potato plants and subsequently inoculated with the sap from PVY^{NTN} infected plants.

CONCLUSION

Despite the importance of the virus's resistance in the potato crop, the progress made in resistance breeding to PVY^{NTN} in the potato is rarely and it should be taken into consideration in the future breeding programs. The spotlight on plant extracts containing RIPs such as *P. americana*, *P. acinosa* and *M. jalapa* by spraying of leave extracts in various crops to prevent or control viral infection.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Kim J, Cha DJ, Kwon M, Maharjan R. *Potato virus Y* (PVY) detection in a single aphid by one-step RT-PCR with boiling technique. Entomological Research, 2016; DOI: 10.1111/1748-5967.12170.
2. Janzac B, Willemsen A, Cuevas JM, Glais L, Tribodet M, Verrier JL, Elena SF, Jacquot E. Brazilian *Potato virus Y* isolates identified as members of a new clade facilitate the reconstruction of evolutionary traits within this species. Plant Pathology, 2015; 64: 799-807.
3. Kehoe MA, Jones RAC. Improving *Potato virus Y* strain nomenclature: lessons from comparing isolates obtained over a 73-year period. Plant Pathology, 2016; 65: 322-33.
4. Wang PG, Tumer NE. Virus resistance mediated by ribosome inactivating proteins. Adv. Virus Res., 2000; 55: 325-55.
5. Van Damme EJM, Hao Q, Chen Y, Barre A, Vandenbussche F, Desmyter S, Rouge P, Peumans WJ. Ribosome-inactivating proteins: a family of plant proteins that do more than inactivate ribosomes. Crit. Rev. Plant Sci., 2001; 20 (5): 395-465.
6. Nielsen K, Boston RS. Ribosome-inactivating proteins: a plant perspective. Ann. Rev. Plant Phys. Plant. Mol. Biol., 2001; 52: 785-816.
7. Lord JM, Hartley MR, Roberts LM. Ribosome inactivating proteins of plants. Semin. Cell Biol., 1991; 2: 15-22.
8. Stirpe F, Barbieri L, Battelli MG, Soris M, Lappi DA. Ribosome-inactivating proteins from plants. Biotechnology, 1992; 10: 405-12.
9. Barbieri L, Battelli MG, Stirp F. Ribosome-inactivating proteins from plants. Biochim. Biophys. Acta, 1993; 1154: 237-82.
10. Mahfouze HA, El-Dougdoug Kh A, Othman BA, Gomaa MAM. Molecular markers in potato cultivars treated with ribosome-inactivating proteins. Pest Technology, 2012; 6 (1):70-4.
11. Osborn RW, Hartley MR. Dual effects of the ricin A chain on protein synthesis in rabbit reticulocyte lysate. Eur. J. Biochem., 1990; 193: 401-7.
12. Irvin JD, Kelly T, Robertus JD. Purification and properties of a second antiviral protein from *Phytolacca americana* which inactivates eukaryotic ribosomes. Arch. Biochem. Biophys., 1980; 200: 418-25.
13. Barbieri L, Aron GM, Irvin JD, Stirpe F. Purification and partia1 characterization of another form of the antiviral protein from seeds of *Phytolacca americana* L (pokeweed). Biochem. J., 1982; 203: 55-9.
14. Chen ZC, Antoniw JF, White RF, Lin Q. Effect of pokeweed antiviral protein (PAI') on the infection of plant viruses. Plant Pathol., 1992; 40: 612-20.
15. Vivanco JM, Querci M, Salazar LF. Antiviral and antiviroid activity of MAP containing extracts from *Mirabilis jalapa* roots. Plant Dis., 1999; 83: 1116-21.
16. Vivanco JM, Flores HE. Biosynthesis of ribosome-inactivation proteins from callus and cell suspension cultures of *Mirabilis expansa* (Ruiz & Pavon). Plant cell Rep., 2000; 19: 1033-9.
17. Corrado G, Delli BP, Ciliento R, Gaudio L, Di Maro A, Aceto S, Lorito M, Rao R. Inducible expression of a *Phytolacca heterotepala* protein leads to enhanced resistance against major fungal pathogen in tobacco. Phytopathology, 2005; 95: 206-15.

18. Picard D, Kao CC, Hudak KA. Pokeweed antiviral protein inhibits *Brome mosaic virus* replication in plant cells. The American Society for Biochemistry and Molecular Biology, 2005; 280(20): 20069-75.
19. Zarling JM, Maran PA, Haffar D, Sias J, Richman DD, Spina CA, Myers DA, Kuebelbeck V, Ledbetter JA, Uckun FM. Inhibition of HIV replication by pokeweed antiviral protein targeted CD4+ cells by monoclonal antibodies. Nature, 1990; 347: 92-5.
20. Frankel A, Schloossman D, Welsh P, Hertler A, Withers D, Johnston S. Selection and characterization of ricin toxin A-chain mutations in *Saccharomyces cerevisae*. Molecular Cell Biology, 1989; 9: 415-20.
21. Taylor S, Massíah A, Lomonossoff G, Roberts LM, Lord JM, Hartley M. Correlation between the activities of tobacco ribosome-inactivating proteins in depurination of tobacco ribosomes and inhibition of *Tobacco mosaic virus* infection. Plant J., 1994; 5: 827-35.
22. Lodge JK, Kaniewski WK, Tumer NE. Broad-spectrum virus resistance in transgenic plants expressing pokeweed antiviral protein. Proc. Natl. Acad. Sci. USA, 1993; 90: 7089-93.
23. Mahfouze SA. Diagnosis of some plant viruses using modern techniques. M.Sc Faculty of Agriculture, Ain Shams University, 2003; 179.
24. Mahdy AMM., Hafez MA, EL-Dougdoug Kh A, Fawzy RN, Shahwan ESM. Effect of two biotic inducers on salicylic acid induction in tomato infected with *Cucumber mosaic cucumovirus*. Egyptian J. Virol., 2010; 355-72.
25. Clark MF, Adams AN. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 1977; 34: 475-83.
26. Gopal J, Kumar R, Kang GS. The effectiveness of using a minituber crop for selection of agronomic characters in potato breeding programmes. Potato Res., 2002; 45: 145-51.
27. Mohsenin NN. Physical properties of plant and animal materials. 2nd Ed. Gordon and Breach Science publ., New York, 1986; 20-89.
28. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus, 1990; 12: 13-5.
29. Kang TJ, Yang MS. Rapid and reliable extraction of genomic DNA from various wild-type and transgenic plants. BMC Biotechnology, 2004; 4(20): 1-12.
30. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 1979; 76(10): 5269-73.
31. Sneath PHA, Sokal RR. Numerical taxonomy. In: Freeman WH and Co, editors. The principles and practices of classification, San Francisco. 1973; 588 p. ISBN 0716706970.
32. Rohlf FJ. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.02 Exeter Software, Setauket, N.Y. 2000.
33. Snedecor GW, Cochran WG. Statistical Methods (6th Edn), The Iowa State University Press, Ames., Iowa, USA, 1972; 593 pp.
34. Yoonkang H, Han CT, Maeng J. Expression characteristics of pokeweed antiviral (PAPs): two distinct types of proteins. Journal of Plant Biology, 1997; 40 (1): 53-60.
35. Poyet JL, Radom J, Hoeveler A. Isolation and characterization of a cDNA clone encoding the pokeweed antiviral protein II from *Phytolacca americana* and its expression in *E. coli*. FEBS let., 1994; 347: 268-72.
36. Hemmat G, Kashani A, Vazan S, Hasani F. Evaluation of some quantitative properties of potato mini-tubers affected by genotype, different planting bed composition and pot size. Int. J. Biosci., 2014; 4(2): 55-62.
37. Rezaii A, Soltani A. Potato crop (Jahad-e-Daneshgahi) University of Mashhad, 1996; 45-92.
38. Shiran B, Amirbakhtiar N, Kiani S, Mohammadi SH, Tabatabaei BES, Moradi H. Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Sci. Hortic., 2007; 111: 280-90.
39. Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ. Trends in genetic and genome analyses in wheat: a review. Aust. J. Agric. Res., 2001; 52(12): 1043-77.
40. Williams JGK, Kubelik AR, Leavk KJ, Rafaski JA, Tingey SV. DNA polymorphism amplification by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 1990; 18: 6531-5.
41. Welsh J, Mccelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 1990; 18: 7213-8.
42. Casiva PV, Saidman BO, Vilardin JC, Cialdella AM. First comparative phonetic studies of Argentinean species of *Acacia* (Fabaceae) using morphometric isozymal and RAPD approaches. Am. J. Bot., 2002; 89: 843-53.
43. Karp A, Kresovich S, Bhat KV, Ayad WG, Hodgkin T. Molecular tools in plant genetic resources conservation: a guide to the technologies. In: IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy, 1997.
44. Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, Van Dewiel C, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschneider R, Bettini P, Buiatti M, Maestri E, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A, Karp A. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed., 1997; 3: 381-90.
45. Swaileh KM, Hussein R, Ezzughayyar A. Evaluating wastewater-induced plant genotoxicity using Randomly amplified polymorphic DNA. Environ. Toxicology, 2008; 23(1): 117-22.
46. Raj A, Kumar S, Haq I, Kumar M. Detection of tannery effluents induced DNA damage in Mung Bean by use of Random amplified polymorphic DNA markers. ISRN Biotechnology, 2014; 1-8.
47. Mahfouze SA, Mahfouze HA. Genotoxicity evaluation of salicylic acid in potato cultivars inoculated with *Potato virus* Y^{NTN} using ISSR markers. Int. J. Pharm Bio Sci., 2016; 7(3): (B) 230-40.