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RESEARCH

Incidence, serologic and molecular characterization of *Potato virus S* from commercial potato in Egypt

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ABSTRACT

Background: In Egypt potato crop is affected heavily by a wide variety of viruses, viz. PVY, PLRV, and PVX. Potato virus S (PVS), family Betaflexiviridae; genus Carlavirus) is one of the most common potato (Solanum tuberosum L.) viruses found infecting potato worldwide. PVS was detected only once in Egypt in 1988. However no further studies have been made on it in Egypt recently. PVS-infection to potatoes causes mild symptoms, viz. rugosity, mosaic, stunting, leaf bronzing, and early leaf dropping. Heavy losses; however, can occur upon mixed infection with PVS and PVX or PVM.
Objective: The objective of this study is to characterize the incidence of PVS serologically with authentic antiserum for PVS and, molecularly using specific primers for PVS.
Methods: In-direct ELISA (I-ELISA) and dot blotting immunobinding assay (DBIA) were used for detection of the virus in commercial potato plants. IC RT-PCR amplified 187 bp of the virus coat protein using antiserum for PVS and the specific primer 5'TGGCGAACACCGAGCAAATG3' (sense) and 5'ATGATCGAGTCCAAGGGCACTG3' (antisense).
Results: A specific antiserum for PVS detected PVS in commercial potato plants with I-ELISA and DBIA. Further, IC RT-PCR confirmed the presence of PVS in Egypt for the second time; yielding 187 bp of the virus coat protein.
Conclusion: Presence of PVS was reconfirmed in Egypt using both serology and IC RT-PCR. Previous finding showed that PVS alone causes 3 to 20% tuber yield loss. However, heavier losses (40%), through synergistic interactions, can occur upon its mixed infection with PVX or PVM. Therefore, PVS indexing in potato should be put into action along with other potato viruses in Egypt.

Keywords: PVS; IC RT-PCR; DBIA; *Solanum tuberosum*

BACKGROUND

Potato (*Solanum tuberosum* L.) is one of the most widely grown field crops worldwide. In Egypt potato production was 2,688,649 tonnes in 2013 (http://faostat3.fao.org).

More than 30 viruses infect potato worldwide (Salazar, 1996). *Potato leafroll virus* (PLRV), *Potato virus S* (PVS), *Potato virus X* (PVX) and *Potato virus Y* (PVY) are the most common found viruses affecting potato crops.

Potato virus S (PVS) family *Betaflexiviridae*; genus *Carlavirus, named after Carnation latent virus,*(Adams *et al.,* 2011) is one of the most common potato (*Solanum tuberosum* L.) viruses economically affecting potato production worldwide (lambert and Scott, 2012). The virus particles are flexuous, 650 x 12 nm, containing single stranded RNA genome of 7.5 kb (Wetter, 1971). PVS has two major strains identified as the Andean strain (PVS^A), from the Andean rejoin, South America, and the ordinary strain (PVS^O) (Jones *et al*., 1981). The two strains can be identified biologically through inoculation on leaves of *Chenopodium* spp. PVS^A induces systemic symptoms, While PVS^O induces local lesions on leaves of *C. amaranticolor* and *C. quinoa* (Jones *et al*., 1981). The two PVS strains could be differentiated on the molecular level through amino acid sequence of the 7K protein, coat protein, and the 11K –nucleotide binding

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protein (Matoušek *et al.,* 2000). PVS is transmitted by several species of aphids in a non-persistent manner to several members of the *Solanaceae* and *Chenopodiaceae* and through natural mechanical and vegetative transmission (Jones, 1981; Franc and Banttari, 1996; Raigond *et al*., 2013). Usually infection of potato with PVS is symptomless but in susceptible varieties especially in secondary-infected potato crop PVS infection may develop symptoms, including stunting, chlorotic spots, bronzing, rugosity, waving of leaf margins, deepening of areas between veins on the lower leaf surface, and premature leaf dropping (Dolby and Jones, 1987; Burrows and Zitter, 2005; Pappu *et al.,* 2007; Lin *et al.,* 2009; lambert and Scott, 2012). Infection with PVS alone can cause 3-20% tuber-yield loss. Heavy losses up to 40-50% in tuber yield; however, can occur upon mixed infection with PVS and PVX or PVM (Khurana, 2000; lambert and Scott, 2012). Infection of potato with PVS enhances susceptibility of potato genotypes to late blight caused by *Phytophthora infestans* (Singh *et al*., 1986).

PVS was detected once in Egypt by Khalil *et al.* (1988). However, no additional reports on PVS presence on potato have been reported ever since. During recent field visit to some commercial potato fields in Giza governorates poor growth was observed on some potato plants where leaves showed rugosity, bronzing and waving of leaf margins. The formed tubers were considerably small in size. The purpose of the present study is to characterize the virus(s) associated with the disease symptoms.

MATERIALS AND METHODS

Plant materials and source of antiserum:

Commercial potato tubers of unknown variety were brought from potato farms in Giza governorates where potato plants showed symptoms suspected for PVS infection. Potato tubers were planted in 12 rows using 5 tubers per row. Samples of leaves from each raw were collected and used immediately for analysis or preserved frozen. Random samples of tubers were also collected and preserved at 4^{o}C for further experiments. Antiserum for PVS was obtained from the All Union Res. Inst., Plant Prot. Saint Petersburg, Russia.

Serologic studies:

Indirect ELISA (I-ELISA):

The technique of I-ELISA described by Converse and Martin (2000) was followed. Plant sap was diluted 10^{-1} (w/v) with coating buffer, pH 9.6, and tested for the presence of PVS in infected leaves. PVS primary antiserum was used at 10^{-3} dilution. Goat anti rabbit alkaline phosphatase conjugate was used at 10^{-4} dilution. Samples were measure with ELISA reader at 405 nm. Samples were considered positive if optical density of PVS-infected samples measured more than three times the value of the healthy sap.

Dot blot immune binding assay (DBIA):

The technique of DBIA described by Abdel-Salam (1999) was followed. PVS- primary antiserum was used at 10^{-3} dilution for detection of the virus in leaves and tubers of infected potato. Goat anti rabbit alkaline phosphatase conjugate was used at 10^{-4} dilution.

Molecular studies:

Nucleic acid extraction and reverse transcription polymerase chain reaction (RT-PCR):

Total nucleic acid was extracted according to the silica captured method described by Boom *et al*. (1990) using 100 mg tissues per 1 ml extraction buffer.

Two-step RT-PCR procedure was followed. For the reverse transcription of RNA extract, 5 µl of nucleic acid was incubated at 65^{o}C for 8 min and then chilled in ice to denature RNA. The reverse transcription (RT) mixture was added to a final concentration of 40 ng of reverse primer, 50 mM Tris-HCl buffer, pH 8.3, 75 mM KCl, 10 mM dithiothritol , 2.5 mM $MgCl_2$, 2.5 mM of each dNTPs, 20U RNasin (Promega, Madison, WI), and 2 U Moleney Murine Leukemia virus (MMLV)–reversze transcriptase (SibEnzyme Ltd). Samples were incubated at 42^{o}C for one hr for RT and subsequently incubated at 94^{o}C for 5 min to terminate the RT reaction.

PCR was carried out using aliquots of the 2 µl cDNA mixture in 23 µl containing 1X GoTaq buffer (Promega, Maison, WI), 1.5 mM $MgCl_2$, 0.2 mM of each of dNTPs, 0.4 µM of each PVS primer pair (Matousek *et al*., 2000) [sense (5'-TGG CGA ACA CCG AGC AAA TG-3') and antisense (5'-ATG ATC GAG TCC AAG GGC ACT G-3')], 1.25 U Taq polymerase, and DEPC-treated water. Samples were amplified 40 cycles using a denaturation at 92^{o}C for 1 min, primer annealing at 57^{o}C for 1 min, primer extension at 72^{o}C for 1 min, and final extension of 10 min at 72^{o}C. Eight µl of amplified products were electrophoresed in 2% agarose gel. Gel was viewed under UV illumination after staining in 0.5 µg/ml ethidium bromide.

Immunocapture (IC) RT-PCR:

The technique described by Moury *et al.* (2000) was followed. Sterile polypropylene tubes (0.2 ml) were coated with PVS antiserum, diluted 10^{-2} with phosphate buffer saline (PBS), pH 7.4, and incubated 2 h at 37^{o}C. The tubes were washed once with PBS plus 0.05% Tween 20 (PBST). The tubes were low-speed centrifuged after removing the wash; then loaded with plant extracts (50 µl aliquot per tube), prepared with PBST, and incubated overnight at 4^{o}C. The tubes were washed twice (50 µl per tube) with PBST containing 2 % (w/v) poly vinyl pyrrolidone (40,000 mw) and 20 mM dietyldithiocarbamate and briefly centrifuged to remove the remaining droplets. The tubes were then preheated for 15 min at 65^{o}C before adding the RT mixture composed of 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.3% (v/v) Triton X-100, 250 µM each dNTP, 0.4 µM of each primer pair, 100 U of MMLV-reverse transcriptase (SibEnzyme Ltd) and 1.25 U of Taq polymerase (Promega). The RT-PCR conditions included one h at 46^{o}C, five min at 94^{o}C, followed by 40 cycles of amplification as described above. The amplicons were loaded in 2% agarose gel, electrophoresed, stained and examined under UV illumination.

RESULTS

Infected plants are generally stunted. Leaves show surface rugosity and waving of leaf margins. (Fig.1-A). These symptoms are followed by leaf bronzing (Fig. 1-B) and deepening of the areas between veins on the lower surface of the leaves (Fig. 1-C). Chlorotic spots may develop on lower leaves and may coalesce to form yellow patches (Fig. 1-D) which become necrotic upon disease development. The formed tubers are drastically reduced in size (Fig. 2).

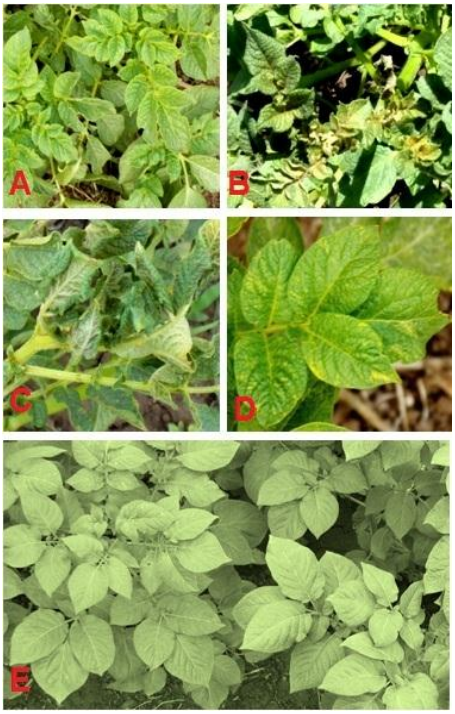

Fig. 1: PVS disease symptom manifestation on potato in the field. A, marginal waving of leaves; B, bronzing; C, deepening of areas between veins; D, chlorotic blotches; E, healthy control.

Fig. 2: Effect of PVS infection in reduction of potato tuber size

Serological studies:

I-ELISA:

I-ELISA detected PVS in leaves of infected plants. Results in Table (1) showed that the tested materials for infectivity with PVS had $O.D_{405\ nm}$ values more than thrice the corresponding healthy control.

Table 1: Indirect ELISA for testing infected potato leaves against the presence of PVS

Optical density at 405 nm•													
Tested samples••												Controls	
R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	Healthy	Buffer
2.2	1.07	1.35	1.26	1.31	1.34	1.61	1.68	1.78	1.80	1.66	1.65	0.05	0.04

•Tested samples with reading values 3 times more the healthy control were considered positive. ••Data represent the mean of five replicates per rows (R) used for planting potato tubers. LSD (least significant difference) measured at $p = 0.05$ using the ANOVA: Single Factor Program = 0.644

DBIA

The DBIA detected the presence of PVS upon testing leaves and tubers of symptomatic potato plants (Fig. 3). Infected sap reacted positive with the Naphthol/Fast Red complex stain forming red color. Whereas, healthy control sap remains green in colour.

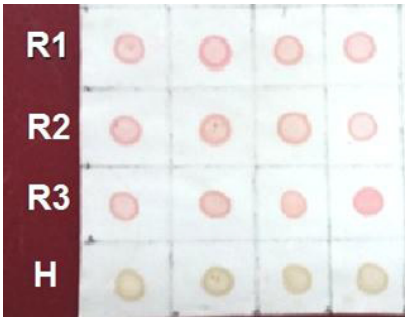

Fig. 3: DBIA on nitrocellulose membrane (NCM) showing the detection of PVS in leaves (R1 row) and tubers (R2, R3 rows). H, Healthy control. NCM was stained with Naphthol/Fast Red complex

Molecular studies:

RT-PCR

RT-PCR analysis succeeded in amplifying 187 bp DNA amplicons from the coat protein (CP) of PVS infecting potato leaves as shown in Fig. (4).

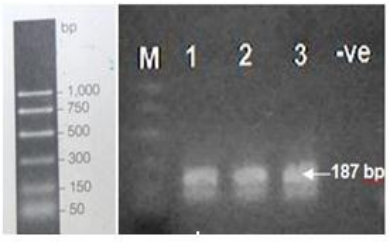

Fig. 4: 2% agarose gel of RT-PCR for the 187 bp fragment of PVS coat protein. M, PCR Markers; 1. 2. 3, DNA amplicons; -ve, negative control, H2O.

IC RT-PCR:

The IC-RT-PCR detected DNA amplicons of 187 bp for PVS coat protein in infected potato leaves (Fig.5).

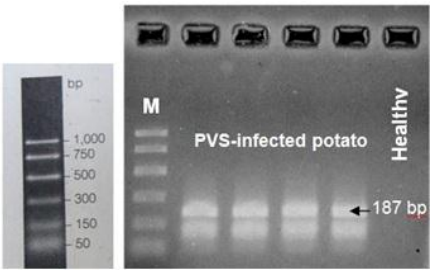

Fig. 5: 2% agarose gel of IC PT-PCR showing the migration of 187 bp fragment of the CP of PVS particles entrapped with PVS antiserum. M=PCR Markers.

DISCUSSION

PVS usually is symptomless on resistant potato varieties Therefore it was not indexed in potato tubers comparing to what is being done for PLRV, PVY and PVX. PVS was recorded once in Egypt (Khalil *et al.,* 1988). However, recent observations of PVS-induced symptoms on potato in Giza governorate have suggested the possibility of introducing potato varieties susceptible to PVS or the introduction of severe isolates of the virus through migrating aphids. Additionally the co infection of potato with PVS along with PVX or PVM is common and is known to aggravate symptom expression (Khurana, 2000; lambert and Scott, 2012).

Serologic and molecular tools are the most two efficient tools for detection of PLRV, PVY and PVX in potato leaves and tubers. Therefore in order to confirm the presence of PVS it was necessary to use symptomatology, serology, and molecular diagnosis to confirm the presence of PVS.

The described disease symptoms resulting from PVS infections included stunting , poor vegetative growth, rugosity of leaf surface, marginal waving of leaf lamina, deepening of areas between veins, chlorotic blotches, and necrosis of leaf, premature dropping of leaf, and drastic reduction in tuber size. All the described above symptoms were recorded in this study and resemble those symptoms described by several investigators for PVS infection on potato (Dolby and Jones, 1987; Burrows and Zitter, 2005; Pappu *et al.,* 2007; Lin *et al.,* 2009; lambert and Scott, 2012).

PVS was detected successfully in potato foliage using antiserum for PVS with I- ELISA, performed either in microplates or on nitrocellulose membranes in DBIA test. I-ELISA detected PVS in all tested potato samples which indicates that PVS has wide distribution in commercial potato fields. Additionally DBIA confirmed PVS incidence in leaves and potato tubers. Immunoblotting assays have been used commonly for detection of potato virus (Lizaragga and Northcote, 1989).

Both RT-PCR and IC RT-PCR are used routinely for the detection of potato viruses. Bostan and Peker (2009) detected PLRV, PVS and PVX in dormant potato tubers using triplex RT-PCR. Also Lambert *et al*. (2003) used IC RT-PCR for detection of *Potato mop top virus* in potato tubers. Additionally, Weildemann and Maiss (1996) detected the potato tuber necrotic ringspot strain of potato virus Y (PVY^{NTN}) by reverser transcription and immunocapture polymerase

chain reaction. In the present study both RT-PCR and IC RT-PCR detected the187 bp fragment of the PVS coat protein upon using specific primers for PVS described by Matoušek *et al*. (2000). This primer pair was used previously to detect PVS in both leaves and tubers of infected potato plants (Nie and Singh, 2001; Bostan and Elibuyuk, 2010). IC RT-PCR surpasses however, RT-PCR in eliminating the need for RNA extraction and therefore saving time and effort during analysis. Further IC-RT-PCR increases the yield of DNA amplicons through increasing the entrapped virions on the surface of the PCR microtubes.

CONCLUSION

The present study confirmed the presence of PVS in Egypt for the second time. The developed symptoms and the economic loss induced by the current PVS isolate, either alone or with possible association with other potato viruses, necessitate that PVS should be put through post-harvest potato indexing program. Additionally, the finding that PVS infection renders several potato genotypes prone to late blight fungal infection assessed the need for PVS indexing

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REFERENCES

Abdel-Salam, A.M. 1999. Isolation and partial characterization of a whitefly-transmitted geminivirus associated with the leaf curl and mosaic symptoms on cotton in Egypt. Arab .J. Biotech. 2(2):193-218.

Adams, M. J., Candresse, T., Hammond, J., Kreuze, J. F., Martelli, G. P., Namba, S., Pearson, M. N., Ryu, K. H., Saldarelli, P., and Yoshikawa, N. 2011. Family Betaflexiviridae. in: Virus taxonomy: ninth report of the international committee on taxonomy of viruses, Pages 920-941, (A. M. Q. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz, eds), ElsevierAcademic Press, San Diego, CA.

Boom, R., Sol., C.J.A., Salimans, M.M.M., Hansen, C.L., Werthein-van Dillen, P.M.E., and Van Der Noordaa, J. 1990. Rapid and simple method for purification of nucleic acids. J. Clinic. Microbiol. 28(3):495-503.

Bostan, H. and Elibuyuk, I.O. 2010. A simple and rapid nucleic acid preparation method for reverse transcription polymerase chain reaction (RTPCR) in dormant potato tubers. African J. Biotechnol. 9(13:1982-1986.

Bostan, H. and Peker, P.K. 2009. The feasibility of tetraplex RT-PCR in the determination of PVS, PLRV, PVX and PVY from dormant potato tubers. African J. Biotechnol. 8(17):4043-4047.

Burrows, M.E. and Zitter, T.A. 2005. Virus Problems of Potatoes. http://vegetablemdonline.ppath.cornell.edu/NewsArticles/Potato_Virus.htm

Converse, R. and Martin, R. 1990. Enzyme-linked immunosorbent assay (ELISA). In: Serological Methods for Defection and ldentification of Viral and Bacterial Plant Pathogens, Pages179-196, (R Hampton, E Ball, S de Boer, eds.), APS Press, Saint Paul, MN, USA.

Dolby, C. A., and Jones, R. A. C. 1987. Occurrence of the Andean strain of *Potato virus S* in imported potato material and its effects on potato cultivars. Plant Pathol. 36:381-388.

Franc, G.D., and Banttari, E.E. 1996. Translocation and mechanical spread of a Minnesota isolate of *Potato virus S* in potatoes. Am. Pot. J. 73:123-133.

Jones, R.A.C. 1981. The ecology of viruses infecting wild and cultivated potatoes in the Andean regions of South America. In: Pests Pathogens and Vegetation, (J. M. Thresh, ed), Pages 89-107, Pitman, London.

Jones, R. A. C., Fribourg, C. E., and Slack, S. A.1981. Potato virus and virus-like diseases. in: Plant virus slide series, no. 2. O. W., (Barnett and S. A. Tollin, eds.), Clemson University, SC. USA.

Khalil. E.M., Ibrahim, I.A., and El-Banna, O.M. 1988. Comparison between the test plant, microprecipitin test and enzyme-linked immunosorbent assay for the detection of potato virus S. Egypt. J. Phytopathol. 20(1):63-71.

Khurana, S.M.P. 2000. Potato viruses: detection and management. in, African Potato Association Conference Proceedings vol. 5, Pages 257–269, (E. Adipala, P. Nampata, and M. Osiru., eds.)

Lambert, D.H., Levy, L., and Mavrodieva, V.A., Johnson, S.B., Babcock, M.J., and Vayda, M.E. 2003. First report of *Potato mop-top virus* on potato from the United States. Plant Dis. 87(7):872.

Lambert, S.J. and Scott, J.B. 2012. Strain Characterization of *Potato virus S* Isolates from Tasmania, Australia. Plant Dis. 96:813-819.

Lin Y.H., Druffel, K.L., Whitworth, J., Pavek,. H.R. 2009. Molecular characterization of two potato virus S isolates from late blight resistant genotypes of potato (Solanum tuberosum). Arch Virol 154:1861–1863

Lizarrage, C. and Northcote, F. 1989. Detection of potato viruses X and Y in sap extracts by a modified indirect enzyme-linked immunosorbent assay on nitrocellulose membranes (NCM-ELISA). Plant Dis. 73(1):12-14.

Matoušek, J., Schubert, J., Dědič, P., and Ptáček, J. 2000. A broad variability of potato virus S (PVS) revealed by analysis of virus sequences amplified by reverse transcriptase - polymerase chain reaction. Can. J. Plant Pathol. 22:29-37.

Moury, B., Cardin, L., Onesto J.P. Candress, T. and Poupet, A. 2000. Enzyme-linked immunosorbent assay testing of shoots grown in vitro and the use of immunocapture-reverse transcription-polymerase chain reaction improve the detection of *Prunus necrotic ringspot virus* in Rose. Phytopathol. 90(5):522-528.

Nie, X. and Singh, R.P. 2001. A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers. J. Virol. Methods 91:37-49.

Pappu, H. R., Druffel, K. L., Whitworth, J., and Pavek, M. J. 2007. Incidence, transmission and molecular characterization of Potato virus S from selected potato cultivars in Washington State. Phytopathol. 97:S89.

Raigond, B., Sharma, M., Chaauhan, Y., Jevalatha, A., Singh, B.P., and Sharma, S. 2013. Optimization of duplex RT-PCR for simultaneous detection of Potato virus Y and S. Potato J. 40(1):22-28.

Salazar, L.F. 1996. Potato Viruses and Their Control. CIP, Lima. P214.

Singh, K., Rai, R.P., Srivastava, S.N.S., and Rampal. 1986. Dates of planting and haulm cuttings for disease-free seed potato production in eastern Gangetic plains. J. Indian Potato Assoc. 13:31-41.

Weildemann, H.L. and Maiss, E. 1996. Detection of the potato tuber necrotic ringspot strain of potato virus Y (PVYNTN) by reverser transcription and immunocapture polymerase chain reaction. J.Plant Dis Protect.103(4):337-345.

Wetter, C. 1971. Potato virus S CMI/AAB Description of Plant Viruses No. 60, 3 pp.

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