MOLECULAR STUDIES ON GENE TYPES RESISTANT TO POTATO VIRUS Y

By

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B.Sc. Agric. Sc. (Agric. Microbiology), Ain Shams University, 2002
M.Sc. Agric. Sc. (Virology), Ain Shams University, 2008

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ABSTRACT

Heba A. Mahfouze: Molecular Studies on Resistant Gene Types to Potato Virus Y. Unpublished Ph.D. thesis, Dept. Agric. Microbiology, Faculty of Agric., Ain Shams University, 2013.

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').

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*). Leaf extracts from *Phytolacca* sp. and *M. jalapa* leaves were blended in dsH_2O (1: 5, w/v) 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 infected potato cultivars through the quantitative and qualitative determination of total proteins. Monomorphic band with molecular weights 11 kDa appeared in AVP-treated potato leaves. In addition, another common band at 28.5 kDa induced in potato tubers resulted from AVP-treated potato plants and disappeared in non-AVP-treated potato plants and the control. On the other hand, the highest PPO and POD activities appeared in AVP-treated potato plants of Cara (three bands), the lowest isozyme activities showed into Spunta (one), whenever the other cultivars were equally in number of bands (two). Otherwise, the maximum PPO and POD activities appeared in non-AVP-treated potato plants of Spunta (six bands). Followed by, Nicola (four), the minimum

isozyme activities showed into Selan and Cara (two), whenever Diamond cultivar was scored (three) bands. Changes in DNA caused by AVP-treated potato cultivars resulted genetic variations detected by ISSR-PCR analysis were performed using five random primers compared to PVY^{NTN} infected plants and the healthy control. A total of 63 scorable amplified DNA fragments ranging from 90 to 1105 bp were observed using these primers, 43 of which were polymorphic while the others were monomorphic. The five primers showed mean polymorphic percentage of 68.25%. The extent of polymorphism per primer ranged from 87.50% (ISSR-1) to 33.33% (ISSR-4). Among the 43 polymorphic bands, 20 bands were unique markers with a total average of 31.74%. The AVP-treated potato cultivars varied considerably in banding patterns using the five ISSR-PCR primers. 'Selan' had the highest number of unique markers (10), followed by 'Nicola' (9), 'Spunta' (5), 'Diamond' (1) and 'Cara' (0). Leaf extracts from *P. americana, P. acinosa* and *M. jalapa* could be used in simple crop-protection agricultural systems by spraying these extracts on leaves of various crops to prevent or control viral infection. The fragments of *PAP-I* gene isolated from *P. americana* and *P. acinosa* leaves using specific primers A and B were 1188 and 868 bp, respectively. The RNAs were reverse transcribed by MMLV reverse transcriptase. The resulting cDNA was amplified by PCR after adding specific primer-C for *PAP-II* gene. DNA amplifed from *P. americana* and *P. acinosa* leaves was 855 bp. The PCR product (868 bp) of *PAP-I* gene amplified from the genomic DNA of *americana* leaves using specific primer-B was eluted from the gel, purified, further amplified and cloned in a pTZ57R/T vector and mobilized into *E. coli* DH5 α competent cells. White ampcillin colonies were selected for plasmid minipreparation using the plasmid minipreparation techniques.

Key words: *Phytolacca americana, P. acinosa, Mirabilis jalapa,* SDS-PAGE, Polyphenol oxidase, Peroxidase isozymes, ISSR-PCR, *PAP* genes, nucleotide sequence.

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LIST OF ABBREVIATIONS

	(A)
AFLP	Amplified Fragment Length Polymorphism
	(B)
BLAST	Basic Local Alignment Search Tool
βME	β-Mercaptoethanol
bp	base pair
BSA	Bovine serum albumin
	(C)
$^{\circ}C$	Temperature
cDNA	Complementary deoxynucleic acid
cm	Centimeter
cv.	Cultivar
CMV	*Cucumber mosaic virus*
CTAB	hexadecyltrimethyl-ammonium bromide.
C	Control
	(D)
DAS-ELISA	Double antibody sandwich- Enzyme linked immunosorbent assay
DNA	Deoxy ribonucleic acid
$dNTP_s$	Dideoxy nucleotide triphosphate
dsH_2O	distilled water
DTT	Dithiothreitol
	(E)
EDTA	Ethylene diamine tetraacetic acid.
E. coli	*Escherichia coli*
	(F)
Fig.	Figure
	(G)
G	gram(s)