Pest Technology ©2012 Global Science Books

Inhibition of *Potato Virus Y* by Ribosome Inactivating Proteins (RIPs)

Heba Mahfouze[1*] • Khalid El-Dougdoug[2] • Badawi Othman[2] • Mostafa Gomaa[1]

[1] Genetic Engineering and Biotechnology Division, Genetic and Cytology Department, National Research Center, Dokki, 12622, Egypt
[2] Agriculture Microbiology Department (Virology Laboratory), Agriculture Faculty, Ain Shams University, Cairo, Egypt

Corresponding author: * **hebaamn@yahoo.com**

ABSTRACT

Antiviral proteins (AVPs), also referred to as ribosome inactivating proteins (RIPs), are an extended, fairly heterogeneous group of plant proteins which confer resistance against different viruses when applied exogenously or expressed in transgenic lines. These have been identified in a number of plant species such as pokeweed (*Phytolacca americana*, *P. acinosa*), and "the marvel of Peru" (*Mirabilis jalapa*). The primary objective of this study was the development of an easily adaptable technology for controlling *Potato virus Y* (PVY^{NTN}) by the pre-inoculation application of *Phytolacca* sp. and *M. jalapa* extracts on five potato (*Solanum tuberosum* L.) cultivars ('Selan', 'Spunta', 'Cara', 'Diamond', and 'Nicola'). In addition, we aimed to investigate the functional expression of PVY^{NTN} resistance in these potato cultivars by comparing protein composition using SDS-PAGE and polyphenol oxidase and peroxidase isozymes. Leaf extracts from *Phytolacca* sp. and *M. jalapa* leaves were blended and diluted (1: 5, w/v) in distilled water and sprayed on the five potato cultivars before virus inoculation, inhibiting infection by almost 100%, as corroborated by DAS-ELISA. SDS-PAGE was used to detect antiviral proteins in *P. americana*, *P. acinosa* and *M. jalapa*, in addition to studying genetic variability among healthy, resistant and susceptible potato cultivars through the quantitative and qualitative determination of total proteins. Monomorphic bands with molecular weights 11 and 28.5 KDa appeared in AVP-treated leaves in addition to another common band at 28.5 KDa induced in AVP-treated tubers and disappeared in non-AVP-pretreated potato plants and the control. On the other hand, polyphenol oxidase isozyme activity in the non-AVP-pretreated potato cultivars (PVY-infected) was higher than in AVP-pretreated cultivars. In addition, one unique peroxidase marker appeared at an *Rf* value 0.280 in all the potato cultivars except for 'Spunta'. Also, in non-AVP-pretreated potato plants a monomorphic band appeared at an *Rf* value 0.945 in all five potato cultivars.

Keywords: *Mirabilis jalapa*, *Phytolacca americana*, *P. acinosa*, peroxidase and polyphenol oxidase isozymes, SDS-PAGE

INTRODUCTION

Various plants contain enzymes called ribosome inactivating proteins (RIPs), officially called rRNA *N*-glycosidases, which inhibit protein synthesis by cleaving a single N-glycosidic bond of 28S rRNA (Barbieri *et al.* 1993; Ferreras *et al.* 2011). RIP have been isolated from many different plant species such as pokeweed (*Phytolacca americana*, *P. acinosa*) and "the marvel of Peru" (*Mirabilis jalapa*). RIPs are divided into two categories depending upon the conformation of their subunits. Type-I RIPs have a single polypeptide chain, whereas type-II consists of two polypeptide, an active A chain and a B chain which is a galactose-binding lectin (Vargas *et al.* 2009).

RIPs have shown broad spectrum antiviral activity against RNA, DNA, and plant and animal viruses (Picard 2005) including tobacco and potato mosaic viruses, poliovirus, herpes simplex virus, cytomegalovirus, influenza virus, and human immunodeficiency virus (HIV)-1 (Lee-Huang 1991). In contrast, RIPs were found to have no effect on protein synthesis in uninfected plant cells, which suggests that intact, uninfected cells, however it has been suggested that RIPs entry is mediated by changes in the cellular membrane induced by the adsorption of viral particles on the cell surface (Poyet *et al.* 1994). In this process, virus entry is not required. A change in the cell membrane integrity could lead to the entry of RIPs and thus would provide a mechanism of cellular suicide. Because of this dual inhibitory activity, RIPS have become the subject of a wide range of investigations concerning their potential application as novel therapeutic agents and as putative protective proteins used by plants as a defense against viruses (Poyet *et al.* 1994).

The primary objective of this study was development of an easily adaptable technology for controlling *Potato virus Y* (PVY^{NTN}) by the pre-inoculation application of Pokeweed and Peruvian extracts on potato cultivars. In addition to, investigate functional expression of potato cultivars resistance of PVY^{NTN} by protein composition using SDS-PAGE and polyphenol oxidase and peroxidase isozymes.

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. Fifty-seeds were sowed in 14 cm- pots and kept in a greenhouse for approximately three weeks until germinated. Temperature in the greenhouse was kept at 21-25°C.

Source of PVY^{NTN} strain

The necrotic strain of *potato virus Y* (PVY^{NTN}) was obtained from the Virology Laboratory, Agriculture Microbiology Department, Faculty of Agriculture, Ain Shams University which were previously isolated and identified from systemically infected potato plants (Mahfouze 2003). The isolate was maintained in thorn apple (*Datura metel* L.) plants. Systemically infected leaves were used as sources of inoculum in all experiments.

Received: 17 July, 2011. Accepted: 23 September, 2011.

Original Research Paper

Preventive treatment with *P. americana*, *P. acinosa* and *M. jalapa* extracts

The five potato cultivars ('Selan', 'Spunta', 'Cara', 'Diamond', and 'Nicola') were tested for potential infection with free potato viruses by DAS-ELISA technique. Random complete design block was used, 25 tubers from each cultivar were planted in the open field for three winter seasons (2009-2011).

P. americana, *P. acinosa* and *M. jalapa* leaves were blended and diluted (1:5, w/v) in distilled water. The procedure used in this experiment consisted of pretreatment of the potato leaves with *P. americana*, *P. acinosa* and *M. jalapa* extracts (100 µg/ml) by hand rubbing, followed by viral inoculation. PVY^{NTN} were mechanically inoculated to plants dusted with carborundum, 600-mesh, as an abrasive. The inoculum consisted of virus in leaf sap diluted 1:2 (w/v) in 10 mM phosphate buffer (pH 7.2). Systemic symptoms were recorded 21-30 days after virus inoculation. Control treatments consisted of plants inoculated with the sap from virus-infected plants without any pretreatment. The percentage of viral inhibition in the treatments was analyzed utilizing a random distribution model, using each plant as an experimental unit. Potato leaves and tubers of healthy plants were collected after 30 days of inoculation respectively and stored at 4°C for biochemical marker analysis.

Enzyme linked immunosorbent assay (DAS-ELISA)

All the samples were tested for the presence of PVY^{NTN} by the double antibody sandwich enzyme linked immunosorbent assay technique (DAS-ELISA) as described by Clark and Adams (1977). PVY ELISA kit provided from Sanofi Company Sante Animal Paris, France.

Electrophoretic analysis of protein by SDS-PAGE

SDS-PAGE was used for detection of antiviral proteins in *P. americana*, *P. acinosa* and *M. jalapa*, in addition to studying genetic variability among healthy, resistant and susceptible potato cultivars via determination quantitative and qualitative of the total proteins. This method was done according to Laemmli (1970) as modified by Studier (1973).

Electrophoresis of peroxidase (POD) and polyphenol oxidase (PPO) isozymes

Detection of POD and PPO isozymes among healthy, resistant and susceptible potato cultivars were recognized by using benzidine dihydrochloride and *O*-catechol according to Vallejos (1983) and de Ascensão and Dubery (2000), respectively.

Gel analysis

The gel analysis was applied by a programme (UVI Geltec version 12.4, 1999-2005, USA).

RESULTS AND DISCUSSION

Expression characteristics of PAP in *Phytolacca* sp. and *Mirabilis jalapa* plants

Pokeweed antiviral proteins (PAP-I) and PAP-S could be expressed in the spring leaves and seeds, respactively of *P. americana* and *P. acinosa* with molecular mass of 29 KDa (Hur *et al.* 1997) (**Fig. 1**), whenever leaves and seeds of plant *Mirabilis jalapa* L. were found to contain an antiviral protein (MAP) belong to type-I RIP (PAP-I), with a molecular weight 24.2 and 29 KDa, respectively as determined by SDS-PAGE (Vivanco *et al.* 1999) (**Fig. 2**).

Inhibitory activity of *Phytolacca* sp. and *M. jalapa* extracts against PVY

P. americana, *P. acinosa* and *M. jalapa* leaf extracts were applied to the leaves of five cultivars of potato. Results

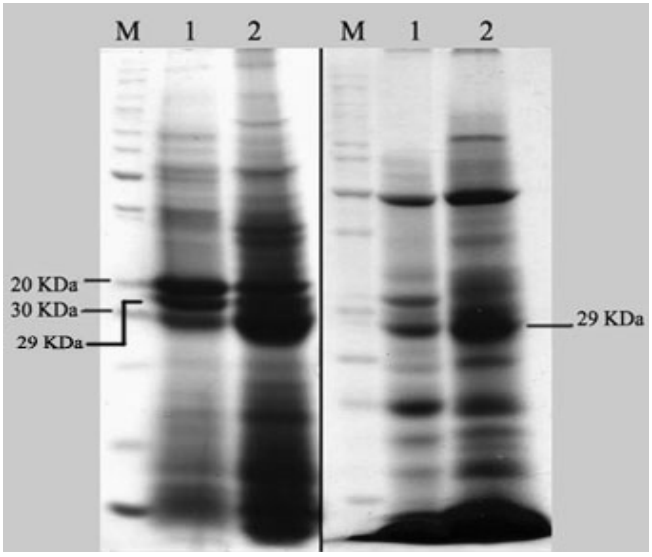

Fig. 1 Total soluble-protein extracted from leaves (left gel) and seeds (right gel) of *Phytolacca* sp. in spring and tissue-specific expression of PAP-I and PAP-S determined by SDS-PAGE. M= marker; Lane 1 = *P. Americana*; Lane 2 = *P. acinosa.*

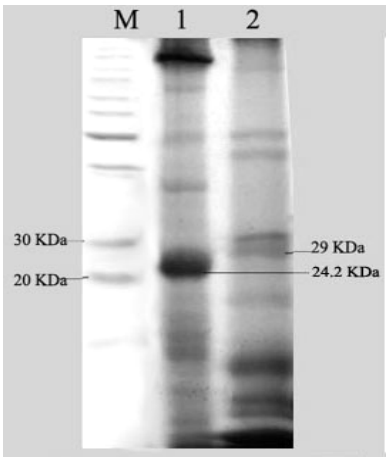

Fig. 2 Total soluble-protein extracted from leaves (lane 1) and seeds (lane 2) of *Mirabilis jalapa* L. in spring and tissue-specific expression of PAP-I and PAP-S determined by SDS-PAGE. M = marker.

show that the leaf extracts diluted 1:5 (v/v) in distilled water were strongly inhibitory to PVY^{NTN} infection, because almost 100% inhibition was confirmed by DAS-ELISA (**Table 1**). These results were in an agreement with Hur *et al.* (1997) and Vivanco *et al.* (1999) who found that extracts of *Phytolacca* sp. and *M. jalapa*, containing a ribosome inactivating protein (RIP), were tested against infection by *Potato virus X*, PVY, *Potato leaf roll virus*, and *Potato spindle tuber viroid*. The root extracts of *M. jalapa* sprayed on test plants 24 h before virus or viroid inoculation inhibited infection by almost 100%, as corroborated by infectivity assays and the nucleic acid spot hybridization test. Antiviral activity of RIP extracts was observed against mechanically transmitted viruses but not against aphid transmitted viruses. The effect of plant extracts against virus infection suggests a mechanism by which RIP penetrates the upper layers of the epidermis and situates itself in the intercellular spaces. When the plants are wounded during virus infection, RIP is able to penetrate epidermal and other leaf cells, where it deglycosilates the 28S rRNA. This prevents viral replication at an early stage by deactivating the cell protein synthesis machinery (Barbieri *et al.* 1993). When viral inoculation is performed, the virus penetrates the cell along with RIP. Once inside the cell, RIP and the virus compete for the active sites on the ribosomes. RIP depurinates the 28S rRNA and thus inactivates the protein chemistry of the cell. If this

Table 1 Specific protein markers induced in AVP-treated leaves and tubers of five potato cultivars.

Band No.	MW	Leaves					Tubers				
		Selan AVP	Spunta AVP	Cara AVP	Diamond AVP	Nicola AVP	Selan AVP	Spunta AVP	Cara AVP	Diamond AVP	Nicola AVP
1	175		+								
2	147			+						+	
3	122									+	+
4	105	+									
5	89.5							+			+
6	84.5							+	+	+	+
7	75.5					+					
8	50.5		+								
9	50							+			
10	48			+							
11	47.5							+	+	+	+
12	42					+					
13	35.5	+									
14	35									+	
15	33.5	+			+			+			
16	24.5	+									
17	28.5						+	+	+	+	+
18	21						+			+	
19	20.5			+							
20	19							+		+	+
21	16.5			+							
22	14.5					+					
23	13		+								
24	11.5		+								
25	11	+	+	+	+	+					
26	10.3	+									
Total of bands= 26		6	5	5	2	4	2	7	3	8	6

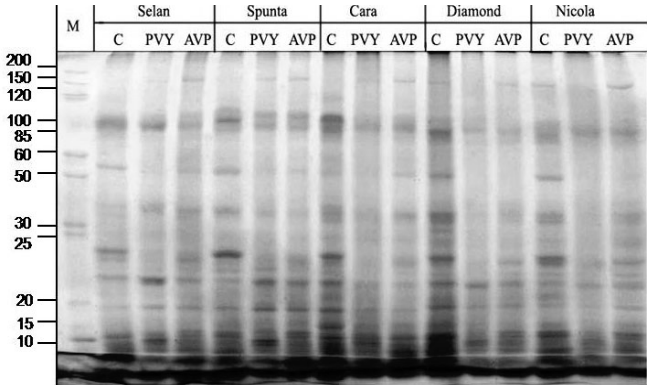

Fig. 3 SDS-PAGE analysis of total soluble protein fractions of AVP-pretreated leaves potato cultivars and non AVP-pretreated ones (PVY^{NTN}-infected) compared with the control (Lane C).

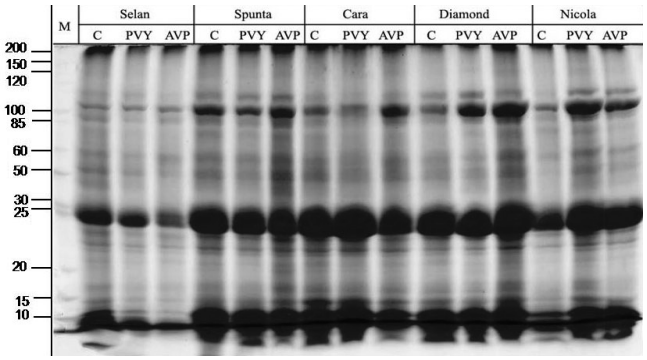

Fig. 4 SDS-PAGE analysis of total soluble protein fractions of AVP-pretreated tubers potato cultivars and non AVP-pretreated ones (PVY^{NTN}-infected) compared with the control (Lane C). Lane M = marker.

is the case, our results suggest that MAP reaches the active site of the ribosomes first, preventing viral infection at an early stage, probably before viral de-encapsidation (Vivanco *et al.* 1999).

Functional expression of AVP-treated potato plants

1. SDS-PAGE of AVP-treated leaves potato cultivars

The SDS-PAGE protein profile of total soluble-proteins extracted from leaves of AVP-pretreated and non AVP-pretreated five potato cultivars showed differences in band patterns when compared with their respective healthy ones (**Table 1; Fig. 3**). AVP-pretreated leaves potato the results of SDS-PAGE revealed a total number of 51 bands with different molecular weights (MWs) ranged from about 270 to 7.5 KDa. The 36 bands were varied in some distinctive either in AVP-treated or non AVP-treated potato and the remaining were common bands. Six protein markers induced in AVP-pretreated 'Selan' with 105, 35.5, 33.5, 24.5, 11 and 10.3 KDa. Four protein bands appeared in 'Nicola' at 75.5, 42, 14.5 and 11 KDa; however 'Diamond' revealed two markers of 33.5 and 11 KDa. On the other hand, 'Spunta' and 'Cara' showed five protein markers with MWs (17.5, 50.5, 13, 11.5 and 11 KDa) and (14.7, 48, 20.5, 16.5 and 11 KDa), respectively. A monomorphic band of 11 KDa appeared in all AVP-treated leaves potato cultivars (**Fig. 3; Table 1**).

2. SDS-PAGE of PVY-resistant tubers potato

The results of SDS-PAGE revealed a total number of 43 bands with different MWs ranged from about 225 to 4.5 KDa. The 23 bands were varied in some distinctive either in AVP-pretreated or non AVP-pretreated five potato cultivars compared with the control and the remaining were monomorphic bands. On the other hand, 'Diamond' showed the highest number from protein markers (eight) with MWs of 147, 122, 84.5, 47.5, 35, 28.5, 21 and 19 KDa followed by 'Spunta', which displayed seven protein bands at 89.5, 84.5, 50, 47.5, 33.5, 28.5 and 19 KDa; however, 'Selan' had the fewest markers (two): 28.5 and 21 KDa. In addition, 'Nicola' and 'Cara' had six and three markers (122, 89.5,

Table 2 PPO and POD isozyme markers of AVP-pretreated potato leaves.

Band No.	*Rf*	PPO					POD				
		Selan AVP	Spunta AVP	Cara AVP	Diamond AVP	Nicola AVP	Selan AVP	Spunta AVP	Cara AVP	Diamond AVP	Nicola AVP
1	0.280						+		+	+	+
2	0.462			+							
3	0.850									+	
4	0.954	+	+	+		+					
Total bands = 4		1	1	2	0	1	1	0	1	2	1

Table 3 PPO and POD isozyme markers of non AVP-pretreated potato leaves.

Band No.	*Rf*	PPO					POD				
		Selan PVY	Spunta PVY	Cara PVY	Diamond PVY	Nicola PVY	Selan PVY	Spunta PVY	Cara PVY	Diamond PVY	Nicola PVY
1	0.300		+								
2	0.352			+							
3	0.380		+		+	+					
4	0.462		+	+							
5	0.847		+			+					
6	0.945						+	+	+	+	+
7	0.965	+	+		+	+					
Total bands = 7		1	5	2	2	3	1	1	1	1	1

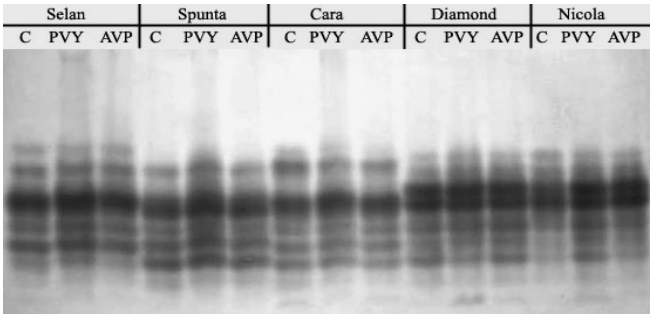

Fig. 5 PPO-isozyme polymorphism profile of five AVP-pretreated potato cultivars and non AVP-pretreated ones (PVY^{NTN}-infected) compared with the control (Lane C).

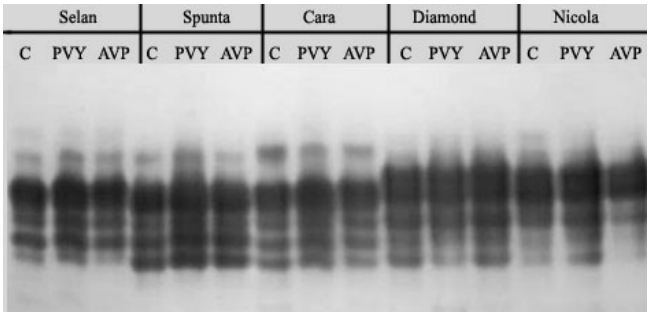

Fig. 6 POD-isozyme polymorphism profile of five AVP-pretreated potato cultivars and non AVP-pretreated ones (PVY^{NTN}-infected) compared with the control (Lane C). Lane M= marker.

84.5, 47.5, 28.5 and 19 KDa) and (84.5, 47.5 and 28.5 KDa), respectively. A monomorphic band of 28.5 KDa appeared in all AVP-pretreated potato cultivars (**Fig. 4; Table 1**). These results were similar to those of Chauduhry *et al.* (1994) and Song *et al*. (2000), who mentioned that many RIPs are sequestered in the cell wall or cellular storage compartments from host ribosomes. It is not known how the RIPs move from these compartments into the cytosol where they can act on either the viral nucleic acid or the host-cell translational machinery. The most probable theory is that RIPs are defense-related proteins regulated by signalling pathways in plants. It has been demonstrated that RIPs from *Phytolacca insularis* and barley are induced *in vivo* by jasmonic acid, a key signalling molecule in the expression of a number of plant defense-related proteins (reference required). Two type-1 RIPs present in *Beta vulgaris* accumulate in response to treatment of plants with salicylic acid and hydrogen peroxide (two mediators of induced acquired resistance) (Girbés *et al.* 1996).

Isozyme profiles of AVP-pretreated potato leaves

1. Polyphenol oxidase isozymes (PPO)

PPO analysis displayed a total of 13 bands at different *Rf* values varying from 0.300 to 0.965, whereas seven bands were variable and the six other bands with an *Rf* value (0.434, 0.530, 0.562, 0.641, 0.726 and 0.801) were found to be monomorphic among the AVP-pretreated potato and non AVP-pretreated potato plants and inoculated with PVY^{NTN} of the five potato cultivars, compared with healthy control as presented in **Fig. 5**. The relative front (*Rf*) value of each band was calculated depending on this. In the resistance AVP-pretreated 'Cara' plants two unique markers with *Rf* values of 0.462 and 0.954 appeared while 'Selan', 'Spunta' and 'Nicola' had only one isozyme marker with *Rf* = 0.954 (**Table 2**). On the other hand, non AVP-pretreated potato plants and PVY^{NTN}-infected plants showed differences in isozyme banding. Five clear extra bands were found in 'Spunta' with *Rf* values of 0.300, 0.380, 0.462, 0.847 and 0.965. Followed by, PVY^{NTN}-susceptible plants of 'Nicola' cultivar revealed three bands with *Rf* 0.380, 0.847 and 0.965. In addition, PVY^{NTN}-susceptible plants of 'Diamond' showed two isozyme markers at *Rf* values 0.380 and 0.965. The diseased plants of 'Cara' and 'Selan' had equal number of markers with one isozyme band with an *Rf* of 0.965 (**Table 3**). Consequently, PPO-isozyme activity in the non AVP-pretreated potato cultivars and PVY^{NTN} infected ones is higher than in AVP-pretreated cultivars.

2. Peroxidase isozymes (POD)

POD analysis displayed a total of 10 bands at different *Rf* values varying from 0.280 to 0.945, whereas 5 bands were variable and the 5 other bands, with *Rf* values of 0.480, 0.575, 0.604, 0.669 and 0.767, were monomorphic among the AVP-pretreated potato and non AVP-pretreated potato plants when compared with the control (**Fig. 6**). One unique marker appeared in all the AVP-pretreated five potato cultivars except for 'Spunta' at *Rf* value = 0.280 (**Table 2**). In addition, another band appeared in resistance plants of 'Diamond' with *Rf* = 0.850. On the other hand, a monomorphic band appeared in non AVP-pretreated potato plants at an *Rf* value of 0.945 in all five potato cultivars (**Table 3**). These results were in an agreement with those of Neog *et al*. (2004), who mentioned that enzymes control biochemical

reactions, and their syntheses are under the control of a specific gene, and any change in the activity of an enzyme would reflect the pattern of gene expression and corresponding metabolic events in the cell. Hence, enzymes can be used as tools to study the induced responses of plants showing disease symptoms at the biochemical level. Micales *et al.* (1986) found that differences in isozyme binding patterns are due to variation in the amino acid content of the molecule, which in turn is dependent on the sequence of nucleotides in DNA. Different bands obtained indicate different electrophoretic mobilities of the isozymes, which are coded by different alleles or separate genetic loci. Therefore, such studies are useful in identifying and characterizing resistance in potato caused by infection PVY^{NTN}. In addition, phenol-oxidizing enzymes such as POD and PPO are associated with many diseases (Pegg 1985). Nadlong and Sequeira (1980) suggested that the increased POD-activity following virus infection was a reflection of physiological changes associated with, but not responsible for, induced resistance whereas up-regulated peroxidases might be responsible for reduction in growth and malformations in virus-infected plants. POD participates in a variety of plant defense mechanisms (Mareschbacher *et al*. 1986) in which H_2O_2 is often supplied by an oxidative burst, a common event in defense responses (Dixon and Lamb 1990). The cell wall of plants appears to be a major site for defense-related peroxidase polymerization reactions such as lignification (Hammerschmidt and Kuc 1982), suberization (Espelie *et al*. 1986) and cross-linking of structural cell wall proteins (Fry 1986). PODs comprise one important class of PR proteins (PR-9) implicated in these "defense responses" in which an important role is to catalyze the formation of phenolic radicals at the expense of H_2O_2 (Gaspar *et al.* 1985). PODs may also oxidize phenolic monomers to form lignin (Grisebach 1981), function in H_2O_2 production, and metabolize indole-3-acetic acid (Mato *et al*. 1988). Each plant species typically displays a unique pattern and number of soluble and wall-bound isozymes that may respond differentially to environmental stimuli. The factor known to influence POD isozyme expression is pathogen infection (Ye *et al.* 1990).

Plant extracts from *P. americana*, *P. acinosa* and *M. jalapa* could be used in simple crop-protection methods agricultural systems, such as the spraying of extracts on leaves of various crops to prevent or control viral infection.

ACKNOWLEDGEMENTS

The authors thank Dr. Jaime A. Teixeira da Silva for significant improvements in the grammar.

REFERENCES

Barbieri L, Battelli MG, Stirp F (1993) Ribosome-inactivating proteins from plants. *Biochimica et Biophysica Acta* **1154**, 237-282

Chaudhry B, Müller-Uri R, Cameron-Mills V, Gough S, Simpson D, Skriver K, Mundy J (1994) The barley 60 KDa jasmonate-induced preotin (JIP60) is a novel ribosome-inactivating protein. *Plant Journal* **6**, 815-824

Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**, 475-483

de Ascensão A, Dubery IA (2000) Panama disease: Cell wall reinforcement in banana roots in response to elicitors from *Fusarium oxysporum* f. sp. *cubense* race four. *Phytopathology* **90**, 1173-1180

Dixon RA, Lamb CJ (1990) Molecular communication in interactions between plants and microbial pathogens. *Annual Review of Plant Molecular Biology* **41**, 339-367

Espelie KE, Franceshi VR, Kolattukudy PE (1986) Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound healing potato tuber tissue. *Physiologia Plantarum* **81**, 487-492

Ferreras JM, Citores L, Iglesias R, Jiménez P, Girbés T (2011) Use of ribosome-inactivating proteins from *Sambucus* for the construction of immunotoxins and conjugates for cancer therapy. *Toxins* **3**, 420-441

Fry SC (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review of Plant Physiology* **37**, 165-183

Gaspar T, Penel C, Castillo FJ, Greppin H (1985) A two-step control of basic and acidic peroxidases and its significance for growth and development. *Physiologia Plantarum* **64**, 418-423

Girbés T, de Torre C, Iglesias R, Ferreras JM (1996) RIP for viruses. *Nature* **379**, 777-778

Grisebach H (1981) Lignins. In: Stumpf PK, Conn EE (Eds) *The Biochemistry of Plants* (Vol 7), Academic Press, New York, pp 457-478

Hur YK, Han CT, Maeng JS (1997) Expression characteristics of poke-weed antiviral proteins (PAPs): Two distinct types of proteins. *Journal of Plant Biology* **40 (1)**, 53-60

Hammerschmidt R, Kuc J (1982) Lignification as a mechanism for induced systemic resistance in cucumber. *Physiology and Plant Pathology* **20**, 61-71

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T_4. *Nature* **227**, 680-689

Lee-Huang S, Huang PL, Kung HL, Li BQ, Huang P, Huang HI, Chen HC (1991) An anti-human immunodeficiency virus protein from *Trichosanthes kirilowii* that is non toxic to intact cells. *Proceedings of the National Academy of Sciences USA* **88**, 6570-6574

Mareschbacher BM, Kogel KH, Noll U, Reisener HJ (1986) An elicitor of the hypersensitivity lignification response in wheat leaves isolated from the rust fungus *Puccinia graminis* f.sp. *tritici*. I. Partial purification and characterization. *Zeitschrift für Naturforschung* **41**, 830-838

Mato MC, Rúa ML, Ferro E (1988) Changes in levels of peroxidases and phenolics during root formation in *Vitis* cultured *in vitro*. *Physiologia Plantarum* **72**, 84-88

Micales JA, Bonde MR, Peterson GL (1986) The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxon* **27**, 407-449

Nadlong L, Sequeira L (1980) Increases in peroxidase activities are not directly involved in induced resistance to *Tobacco mosaic virus*. *Physiological Plant Pathology* **16**, 1-8

Neog B, Yadav RNS, Singh ID (2004) Peroxidase, polyphenol oxidase and acid phosphatase activaties in the stigma-style tissue of *Camellia sinensis* (L) O. Kuntze following compatible and incompatible pollination. *Journal of the Indian Institute of Science* **84**, 47-52

Pegg GF (1985) Life in a black hole: The micro-environment of the vascular pathogen. *Transactions of the British Mycological Society* **85**, 1-20

Picard D, Cheng Kao C, Hudak KA (2005) Pokeweed antiviral protein inhibits *Brome mosaic virus* replication in plant cells. *The Journal of Biological Chemistry* **280 (20)**, 20069-20075

Poyet JL, Radom J, Hoeveler A (1994) Isolation and characterization of a cDNA clone encoding the pokeweed antiviral protein II from *Phytolacca americana* and its expression in *E. coli*. *FEBS Letters* **347**, 268-272

Mahfouze SA (2003) Diagnosis of some plant viruses using modern techniques. MSc thesis, Faculty of Agriculture, Ain Shams University, 179 pp

Song SK, Choi Y, Moon YH, Kim SG, Choi YD, Lee JS (2000) Systemic induction of a *Phytolacca insularis* antiviral protein gene by mechanical wounding, jasmonic acid, and abscisic acid. *Plant Molecular Biology* **43**, 439-450

Studier FW (1973) Analysis of bacteriophage T, early RNAs and proteins of slab gel. *Journal of Molecular Biology* **79**, 237-248

Vallejos CE (1983) Enzyme activity staining. In: Tonskley SD, Orton TJ (Eds) *Isozymes in Plants. Genetics and Breeding Part A*, Elsevier Science Publishers BV, Amsterdam, pp 469-516

Vargas LRB, Martins JN, Bordin D, Salvador M, Schafer AE, de Barros NM, Barbieri L, Stirpe F, Carlini ClR (2009) Type 1 ribosome-inactivating proteins-Entomotoxic, oxidative and genotoxic action on *Anticarsia gemmatalis* (Hu¨bner) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* **55**, 51-58

Vivanco JM, Querci M, Salazar LF (1999) Antiviral and antiviroid activity of MAP-containing extracts from *Mirabilis jalapa* roots. *Plant Disease* **83**, 1116-1121

Yex S, Pan SQ, Kuc J (1990) Activity, isozyme pattern, and cellular localization of peroxidase as related to systemic resistance of tobacco to blue mold (*Peronospora tabacina*) and to *Tobacco mosaic virus*. *Phytopathology* **80**, 1295-1299