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GENOTOXICITY EVALUATION OF SALICYLIC ACID IN POTATO CULTIVARS INOCULATED WITH *POTATO VIRUS Y^{NTN}* USING ISSR MARKERS

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ABSTRACT

Salicylic acid (SA) acts a fundamental function in plant viruses' resistance and hypersensitive cell death. In addition, it is involved in hardening reactions to abiotic stress. The aim of the work was to address the following questions. 1- Is SA treatment causes genotoxicity and DNA damage in potato cultivars and wild species inoculated with *Potato virus Y^{NTN}* (PVY^{NTN})? 2- Is treatment of both SA and PVY^{NTN} affect antioxidant enzymes in potato? In this study, it is shown that total activities of polyphenol oxidase (PPO) and peroxidase (POX) increased upon potato cultivars treatment with a mixture of virus inoculum and SA (SA+V) and/or PVY^{NTN} infection (V). The inter-simple sequence repeats (ISSR) analysis was used to evaluate the genotoxic effect on four potato cultivars and one wild type treated mixture of SA and PVY^{NTN} (SA+V) compared with the control. ISSR profiles showed that SA+V treated cultivars were having low genotoxic effects on potato. This was discernible with appearance/disappearance of bands in the treatments compared with the control. A total of 84 ISSR amplified fragments were obtained using five primers, out of which 28 were polymorphic (33.33%) and 56 were monomorphic (66.67%). SA+V treatment induced 19 new bands (22.62% polymorphism). Nei's genetic similarity index calculated between the treated cultivars and control was clustered in two groups. The first group I: (genetic similarity 0.83 to 0.93) and the second groups II: (genetic similarity 0.75 to 0.92). This indicates that SA+V treatment is less genotoxic. Therefore, it could be concluded that 2 mM SA had not caused significance DNA damage to potato cultivars; it could be used in viral diseases resistance without having harmful effect on human and the environment.

KEYWORDS: ***Solanum tuberosum*** **L., resistant, polyphenol oxidase, peroxidase, molecular marker.**

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INTRODUCTION

Plants have defense mechanisms that provide them to react plastically with altering environmental conditions, such as chemical or physical defenses that are generated quickly in response to pathogen aggression and that therefore, on the contrary to constitutive defenses, avert the waste of valuable resources when enemies are absent.[1,2,3] Two hormones play role in the signaling cascades of induced plant defenses such as jasmonic acid (JA) and salicylic acid (SA; 2-hydroxybenzoic acid). SA is included in plant responses to viral, bacterial and fungal pathogens.[4,5] Many studies have showed that SA is a chemical compound which mediates the stimulation of systemic acquired resistance (SAR).[6,7] SA is a key regulatory compound of pathogens resistance.[5] SA has been proven to intermediate resistance in plant-virus reactions. Relying on the virus, SA could inhibit of virus multiplication and virus movement to long distances.[8] Furthermore, SA acts a necessary role in compatible responses; Whereas, SA induces defense-related genes.[9] In addition, methyl salicylate plays a major role as communication signal for defense response both inside plant.[10,11] It was decided that SA-induces RNA-dependent RNA polymerase has a necessary function in antiviral defense.[12] Inter-simple sequence repeat (ISSR) analysis uses oligonucleotides depend on a simple sequence repeat anchored or not at their 5'- or 3'-end by two to four arbitrarily selected nucleotides. This triggers site-specific annealing and initiates PCR amplification of genomic fragments which are flanked by inversely orientated and closely spaced repeat sequences.[13] The study reported here was depend on an ISSR-PCR technique to detection of genotoxicity leading to genomic instability. The aim of the work was to address the following questions. 1- Is SA treatment causes genotoxicity and DNA damage in potato cultivars and wild species inoculated with *Potato virus* Y^{NTN} (PVY^{NTN})? 2- Is treatment of both SA and PVY^{NTN} affect antioxidant enzymes in potato?

MATERIALS AND METHODS

Plant materials

Four potato cultivars cultivated in Egypt and one wild potato species provided by the Centre for Genetic Resources Netherlands (CGN) were used in the study as shown in (Table 1).

Virus inoculation

The necrotic tuber necrosis strain of *potato virus Y* (PVY^{NTN}) was obtained from the Virology Laboratory, Department of Microbiology, Faculty of Agriculture, University of Ain Shams.[14] The PVY^{NTN} strain was maintained on *Datura metel* L. plants and used as sources of virus inoculum in all experiments. The healthy leaves of potato cultivars and wild type were rubbed gently with a cotton swab dipped into the suspension of virus inoculums.

SA and virus treatment

Tubers of the four potato cultivars and one wild type were tested for free from potential infection with potato viruses PVY^{NTN}, *Potato virus X* (PVX) and *Potato leaf roll virus* (PLRV) by double antibody sandwich enzyme linked immunesorbent assay (DAS-ELISA) technique were provided by Sanofi Company Sante Animal, Paris, France and used as described by Clark and Adams.[15] Thirty healthy tubers from each cultivar or species were planted in the pots in winter season (2012-2013) with randomized complete block designed. Potato plants grown from thirty tubers of each cultivar or wild type were divided into three groups: Group 1: Ten potato plants were treated with dsH_2O (the healthy control) (C). Group 2: Ten potato plants were inoculated with PVY^{NTN} strain (V). Group 3: Ten potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v) (SA+V). Two-three weeks after inoculation, the youngest fully developed leaves from both control and treated plants were collected for biochemical and the molecular analyses.

Detection of PVY^{NTN} virus

All the samples were tested for the presence of PVY^{NTN} strain by DAS-ELISA as described by Clark and Adams.[15]

Polyphenol oxidase (PPO) and peroxidase (POX) isoforms

For analysis of PPO and POX isozymes by native-polyacrylamide gel electrophoresis (Native-PAGE), 500 mg fresh treated potato leaves and control were homogenized in liquid N_2 and 100 µl of 0.2 M phosphate buffer was added (pH 7.0 was adjusted by potassium phosphate, monobasic) and 10 µl of 2-mercaptoethanol before centrifugation at 14000 rpm for 15 min at 4°C. The supernatant was stored at a temperature of -20°C until isozyme analysis.[16] PPO and POX isozymes were detected according to Baaziz *et al*.[17]; Brown.[18]

DNA extraction

Young leaves of treated potato cultivars and control were soaked in liquid nitrogen for DNA extraction using the 2% CTAB (Cetyltrimethylammonium bromide) method modified by Doyle and Doyle[19]; Mahfouze[20] (Fig 3).

Inter-simple sequence repeats (ISSR) profiles

A total of five ISSR primers (Table 3) were used to amplify DNA (Life Technologies, Gaithersburg, Md.). 25 µl amplification reaction consisted of 2.5 µl 10X PCR buffer, 2.5 µl 25 mM $MgCl_2$; 0.5 µl 40 mM dNTPs; 1 µl *Taq* DNA polymerase (1 unit/µl); 2 µl 0.4 µM primer. Amplification was carried out in DNA thermocycler (Biometra, Germany) under the following conditions: one cycle of 94°C/3 min, followed by 30 cycles for 94°C/45 sec, 52°C/30 sec and 72°C/2 min; a final extension for 72°C/6 min. Gel electrophoresis: Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and 0.5 µg/ml ethidium bromide at 90 V. Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

Data analysis

A matrix for ISSR 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 and Li.[21] The data were subsequently used to construct a dendrogram using the un-weighted pair-group method of arithmetic averages (UPGMA)[22] 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.[23] Correlations coefficients were calculated using similarity coefficients obtained from ISSR analysis.

RESULTS AND DISCUSSION

Effects of SA treatment and PVY^{NTN} infection on potato

The four potato cultivars and one wild type appeared different response of PVY^{NTN} infection (V) treatment compared with the healthy control (Table 1 and Fig 1).'Cara' and 'Diamond' cultivars gave mild mosaic, 'Nicola' appeared veinal necrosis, 'Selan' produced yellowing. However, wild *S. andigena* showed no symptom ms. On the contrary, all potato cultivars treated SA+V showed necrotic local lesions (hypersensitivity reaction), except wild *S. andigena* has resistance. Dat *et al.*[24] mentioned that salicylate can increase H_2O_2 concentration in plant. The hypersensitive response to pathogens shows an early 'oxidative burst' of superoxide which quickly dismutates to H_2O_2. In this study, exogenous SA implementation reduced the appearance of symptoms induced by virus infection (Table 1 and Fig 1). Thus, SA decreased the disease severity. Consequently, SA plays a role in induce a resistance against PVY^{NTN}.[25] Our results showed that treatment of potato with mixture from PVY^{NTN} inoculation and SA gave resistant against PVY^{NTN}. These results were agreement with Nie[26] found that 1 to 5 mM SA significantly reduced $PVY^{N:O}$ symptoms in tobacco plants. It has been suggested that viral infection induces stimulation or suppression of gene expression in the host.[27,28,29] Changes in gene expression explain an incorporation of stress and defense reactions, viral pathogenesis and plant symptom developing. The ways controlling host defense such as viral pathogenesis are specific for the virus reaction with host.[30]

Activities of PPO and POX antioxidative enzymes in potato

It is found that ,PPO and POX total activities increased due to treatment with 2 mM SA+V and PVY^{NTN} infection (V) (Fig 2 and Table 2). The highest PPO and POX activities were recorded in wild *S. andigena* treated SA+V (four isoforms), followed by 'Diamond' and 'Nicola' cultivars (three). However, the lowest PPO and POX activities were showed in 'Selan' and 'Cara' (two) (Fig 2 and Table 2). On the other hand, the maximum activities of PPO and POX isoenzyme showed in potato cultivar 'Cara' infected with PVY^{NTN} (three markers), followed 'Nicola' and wild *S. andigena* (two), while the minimum PPO and POX activities displayed in 'Selan' and 'Diamond' cultivars (one). In this study, showing different activities of PPO and POX isoforms due to the SA+V and/or V treatment compared with the control. Consequently, SA induces a generation of H_2O_2. It was observed that, H_2O_2 accumulate in plant cells due to biotic and abiotic stresses when attacked by pathogen.[31] Therefore, undue generation of H_2O_2 is proven to be toxic to both the host and a pathogen. The cells destruction around the infection place would prevent a pathogen of movement from cell to cell. New studies have stated that H_2O_2 induces stress signaling lead to stimulate the resistant inside the host.[32,33] H_2O_2 has been observed to help as a necessary substrate for POX activities.[34] Besides, POX can help in two different mechanisms: (I) showing NADH-oxidase activity, it can produce H_2O_2 and O_2^- in reactions that compose the oxidative burst; (II) functioning as a H_2O_2-consuming enzyme, it shares in the rigidifying of cell wall and consequently the blockage of plant growth.[31,35] The external spraying of SA led to activate the antioxidant system of resistance and susceptible potato cultivars due to various internal and external detoxification systems and can lead to biochemical changes.

Detection of genotoxicity by ISSR

No DNA degradation was observed on agarose gel in potato plants treated SA+V and V as shown in Fig (3). ISSR analysis using five primers differed among PVY^{NTN} inoculated (V), SA+V treated potato cultivars and the control with visible changes in the number and size amplicons (Fig 4 and Table 3). The five ISSR primers amplified 84 alleles ranging from 50 to 3000 bp, out of which 28 were polymorphic (33.33%) and 56 were monomorphic (66.67%). The percentage polymorphism ranged from 23.81% (ISSR-4) to 55.56% (ISSR-5). Primers ISSR-3 and ISSR-4 amplified maximum alleles (21), while primer ISSR-5 amplified only (nine). Primer ISSR-1 gave higher number of specific bands (five) of (1500; 1250; 950; 890 and 290 bp), followed by Primers ISSR-3 and ISSR-4 (four) with molecular sizes (820; 800; 460 and 390 bp) and (1125; 820; 600 and 485 bp), respectively. However, primers ISSR-2 and ISSR-5 scored lower number of unique fragments (three) of (3000; 1000 and 186 bp) and (820; 550 and 135 bp), respectively. The highest cultivar created DNA change and produced more number of specific alleles in PCR amplification, DNA from potato cultivar 'Diamond' treated SA+V (11), followed by 'Nicola' (seven); *S. andigena* (six) and 'Cara' (four) (Table 3). Meanwhile, the lowest number of unique fragments showed in both potato cultivars 'Selan' treated SA+V and 'Cara' inoculated with PVY^{NTN} (V)(three). Followed, potato cultivar 'Selan' and wild *S. andigena* inoculated with PVY^{NTN} (V) (two), then 'Diamond' cultivar infected with PVY^{NTN} (V) (one) (Table 3). In this study, the molecular marker ISSR was used to assess the effect of SA+V treatment on genomic DNA stability in potato cultivars. Our results showed changes in ISSR patterns of SA+V treated potato cultivars were low. Consequently, 2 mM SA reduced DNA damage in potato cultivars. This is due to a low genotoxic effect and mutagenicity of SA+V which induce DNA change through single and/or double strand breaks, modified bases and DNA–protein cross-links.[36] Wolf [37]and Raj *et al.*[38] mentioned that the disappearance of bands in the control plants can be linked to the DNA change (e.g., single- and double-strand breaks, abasic sites, bulky adduct, oxidized and

modified bases and DNA-protein cross links), point mutations, and/or complex chromosomal rearrangements stimulated by genotoxins. Atienzar *et al*.[39] showed that mutation can cause occurrence of new fragments if they happen at same locus in sufficiency cells number (a minimum of 10% of mutations may be wanted to obtain new amplicons obvious in agarose) to be amplified by PCR. Markowitz *et al*.[40] found that DNA change can result from nucleotide sequence modification of the DNA, e.g., a mutation induced throughout DNA repair, gene arrangement, replication of DNA, or chemical change resulting from oxidation and methylation. Microsatellite instability is one among these demonstrations of genomic instability.[41] It corresponds to change in simple repeat sequences size. A finding of microsatellite instability means the presence of mutations in at least one gene implicated in DNA mismatch repair mechanisms.[42] Thus, though a method to detect microsatellite length constitutes an important technique in genetic identification of cultivars, it does not allow one to detect main forms of genomic instability such as molecular aneuploidy arising from deletions, translocations, amplifications, recombination, insertions and chemical change.[43] In this study, ISSR analysis appear to be stable in detection of DNA change in potato cultivars treated SA+V.

Cluster analysis

In the present study, cluster analysis was done to estimate the level of polymorphism between treated potato cultivars and control. Genetic similarity (GDs) among potato cultivars treated SA+V, V and control ranged from 0.75 and 0.93. A dendrogram was constructed using distances matrix by using UPGMA method (Fig 5 and Table 4).The cluster analysis consists from two major groups: The first major group I (genetic similarity 0.83 to 0.93) consisted of two sub-groups: The first sub-group composed of 'Cara' cultivar (SA+V and V). The second sub-group contained on 'Selan' (C, V and SA+V) and 'Cara' (C). The second major groups II (genetic similarity 0.75 to 0.92) divided into the three sub-groups: The first Sub-group had 'Diamond' (C and V), 'Nicola' (C and V). The second Sub-group had *S. andigena* (C, V and SA+V). The third sub-groups involved 'Diamond' and 'Nicola' cultivars (SA+V) (Fig 5). Results indicated the presence of narrow genetic variability which reflects a low level of polymorphism at the DNA level. Therefore, 2 mM SA is a compound which mediates the induction of systemic acquired resistance (SAR) against PVY^{NTN} virus without DNA genome significance damage to potato cultivars. Swaileh *et al*.[38] and Raj *et al*.[44] mentioned that the cluster analysis is considered one of the most efficient methods in numerical analysis regarding band scoring and the analysis of ISSR technique. Sharma *et al*.[45] found that the ISSR markers represent the more effective marker system because of their capacity to give many informative fragments within a single amplification reaction. Generally, ISSR displays a higher capacity to reveal polymorphisms and larger potential to define intra and inter-genomic variability than other arbitrary primer methods such as Random amplified polymorphism DNA (RAPD).[46,47]

Table 1
Effect of PVY^{NTN} inoculation and SA+V treatment on potato cultivars

Cultivars and species	Treatment	Symptoms	Degree of resistant
Cara	SA+V	NLL	Hypersensitivity
	PVY^{NTN} (V)	mM	Susceptible
	Control (C)	NS	-
Diamond	SA+V	NLL	Hypersensitivity
	PVY^{NTN} (V)	mM	Susceptible
	Control (C)	NS	-
Nicola	SA+V	NLL	Hypersensitivity
	PVY^{NTN} (V)	VN	Hypersensitivity
	Control (C)	NS	-
Selan	SA+V	NLL	Hypersensitivity
	PVY^{NTN} (V)	Y	Susceptible
	Control (C)	NS	-
S. andigena	SA+V	NS	Resistant
	PVY^{NTN} (V)	NS	Resistant
	Control (C)	NS	-

C = Potato plants were treated with dsH_2O (control).
V = Potato plants were inoculated with PVY^{NTN} strain.
(SA+V)= Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).
mM = mild Mosaic, NLL= Necrotic local lesions, NS = No symptoms,
VN= Veinal necrosis, Y=Yellowing.

Table 2
POX and PPO isozyme markers of PVY^{NTN} inoculated (V) and SA+V treated potato cultivars.

No.	*Rf*	Selan			Cara			Diamond			Nicola			*S. andigena*		
		C	V	SA +V	C	V	SA +V	C	V	SA +V	C	V	SA +V	C	V	SA +V
PPO																
1	0.183	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0
2	0.195	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
3	0.901	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
4	0.989	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1
Total=4		**0**	**0**	**1**	**0**	**2**	**1**	**0**	**0**	**1**	**1**	**1**	**2**	**0**	**2**	**1**
POX																
1	0.296	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
2	0.551	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0
3	0.637	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1
4	0.742	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total=4		**0**	**1**	**1**	**0**	**1**	**1**	**0**	**1**	**2**	**0**	**1**	**1**	**0**	**0**	**3**
Total of PPO and POX bands=8		**0**	**1**	**2**	**0**	**3**	**2**	**0**	**1**	**3**	**1**	**2**	**3**	**0**	**2**	**4**

0= absence of band, 1= presence of band
C = Potato plants were treated with dsH_2O (control).
V= Potato plants were inoculated with PVY^{NTN} strain.
SA+V= Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

Table 3
ISSR amplified bands, polymorphic bands and unique markers for PVY^{NTN} inoculated (V) and SA+V treated potato cultivars.

Primer name	Polymorphism		No. and Molecular Size of markers (pb)		Potato cultivars/ species									
					Selan		Cara		Diamond		Nicola		*S. andigena*	
	Total	P %			V	SA + V	V	SA + V	V	SA + V	V	SA + V	V	SA + V
ISSR-1 GAG$(CAA)_5$	19	6	5	1500		1				1		1		
				1250						1				
				950						1		1		
				890	1									
				290						1		1		
	31.58%		**26.32%**		**1**	**1**	**0**	**0**	**0**	**4**	**0**	**3**	**0**	**0**
ISSR-2 CTG$(AG)_8$	14	4	3	3000				1				1		1
				1000	1				1				1	
				186									1	1
	28.57%		**21.43%**		**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**2**	**2**
ISSR-3 $(GAC)_6$	21	8	4	820			1					1		
				800				1				1		1
				460		1	1	1						
				390		1								1
	38.10%		**19.05%**		**0**	**2**	**2**	**2**	**0**	**0**	**0**	**2**	**0**	**2**
ISSR-4 $(AG)_8$	21	5	4	1125						1				
				820						1				
				600			1	1		1		1		
				485						1				1
	23.81%		**19.05%**		**0**	**0**	**1**	**1**	**0**	**4**	**0**	**1**	**0**	**1**
ISSR-5 $(AG)_8$YT	9	5	3	820						1				**1**
				550						1				
				135						1				
	55.56%		**33.33%**		**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**	**0**	**1**
Total=	**84**	**28**	**19**		**2**	**3**	**3**	**4**	**1**	**11**	**0**	7	**2**	**6**
Polymorphic=	**33.33 %**		**22.62%**											

* P = Number of polymorphic bands with polymorphic percentages.
** Total = Total number of amplified fragments.
0=absent of marker band, 1 = Presence of marker band.
V= Potato plants were inoculated with PVY^{NTN} strain.
SA+V= Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

Table 4
Jaccard's coefficient of similarity matrix for ISSR data for treated and control potato cultivars.

Cultivars and species		Selan			Cara			Diamond			Nicola			*S. andigena*		
		C	V	SA+V	C	V	SA+V	C	V	SA+V	C	V	SA+V	C	V	SA+V
Selan	SA+V	1.00														
	V	0.90	1.00													
	C	0.88	0.86	1.00												
Cara	SA+V	0.88	0.86	0.89	1.00											
	V	0.83	0.83	0.84	0.89	1.00										
	C	0.84	0.84	0.85	0.85	0.93	1.00									
Diamond	SA+V	0.79	0.81	0.85	0.82	0.87	0.91	1.00								
	V	0.75	0.79	0.78	0.78	0.80	0.84	0.92	1.00							
	C	0.91	0.83	0.82	0.79	0.77	0.80	0.77	0.80	1.00						
Nicola	SA+V	0.88	0.88	0.84	0.81	0.84	0.87	0.90	0.85	0.84	1.00					
	V	0.81	0.83	0.80	0.79	0.82	0.83	0.85	0.89	0.85	0.92	1.00				
	C	0.83	0.78	0.79	0.75	0.77	0.80	0.79	0.83	0.92	0.86	0.87	1.00			
S. andigena	SA+V	0.84	0.81	0.78	0.77	0.82	0.81	0.82	0.86	0.87	0.84	0.91	0.85	1.00		
	V	0.78	0.82	0.79	0.81	0.83	0.79	0.84	0.90	0.81	0.83	0.89	0.81	0.92	1.00	
	C	0.83	0.78	0.77	0.75	0.79	0.80	0.79	0.83	0.86	0.86	0.87	0.89	0.90	0.86	1.00

C = Potato plants were treated with dsH_2O (control).
V= Potato plants were inoculated with PVY^{NTN} strain.
SA+V= Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

Figure 1

Symptoms of PVY^{NTN} infection and SA+V treatment on potato cultivars, compared with the control.
1-The healthy control cv. Diamond, 2- Potato plants cv. Diamond infected PVY^{NTN}, 3- Potato plants cv. Diamond treated SA+V, 4-The healthy control cv. Selan, 5- Potato plants cv. Selan diseased PVY^{NTN}, 6- Potato plants cv. Selan treated SA+V, 7-The healthy control cv. Nicola, 8- Potato plants cv. Nicola infected PVY^{NTN} and 9- Potato plants cv. Nicola treated SA+V.

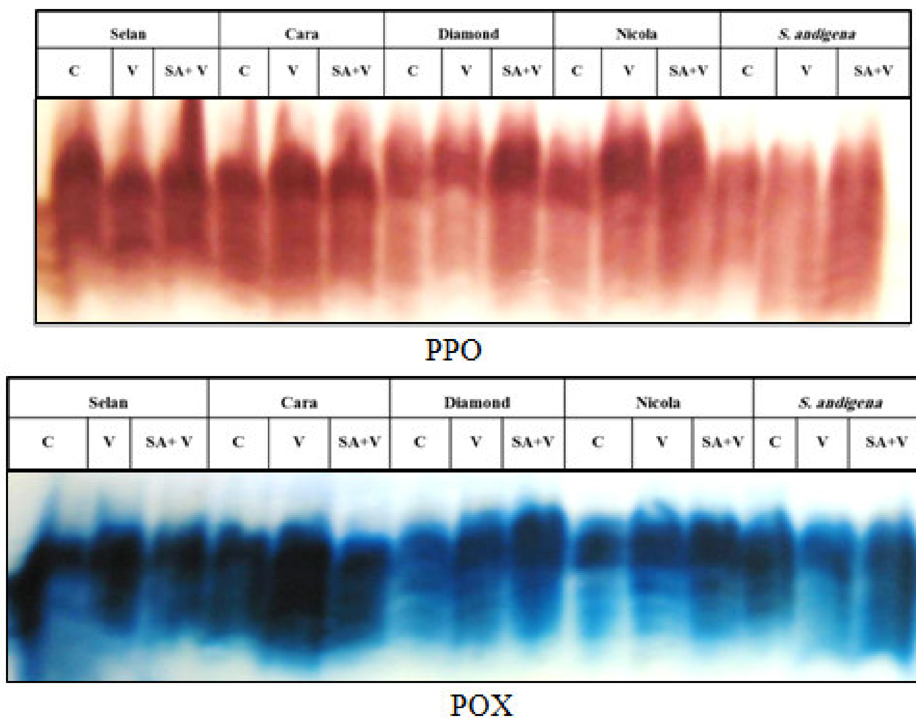

Figure 2

PPO and POX profiles of treated and control potato cultivars.
Lane C = Potato plants were treated with dsH$_2$O (control), lane V= Potato plants were inoculated with PVY^{NTN} strain and lane (SA+V) = Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

Selan			Cara			Diamond			Nicola			*S. andigena*		
C	V	SA+ V	C	V	SA+V	C	V	SA+V	C	V	SA+V	C	V	SA+V

Figure 3

Extraction of genomic DNA from treated and control potato cultivars by 2% CTAB method. Lane C = Potato plants were treated with dsH_2O (control), lane V= Potato plants were inoculated with PVY^{NTN} strain and lane (SA+V) = Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

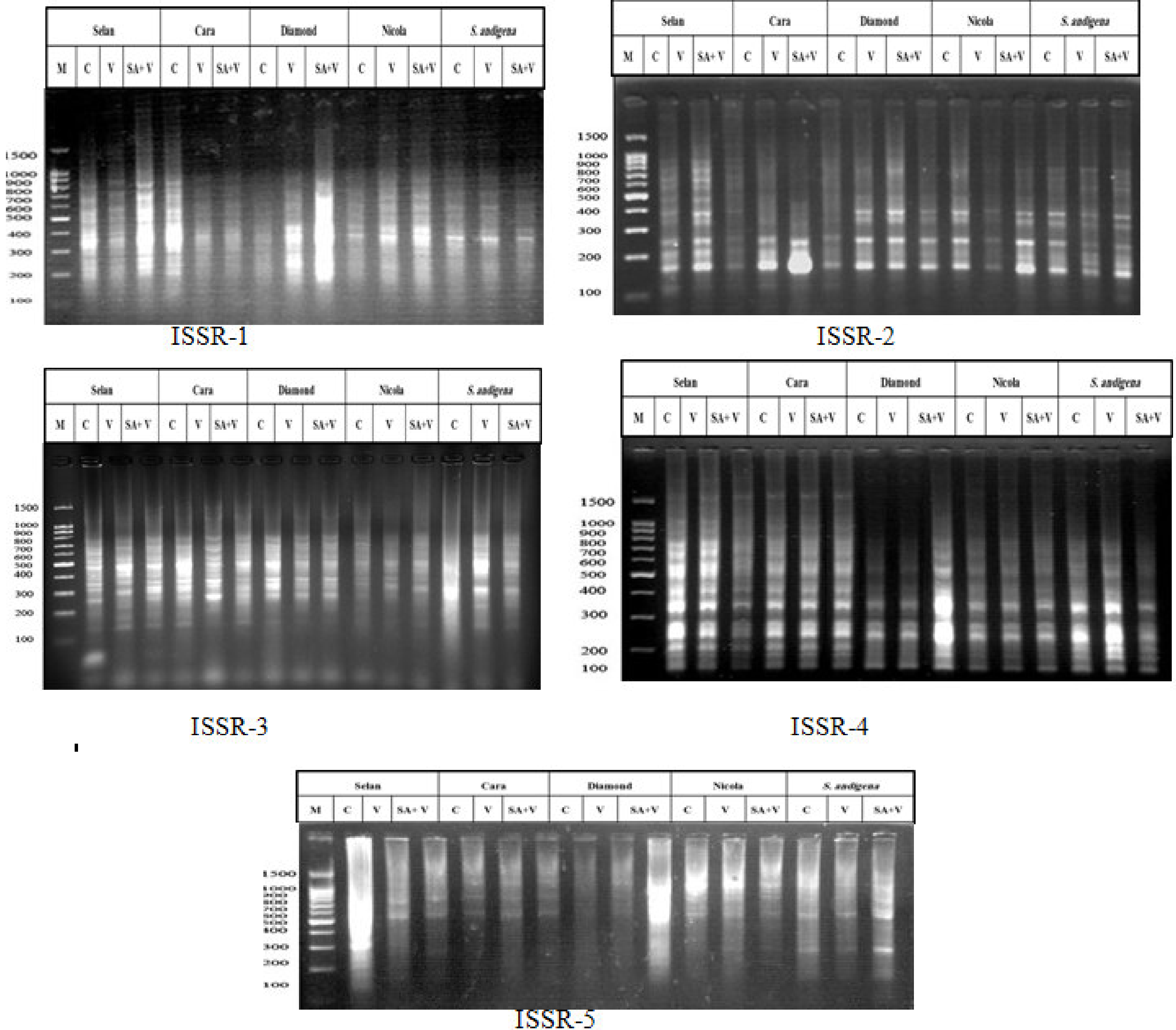

Figure 4

ISSR-PCR profiles using five primers of treated and control potato cultivars. Lane M= DNA ladder 100 bp. Lane C = Potato plants were treated with dsH_2O (control), lane V= Potato plants were inoculated with PVY^{NTN} strain and lane (SA+V) = Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

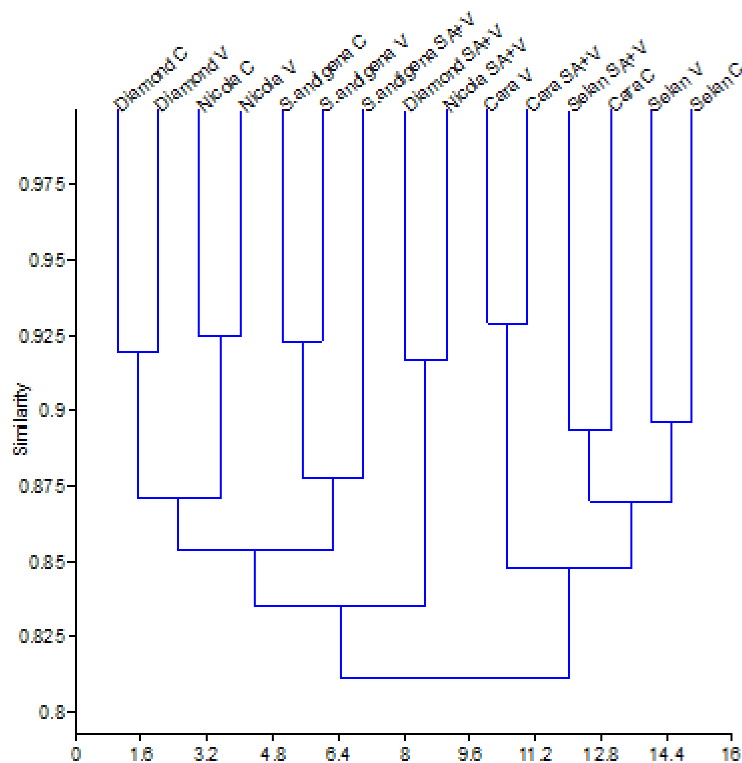

Figure 5
UPGMA dendrogram showing clustering of treated and control potato cultivars.
C = Potato plants were treated with dsH2O (control). V= Potato plants were inoculated with PVYNTN strain. SA+V= Potato plants were sprayed with a mixture of virus inoculum plus 2 mM SA solution (1:1 v/v).

CONCLUSIONS

2 mM SA acts a fundamental function in systemic induced resistance stimulation into plants. It did not cause significance genomic DNA damage to potato cultivars. It could be used in viral diseases resistance without having harmful effect on human and the environment. The comparison between 'control' and 'treated' genomes showed that ISSR analysis could be used to assess how the SA changes DNA structure in plants. On the basis of these observations we suggest that ISSR technique is a powerful tool for detection of qualitative and quantitative genotoxic activity due to SA treatment.

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