Egypt. J. Microbiol. **52,** pp. 141 - 155 (2017)

Physiological and Infectious Characters of *Potato virus Y*-Egyptian isolate

Seham Abdel-Shafi[#], Mohamed F. Ghaly, Khalid El-dougdoug[*] and Mohamed Taha

Department of Botany & Microbiology, Faculty of Science, Zagazig University, Zagazig and [*]Department of Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

POTATO VIRUS Y (PVY) is one of the most destructive aphid transmitted pathogen to potato plants worldwide. In Egypt, PVY infection causing about 80% reduction the global yield of potato. The objective of this study was to characterize *Potato virus Y* (PVY-EG) infecting potato plants, based on biological, serological and molecular properties. Naturally infected potato plants by PVY gave positive reaction with PVY-polyclonal antibodies using DAS-ELISA for virus identification. PVY has a higher stability at 45 h (Longevity), 66°C at 10 min (TIP) at 10^{-6} (DEP), revealing presence of cytoplasmic and crystalline inclusion bodies of the epidermal strips from infected *Datura metel* (diagnostic host) leaves at 12 days post PVY inoculation. The PVY has UV-spectra at λ 235 and at λ 257 nm. PVY yield was 1.25/100 g leaf tissues and 260/280 more than 1 (it was 1.6). The viral particles were rod flexible (helical symmetry) of 11 x 570 nm, with obvious immunogenicity that represented by 1:1024 titer of antibodies. The cDNA fragment of CP gene was 610 bp. Sequence analysis revealed that PVY isolate showed 93-99% similarity with other worldwide PVY isolates.

Keywords: PVY, Potato, *Potyvirus,* ELISA, RT-PCR.

Introduction

The potato (*Solanum tuberosum* L.) is an important economic crop, however, its infection with potato viruses usually causing severe economic damage affecting both seed quality and trade. *Potato virus Y* (PVY) is one of the most important aphid transmitted viral pathogen of potato worldwide. It can affect the production of certified seed and also crops grown for processing or fresh market (Rykbost et al., 1999). PVY is a member of the genus *Potyvirus* (Family *Potyviridae*), the largest group of plant viruses that encompasses 111 recognized and 86 tentative species infecting more than 30 plant families (Fauquet et al., 2005)**.** It has been revealed that different strains of PVY are closely associated with different degrees of pathogenicity where the most important and common are known to be recombinant (Visser et al., 2012). Primary symptoms of PVY are necrosis or yellowing of leaflets, leaf dropping or sometimes premature death. PVY^{O} (common strain) gave mild mosaic, mottle, bronzing and rugosity can arise from mixed infection with PVA, PVX and PVS (Eraky et al., 2014).

In Egypt, PVY was isolated from different crops such as potato, tomato, gladiolus and pepper (Ahmad, 2005 and Al-Nagar, 2007). Also, Mahfouz et al. (2004) isolated three isolates of PVY, four isolates of PVX and three isolates of PVS from naturally infected potato plants cvs. Nicola and Diamont at different farms in Egypt.

The most important vectors of potato virus Y and establishment their relative efficiencies of transmission was recorded by Alyokhin et al. (2002). *Myzus persicae*, *Phoredon humuli* and *Aphis* spp. accounted for 90%, meanwhile, *Bracycaudus helichrysi* represents alone 52% of transmission, respectively.

Stability of *Potato virus Y* was determined in sap of infected potato plants, the thermal inactivation point (TIP) for PVY^{N} was 55-60°C, while, its dilution end point (DEP) was 10^{-3} in sap extracted from potato leaves showing mild symptoms (Hossain & Ali, 1992). DEP 10^{-2}-10^{-3}, TIP 60-65°C, LIV 5-15 days, one type of an antigen with MW c.34 KDa, serological activity and symptoms caused on tobacco cv. Samsun. The TIP of *Potato virus Y* was ranged from 55-60°C, DEP was 10^{-4}

[#]Corresponding author email: hegazyseham@yahoo.com (Seham Abdel-Shafi)
DOI :10.21608/ejm.2017.1725.1029

and LIV at 20°C was within 24 h in sap extracted from infected *Wedelia trilobata* (Linno) (Sheikh et al., 2012).

The virions of the viruses belonging to the *Potyvirus* genus are rod-shaped flexuous filaments 680-900 nm long and 11-13 nm wide. The virions have a monopartite, positive-sense single-stranded RNA, that is about 9.7 kb. Approximately 2000 subunits of a single coat protein are organized around the viral genomic RNA in a helical arrangement (Shukla et al. 1994).

Frequency and severity of PVY, methods of viral detection are needed. Therefore, the objective of this work was aimed to detect and isolate PVY infecting potato plants in Egypt. As well as its biological, serological and molecular characterization.

Materials and Methods

Collection of leaves samples and detection of potato viruses

The PVY was isolated from naturally mixed infection potato plants (50 samples) cultivated in different locations in El-Sharkia, Egypt, during spring, 2011. Different distinct viral systemic symptoms on plant leaves were recorded. The potato viruses (PVY, PVX, PLRV) were detected in naturally infected potato plants using polyclonal antibodies kit (Sanofi, Sante, Animal, Paris, France) by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark & Adams, 1977).

Isolation of PVY

The infected potato plants gave positive results with PVY specific antibodies were used for PVY isolation and identification. Differential host, *Datura metel* was mechanically inoculated with infectious sap of infected potato for PVY isolation. Indicator plant, *Chenopodiumam amaranticolor* L., was inoculated with virus isolate for single lesion isolation and grown under a greenhouse conditions. The five local lesions morphological identical were separated and crushed in 0.1 M phosphate buffer (pH 7)between two slide glass. The crude extract was mechanically inoculated on *Nicotiana tabacum* cv. white burly for virus propagation. The inoculated plants were kept under greenhouse conditions for symptoms development at 26±2 °C.

Propagation of PVY-isolate

The external symptoms were observed and confirmed by DAS-ELISA (Nerway & Kassim, 2014). The homologous local lesions were separated and grinded in mortar with (1:1 w/v) 0.1 ml phosphate buffer containing 0.1 M sodium phosphate pH 7.5, 10 mM Na_2SO_4 and 2 mM EDTA. The extracted sap were squeezed and filtered through two layers of cheesecloth. The filtrated crude sap containing viral particles (infectious sap) was ready for inoculation. The PVY-infectious sap was mechanically inoculated on *Nicotiana tabacum* cv. White Burley for propagation of PVY-isolate (15 plants). The inoculated plants were maintained under insect-proof cages in greenhouse conditions at 26±2°C for 14 to 21 days before use for either biological testing or purification of the virus.

Determination of host range

Twenty-three plant species and cultivars, belonging to six families (Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Fabaceae and Solanaceae) were tested after (Mahfouz, 2003). The tested plants were mechanically inoculated by infectious sap of the isolated PVY isolate. The inoculated plants were kept under greenhouse conditions for symptoms development. The external symptoms were observed and confirmed by DAS-ELISA using polyclonal antibodies specific for *Potyvirus* group.

Mode of transmission:

Mechanical sap transmission

Healthy potato cvs.Diamont and spounta (30 days) and differential hosts, *Ch. amarnticolor* L., *Capsicum annuum cv*. California wonder, *Datura. metel* L., *Lycopersicon esculantum* L. cv. Castle rock, *N. tabacum* cv. Samsun NN. (Mahfouz, 2003) were mechanically inoculated with infectious sap of PVY-inoculum isolate. The inoculated hosts were kept under insect-proof cages in a greenhouse conditions, at 26±2°C for 14 to 21 days, till the developed external symptoms and confirmed with DAS-ELISA.

Aphid transmission

It was carried out by using colonies non-viruliferous aphids *Myzus persicae* Sulz. Aphids were collected from the potato field and identified by Plant Protection Dept. Fac. Agri., Ain Shams Univ. The collected aphids were reared on cabbage seedlings under insect-proof cages. Filial generations, nymphal and adult stages were used in non-persistent manner transmission.

Aphids were fasted 15 min before transferring to infected potato cv. Diamont plants. The aphids (10 insects/plant) were left to feed on the infected

leaves with PVY-isolate for 5, 10, 20 and 30 min. and then transferred to 3 healthy potato plants cv. spounta. All the insects were allowed to feed for one hour, and then killed by spraying malathion at conc. 0.2%. Inoculated plants were kept under insect-proof cages in a greenhouse conditions under observation till 25 days post-inoculation and observed the developed external symptoms. The inoculated plants were tested for PVY infection using *Ch. amaranticolor* L**.** and confirmed by DAS-ELISA (Nerway & Kassim, 2014).

Graft transmission

Bud eye from potato tubers of the infected cv. spounta were grafted on healthy ones. Grafted eye tubers and scions were tied together with paraffin wax and cultivated on pots under insect proof in greenhouse conditions. One to two months after grafting all plants were serologically tested using ELISA and investigated to PVY distinct symptoms.

In vitro stability of PVY-isolate

To study the i*n vitro* stability of PVY-isolate (TIP, DEP and LIV), infectious crude sap of PVY-infected *N. tabacum* cv. White burly leaves were extracted 4 weeks post-inoculation. Leaves of *Ch. amaranticolor* L., plants were mechanically inoculated with PVY-infected treated sap and kept under a greenhouse conditions (Walkey, 1985).

Light microscope examination

For crystalline and amorphous viral inclusions in epidermal strips of the leaves systemically PVY-infected *N. tabacum* cv. Samsun NN were examined as described by Jordan & Baker (1955).

Purification of PVY isolate

Virus purification was done according to Rupar et al. (2013). The purity of PVY isolate was evaluated, biologically, spectrophotometrically and electron microscopy.

Infectivity assay

Infectivity assay of PVY purified isolate was done on *Ch. amaranticolor* leaves by rubbing the purified virus inoculums. Ten fold dilutions up to 10^{-8} was applied. Five replicates for each diluted was carried out for appearance of chlorotic local lesions.

Ultraviolet absorption spectrum

Purified virus was scanned in a Shimadzu UV 1201 spectrophotometer UV absorption at UV range from 220 to 300 nm (intervals 5 nm) maximum, minimum, A260; 280; 260/280 and 280/260 values were calculated.The virus yield was calculated according to Noordam (1973) as follows:

$$\text{Virus yield} = \frac{\text{O.D.(260) X Dilution factor}}{\text{E.C.}}$$

whereas:

O.D. = optical density at 260 nm

E.C.=extinction coefficient 2.8 (mg/ml^{-1} cm^{-1})

Transmission electron microscope

Small drops of purified virus were placed on carbon-coated grids for one minute and dried. Virus particles existed on grids were stained using 2% uranyl acetate and dried then examined using electron microscope in National Research Centre, Egypt (Sharma et al., 2013).

Molecular characters

Reverse transcription- polymerase chain reaction (RT-PCR)

Total RNAs were prepared from infected *N. tabacum* cv. white burly leaves with PVY-isolate according to the instruction manual of High Pure RNA tissue kit (Version 1, 2000) from Roche diagnostics GmbH, Germany. The eluted RNA was stored at -80ºC for later analysis. Agarose electrophoresis and spectrophotometry were performed (Mahfouz, 2003). Two oligo-nucleotide primers were used to generate cDNA and amplify the *PVY-CP* gene. The 3`-end primer sequence 5`- ggatccacatgt t (c/g)tt (c/g) act ccaag (t/c) ag-`3, was based on the complete nucleotide sequences of the previously published sequence of the $PVY^N$ and partially degenerate. The 5`-end primer sequence 5`- ggatccatg g(g/c) aaatgacacaat (t/c) gat gca –`3 (Sudarsono et al. 1993). The oligo-nucleotide primers were synthesized in Thermo Hybaid GmbH, Germany. Viral cDNA was synthesized and amplified as procedure according to Hadidi et al. (1993). One µg of RNA, 3 ml of the primers, 6 µl of 5X first strand cDNA buffer (250 mMTris-HCl, pH 8.3; 500 mMKCl; 15 mM $MgCl_2$), 3 µl of 0.1 Mdithiothreitol (DTT), were added to a final volume of 30 µl by deionized water. The mixture was heated for 5 min at 95ºC, and directly chilled in ice for 2 min, then incubated at room temperature for 1 h to allow primer annealing to the RNA template. Twenty µl of reaction solution (4 µl of 5X first strand cDNA buffer, 5 µl of 0.3 M β-mercaptoethanol, 2.5 µl of 10 mM each deoxynucleotide triphosphate (dNTPs), 1 µl of RNasin (40 units/µl), 2 µl of 0.1 Mdithiothreitol (DTT), 4.5 µl deionized water, and 1 µl (10.000 units/µl) of Moloney Murine Leukamia Virus reverse transcriptase (MMLV–RT) (Promega, Co) were mixed with annealing

reaction mixture, incubated for 1 h at 42ºC. Amplification was performed in thin walled PCR tubes. Each tube containing the following reaction mixture: 5 µl of 10xPCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl and 0.001 % gelatin), 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 5 µl of 10 pmol each downstream and upstream primer for PVY-CP, 2.5 units of Taq DNA polymerase, and sterile water to a volume of 50 µl, and ' hot start ' at 94 °C for 5 min in a programmable thermocycler. Five µl of the cDNA mixture was added to the PCR reaction and amplified with the following cycling parameters: The amplification proceeded through 35 cycles of denaturation at 94°C for 1 min followed by annealing at 57°C for 1 min. and primer extension at 72°C for 2 min, with a final extension step of 72°C for 7 min. PCR amplification was performed in an Thermoblock cycler PCR (Biometra) **(**Mahfouz, 2003). The amplified DNA products of the *CP gene* 1 was visualized on UV transilluminator (λ 254 nm) and photographed by Gel Documentation System (GELDOC 2000, BioRad, USA). pGEM DNA marker (Promega) was used to determine the size of RT- PCR amplified cDNA products.

Sequencing and computer analysis

Partial nucleotide sequencing of the PCR product of coat protein gene for PVY isolate, which purified from the low melting point agarose gel using QlA quick gel extracted kit cat. No. 28702. The nucleotide sequencing was carried out at the Gene Analysis Unit, Egyptian Company for Blood Transfusion Services (VACSERA) by ABI PRISM Sequencer model 310 version 3.4. The sequence data, multiple alignment, phylogenetic relationship and antigenic index were translated and analyzed by DNAMAN program (Wisconsin, Madison, USA).

Results

The current study was undertaken to PVY isolation, identification and molecular characterization.

Field inspection

Potato viruses diseases were diagnosed in naturally infected potato plant, (*Solanum tuberosum* cv. spounta) grown under open field conditions. The naturally infected potato leaves showed systemic viral symptoms mild and severe mosaic, rugosity, vein necrosis, leaf narrow, leaf roll, chlorosis, epinasty, yellowing, shortening of internodes, mottling and plant stunting (Table 1).

TABLE 1. Frequency of potato viruses infected potato plants grown under field conditions.

Potato viruses	No. of infected plants	Frequency %
PVX	4	16
PVY	8	32
PLRV	2	8
PVX+PVY	6	24
PVX+PLRV	2	8
PVY+PLRV	2	8
PVX+PVY+PLRV	1	4
Total number of infected plants.	25	

Potato viruses detection

Potato viruses PVY, PVX and PLRV were detected in naturally infected potato plants exhibited distinct viral symptoms, i.e. mosaic, necrosis, leaf roll on middle leaves of plants by following the method of DAS-ELISA using specific polyclonal antibody of PVY, PVX and PLRV (Table 2).The obtained results indicated that, the leaf samples of cv. spounta naturally infected with PVY, PVX and PLRV individually and their mixture which gave positive reaction with polyclonal antibodies specific PVY, PVX and PLRV.

PVY isolation

PVY was isolated biologically on *Ch. amaranticolor*. L. from the selected DAS-ELLSA positive potato plants. After 5-10 days from inoculation localchlorotic lesions symptoms with the same morphological characters (small chlorotic lesion without halo) were developed (Fig 1). The typical systemic external symptoms

of leaf crinkle, mottling, severe mosaic produced till it gives deformation and stunting of plant growth *N. tabacum* cv. white burly after 20 to 30 days from inoculation. These plants were kept under insect proof into greenhouse conditions for virological studies.

TABLE 2. Detection of potato viruses in naturally infected potato plants cv .spounta using DAS-ELISA Techniq UE.

Leaf symptoms	DAS-ELISA		
	PVY	PVX	PLRV
mM, N, E, LN	+0.475	0.189	0.174
SM, N, LN	+0.572	+0.421	0.125
SM, E, D	0.175	+.0521	0.151
SM, VN, LN	+0.385	+.0375	0.195
MO, LR, VN	+0.175	0.257	+0485
VN, M, E, D	+0.452	0.172	.0175
E, R, RU, MO	0.0225	+0472	0.128
RU, MO, LR	0.185	+0.381	+0.312
N, LN, MO	+0.625	+0.423	0.195
S, LR, D,E	0.225	0.172	+0.325
E, Mo, N	+0.453	+0.421	0172
E, N, LN	+0.521	0.211	0.192
VN, mM, Cl, LR	+0.586	+0.410	+ 0.320
N, E, Ci	+0.520	0.212	0.172
VN, Y, D	+0.721	0.205	0.191
E, LR, SI, S	0.210	0.159	+0.320
mM, VN	+0.420	+0.521	0.212
NS	0.251	+0.321	0.173
E, M, LR	0.210	+0.425	+0.325
SI, SM	0.125	+0.310	0.192
VN, Ch, D	+0.521	0.182	0.200
Y, VN, CI	+0.531	0.193	+0.310
M, SI, VN	+0.425	0.212	0.210
E, CI, N, M	+0.452	+0.421	0.177

CI = Curled inward; LR = Leaf roll; RU= Rossette linke growth; Ch = Chlorosis
mM = Mild mosaic; SM = Severe mosaic; E = Epinasty; Mo = Mottling; S= Stunting
LN = Leaf narrow; N = Necrosis; SI = Shortening of internodes; M = Mosaic; Ns= No symptoms
SNS = Systemic necrotic spots; VN = Veinal necrosis; R= RugosityY=Yellowing
D= deformation; -ve control = 0.173; +ve control = 0.651

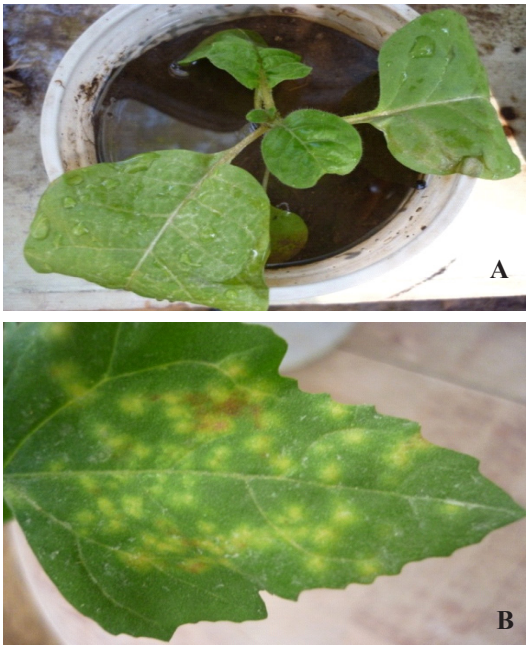


Fig. 1. (A) *Ch. amaranticolor* plants inoculated with infectious sap of naturally infected potato cv. spounta showing chlorotic local lesion. (B) *N. tabacum* cv. White Burley inoculated with PVY isolate showing leaf crinkle, mottling, severe mosaic symptoms.

PVY isolate identification

Mode of transmission

PVY isolate was mechanically transmitted from infected potato cv. spounta to healthy potato by 90% and *Datura metel* with 75%. The efficiency of aphid transmission was higher than rubbing with sap (85%) under greenhouse conditions. In addition, it was transmitted with percent 100% through graft healthy tuber cv. spounta with bud eye from infected tuber. The results were confirmed by DAS-ELISA using specific PVY polyclonal antibodies.

Virus symptomology

The tested plants, Table 3 showed different susceptibility to the PVY isolate when inoculated by rubbing infectious sap. PVY isolate was reacted with three categories with these plants (Table 3 and Fig. 2). Two species out of twenty-three tested plants were (*Ch. amaranticolor* and *Ch. murale* were reacted with local infection which appeared chlorotic local lesions (Fig. 2). Twelve plants belong to five families reacted systemically with PVY isolate. Nine plant species not reacted with PVY isolate. These results were confirmed by DAS-ELISA using polyclonal antibodies specific potyvirus group (Table 3).

TABLE 3. Reaction between host plants and tested PVY isolate using rubbing sap inoculation and confirmed by DAS -ELISA.

Differential host plants		Symptoms	Days-post inoculation (days)	DAS-ELISA
Plant family	**Plant species**			
Amaranthaceae	*Gomphorena globosa*	M, VB	21	0.325
Chenopodiaceae	*Chenopodium amaranticolor*	L. Chl	9	0.358
	Ch. quinoe.	NS	-	0.110
	Ch. mural L.	L.Chl	10	0.243
Compositae	*Zinnia elegans* L.	NS	-	0.120
	Chrysanthemum morifolium	M, VB	22	0.312
Cucurbitaceae	*Cucumber sativus* cv. Beutalpha	NS	-	0.130
	Cucurbita pepo cv. Eschandarani	NS	-	0.175
	Phaseolus vulgaris cv. Pronko	M, VB, CL	21	0.313
Fabaceae	*Vicia faba* cv. Giza 3	NS	-	0.105
	Vigna unguicuta L.	NS	-	0.120
	Capsicum annuum cv. Chilli	VB, Cr, CP	15	0.425
	Datura innoxia L.	NS	-	0.131
	D. metel L.	SM, D, Mo	17	0.521
	D. stramonium	NS	-	0.140
Solanaceae	*Lycopersicon esculentum* cv. Castle rock	SM, D, Cr, CP	15	0.435
	Nicarchia physaloides L.	SM, D.	14	0.311
	Nicotiana Clevelandii L.	NS	-	0.115
	N. glutinosa L.	M, VN	17	0.345
	N. rustica	M	19	0.437
	N. tabacum Cv. White burly	M	18	0.375
	Petunia hybrid L.	SM, D	15	0.412
	Physdlis floridana L.	M	12	0.425

Negative control = 0.112 Positive control = 0.527
M = Mosaic Lchl = local chlorotic lesion
VB = Vein banding NS = No symptoms
CL = Leaf curl D = Deformation
Cr = Leaf crinkle N = Necrosis
SM = severe mosaic VN = Veinal necrosis
CP = Cup shape Mo = Mottling

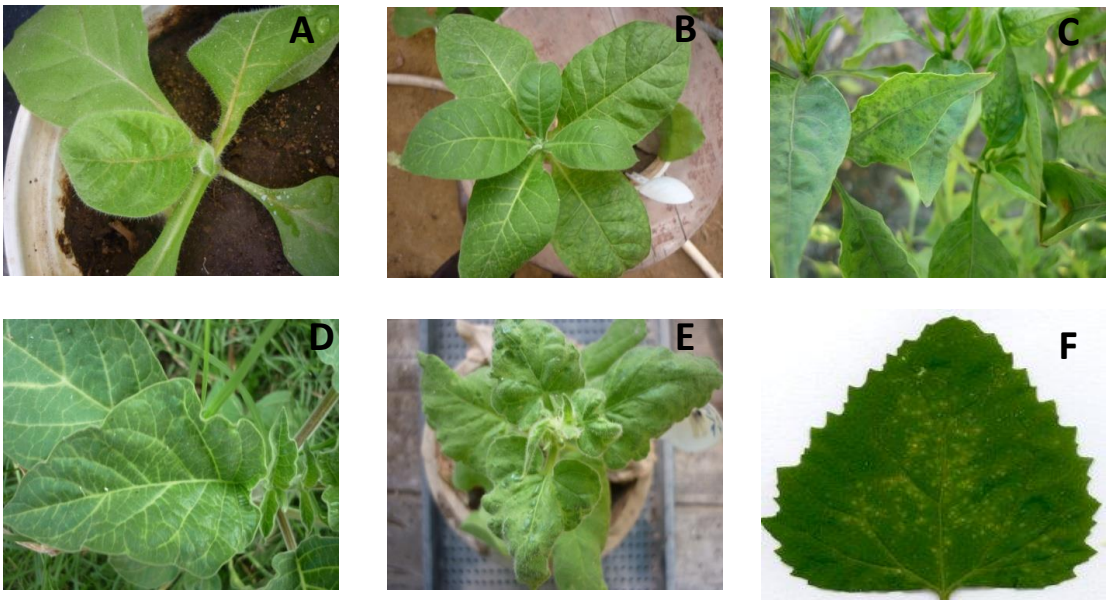


Fig. 2. Host plants inoculated with PVY isolate showing different symptoms.
(A) *N. tabacum* cv. White Burley; (B) *Nicotiana rustica;* (C) Pepper; (D) *D. metel*; (E) *N. glutinosa;* (F) *Ch. amaranticolor.*

Inclusion bodies

The crystalline inclusions are observed in epidermal cells as well as amorphous inclusions stained by bromophenol blue and mercuric chloride (Fig. 3).

PVY isolate stability

The result of TIP showed that PVY isolate was completely inactivated in crude sap when heated to 66°C for 10 min PVY has DEP at $10^{-6}$and was completely inactivated when diluted up to 10^{-7} at room temperature.The result revealed that, PVY was completely inactivated after 45 h when kept at room temperature (Table 4).

PVY particles morphology

Transmission electron microscope examination of partially purified preparation from infected potato leaves negatively stained with uranyl acetate revealed the rod flexible (helical symmetry) particles of PVY with 11 X 570 nm (Fig. 4).

PVY-CP gene properties

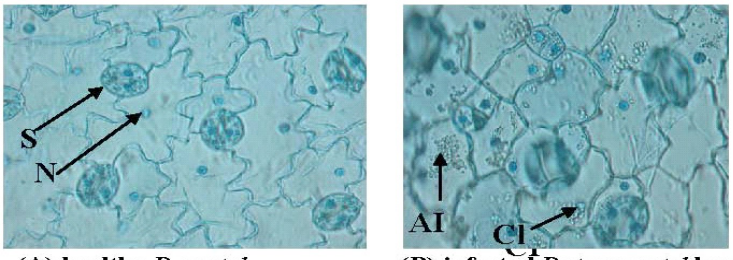
RNA yield

The total RNA extracted from mechanical inoculated *N. tabaccum* cv. white burly with PVY isolate was evaluated before PCR amplification using spectrophotometer at 260 nm and running on agarose (1.5%). The concentration of total RNA was 3.2 ug per 0.5 gm leaves. The RNA fragment appeared on the agarosegel (total RNA extracted from potato leaves cv. spounta) indicating of success of total RNA extraction with the high density (Fig. 5).

The total RNAs from PVY infected *N. tabacum* cv. White Burley, potato cv. spounta leaves were reverse transcribed by RT-PCR using the one oligonucleotide downstream primer for PVY-CP gene. On the other hand, no transcription with the healthy one.The viral cDNA was amplified by PCR using primer sets for PVY/CP-gene. The size of the amplified PCR product of PVY/CP-gene from RNA of infected potato and *N. tabacuum* leaves were estimated by comparing its electrophoretic mobility with those standard DNA ladder (PGEM DNA marker Promega) as shown in Fig. 5**.** The amplified DNA was in the expected sizes calculated (610 bp). But in case of lane (5) no RT-PCR amplified product was observed with uninfected potato healthy leaves.

Nucleotide sequence analysis

The partial sequence of the PCR-amplified fragment for the coat protein (CP) gene of PVY isolate was done to determine the relationship with other recommended PVY isolates. Nucleotide was found to be 610 bp from CP genome sequence (Fig. 5).



(A) healthy *D. metel.* (B) infected *Datura metel* leaves with PVY.

Fig. 3. Light micrography of epidermal strips and hairs PVY infected *Datura metel* leaves (17 days post inoculation) showing cytoplasmic inclusion bodies, Magnification (x - 400)
CI: Crystalline inclusion bodies; AI: Amorphous inclusion bodies; S: Stomata; N: Nucleus.

TABLE 4. Assessment of PVY isolate particles stability in infectious sap at room temperature using local lesion assay.

TIP			DEP			LIV		
Temp.C	**No of L.L.****	**Infectivity*****	**Dilutions**	**No. of L.L.****	**Infectivity**	**Aging (hr)**	**No. of L.L.**	**Infectivity**
Untreated infectious sap*.	**75**	**100**	**Untreated infectious sap.**	**75**	**100**	**Untreated infectious sap.**	**75**	**100**
40	70	93.3	10^{-1}	73	97.3	5	70	93.3
42	60	80.0	10^{-2}	62	82.6	10	50	66.6
44	53	70.7	10^{-3}	50	66.6	15	40	53.3
46	47	62.6	10^{-4}	30	40	20	35	46.6
48	42	56	10^{-5}	21	28	25	30	40
50	35	46.6	10^{-6}	5	6.6	30	20	26.6
52	30	40	10^{-7}	0	0	35	18	24
54	25	33.3	10^{-8}	0	0	40	12	16
56	21	28	10^{-9}	0	0	45	3	4
58	18	24	10^{-10}	0	0	50	0	0
60	13	17.3				55	0	0
62	11	14.6				60	0	0
64	5	6.6				65	0	0
66	0	0				70	0	0
68	0	0				75	0	0
70	0	0				80	0	0
74	0	0				85	0	0

* Infectious sap untreated kept under lab temperature.
** No. of local lesion (L.L) per 100 µl of infectious sap assayed or *Ch. amaranticolor* as indicator plants.
*** % of infectivity was calculated from 5 replicates leaves.

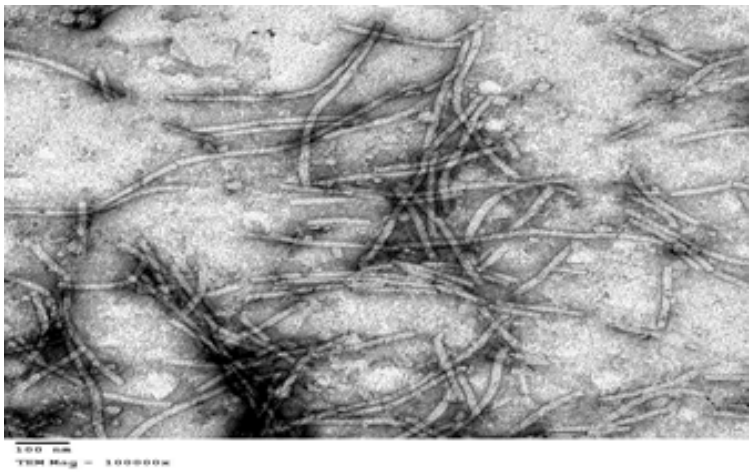


Fig. 4. Electron micrograph showing rod flexible particles (helical symmetry) of PVY isolate negatively stained with uranyl acetate with magnification power 100000x.

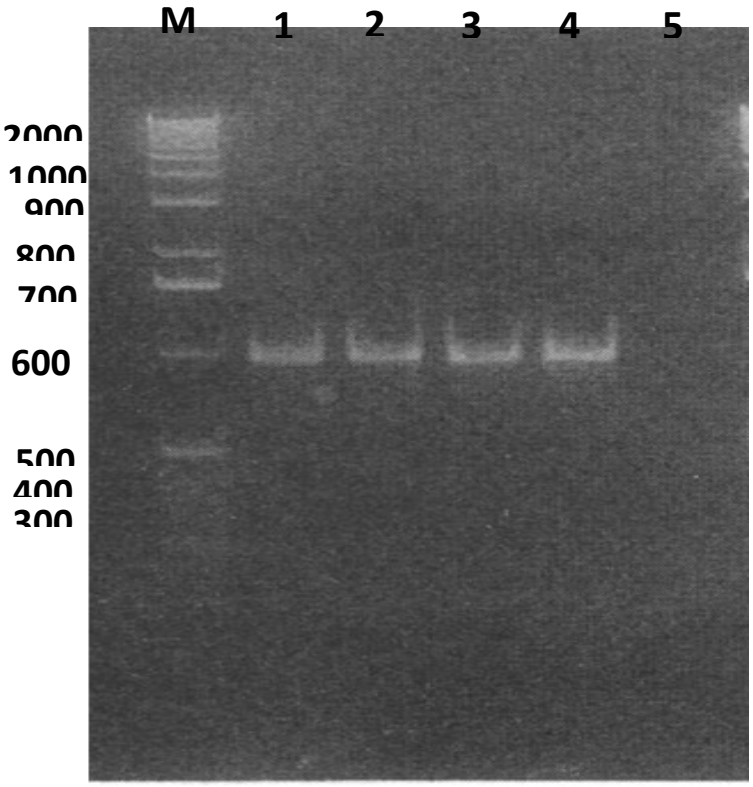


Fig. 5.Electrophoresis agarose gel (1.5%) showing the integrity of total RNA extracted from mechanically infected potato leaves cv. spounta and DNA PCR-products of CP gene of PVY isolate using specific primers.

M: Marker RNA; 1: *N. tabacum* white burly; 2,3,4: Potato plants mechanically infected with PVY from different locations; 5: Potato healthy.

The partial nucleotide sequence of the CP for PVY isolate was aligned with five isolates of PVY (Table 5). A phylogenetic tree of PVY-CP revealed 99% degree of similarity to isolate PVY-2 and 97% to PVY-3. The percentage of nucleotide sequence similarity of PVY-Egyptian isolate and PVY-4, PVY-5 and PVY-6 were 96, 95 and 94%, respectively (Fig. 6).

Nucleotide sequence of CP gene translation:The partial nucleotide sequence of CP gene for PVY isolate was translated into 202 amino acids shown in (Fig. 7).

TABLE 5. Comparison between bases composition of partial nucleotide sequence of the cp gene of the studied pvy isolate with the corresponding sequence of other pvy isolates.

Isolates	MW KDa		A		C		G		T		Similarity
	ssDNA	dsDNA	No	%	No	%	No	%	No	%	
PVY-1	188.92	376.01	172	28.2	106	17.4	141	23.1	191	31.3	
PVY-2	606.53	1205.71	611	31.2	351	17.9	467	23.9	527	26.9	99%
PVY-3	489.87	972.72	493	31.2	275	17.4	394	25	416	26.4	97%
PVY-4	373.65	742.17	371	30.8	209	17.4	297	24.7	327	27.2	96%
PVY-5	330.76	657.72	330	30.9	197	18.5	255	23.9	285	26.7	95%
PVY-2	337.62	742.17	369	30.6	211	17.5	298	24.8	326	27.1	94%

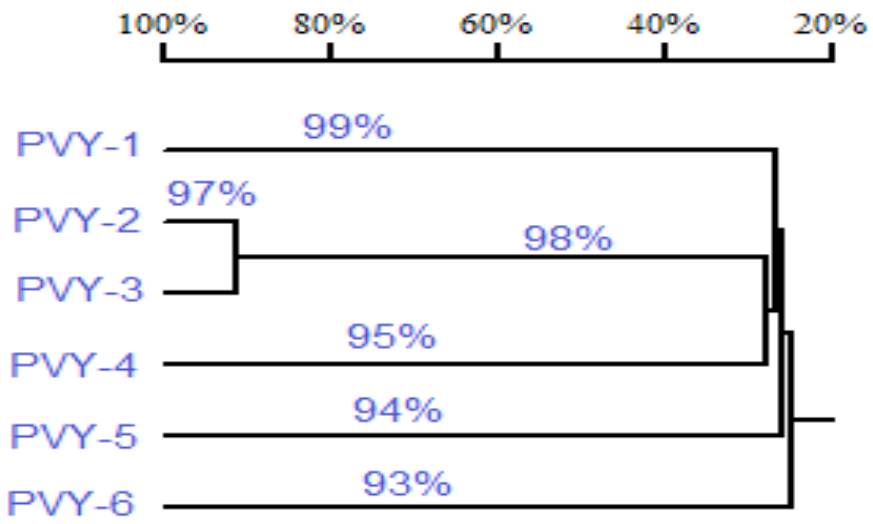


Fig. 6. Phylogenetic tree of PVY isolate based on the nucleotide sequences of the CP gene. The dendrogram displaying the percentage of sequence homology between the PVY/CP and the other five isolates of PVY.

Translation of PVY-EG_Coat_protein(1-202)
Universal code
Total amino acid number: 202

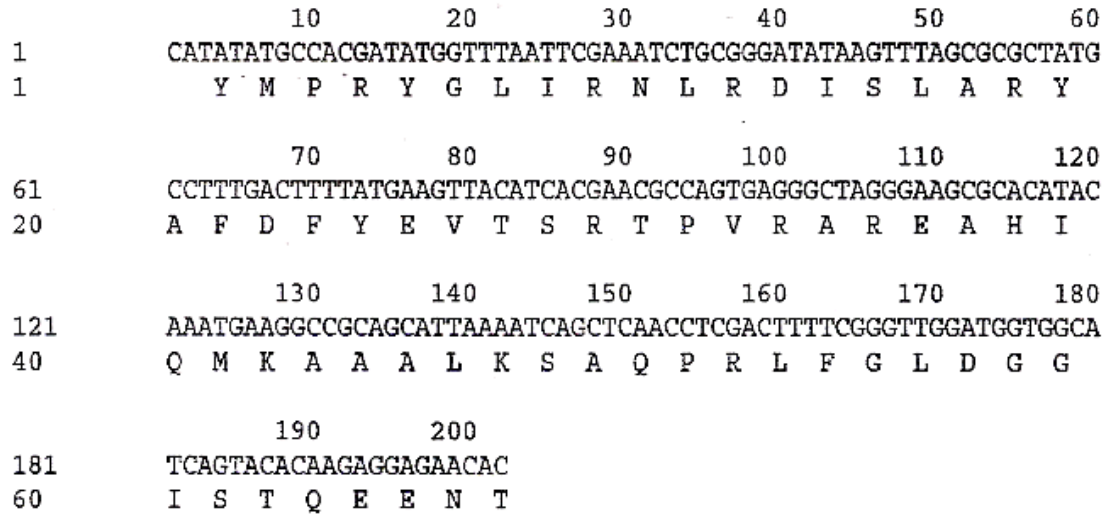


Fig. 7.Translation of partial nucleotide sequence of CP gene for an Egyptian PVY isolate produced 202 amino acids.

Discussion

Visual inspection followed by DAS-ELISA screening indicated that the naturally infected potato plants cvs. spounta were heavily infected with PVY; PVX and PLRV separately and mixed infections with percentage 32%, 16% and 8%, respectively. These results were in agreement with (Mahfouz et al., 2004 and Kandeel, 2014). Detection of PVY was essentially based on distinct potyvirus symptoms (top necrosis, vinal necrosis and severe mosaic on potato leaves) observation followed by DAS-ELISA. This agree with that obtained by Mahfouz (2003) and Nasr El-Din (2007).

The PVY was isolated biologically on *Ch.amaranticolor* and gave small chlorotic lesions without halo. The crude sap containing PVY particles was inoculated on *N. tabacum* cv. white burly. The observed typical external symptoms were leaf crinkle, mottling severe mosaic and stunting meanwhile, Piche et al. (2004) reported that, 58 PVY field isolates transmitted to tobacco, 11 isolates produced a mild mosaic symptom and the remaining 47 field isolates induced varying degrees of leaf necrosis including venial necrosis, severe stunting and leaf death. An Indian PVY^{o}common strain isolated from field-grown potato plants was maintained in *N. glutinosa* in an insect-free green-house (Gawande et al., 2011). The inoculations with single strain of PVY in both potato and tobacco plants results in 100% infection, with every plant inoculated displaying typical symptoms of PVY with mixed infections of PVY strains the success rate of infection varied from 66.6% to 100% (Mallik et al., 2012). High DAS-ELISA readings indicated that high specificity between antibodies and isolated PVY, as well as high virus concentration in infected potato plants. DAS-ELISA has proved to be very efficient for detection and identification of many viruses as reported by Clark & Adams (1977). Because of their sensitivity, specificity and speed, potato tubers cv. spounta was gave DAS- ELISA-positive reaction.

Our results were in accordance with that obtained by many authors (Cojocaru et al., 2009; Alexander et al., 2010; Gwande et al., 2011; Sabir, 2012; and Nerway & Kassim, 2014). The isolated PVY was mechanically transmitted by rubbing and tuber grafting where the virus isolate was parenchyma virus and non-persistent virus. Also the phosphate buffer which releases the virus particles from pigments and organelles of infected plants leading to increasing the virus particles. Mechanical transmission was considered as simple method for transmission of PVY instead of insect transmission. These results were in agreement with Gawande et al. (2011), Sabir (2012) and Nerway & Kassim (2014).

PVY isolate infected some of tested plants of families, Solanaceae, Chenopodiaceae, Fabaceae, Cucurbitaceae, Compositae and Amaranthaceae. The results agreed with Mahfouz, (2003) and Nasr El-Din (2007). PVY isolate showed variation in symptoms and latent period on different tested hosts. The development of the new susceptible

species and the new symptoms to PVY were due to the variation in environmental conditions and the production of new *Potyvirus Y* strains as result of high rate of recombination between species or strains as well as within and a cross genera. PVY purification depends on separation of virus particles from plant constituents. Generally, use of n-butanol in clarification process helps to remove all cell membranes which contain lipid and lipoprotein and their precipitation at low-speed centrifugation with less virus loss. Also, in final purification step including precipitation and differential centrifugation helps to precipitate the virus particles at high-speed centrifugation. The UV-spectrum of the purified virus gave maximal absorption (0.572) at 260 nm, while the absorbency at 280 nm was 0.375. The nucleic acid and protein gave the maximal absorption at 260 and 280 nm, respectively. So, this result indicated a good and successful virus purifying caption. The purity of PVY solution was indicated by the value of A260/A280 ratio which was 1.6. This result showed that the used procedures were successful for separating and purifying PVY from infected potato plant tissues. These results in the range of those obtained by Nerway & Kassim (2014). Dijkstra & de Jager (1998) stated that the value of A260/A280 ratio of elongated viruses is around 1.2 and for those with isometric particles around 1.7. The results showed that the PVY concentration in potato tissues was 1.25 mg virus for each 100 gram of fresh plant tissue. This concentration was calculated by dividing the absorbency at 260 nm (A260) to Extinction coefficient of PVY (2.86). This result indicated the presence of high concentration of the virus particles in diseased plant tissues. TEM revealed the presence of flexible helical symmetry particles with diameter 11 X 570 nm when negatively stained with 2% uranyl acetate pH 7.0. These results were also previously reported by Nerway & Kassim (2014). The spectrometrical tests indicated the good purification of the virus using differential centrifugation of the virus using differential centrifugation, precipitation and filtration techniques. In this concern, the purity of PVY was 0.483 mg per gram of fresh plant tissue was stated by Nerway & Kassim (2014). While, Sharma et al. (2013) reported that, electron microscopy of PVY revealed flexuous filamentous virus particles of 15 X 750 nm.

The detection of inclusion bodies can provide a rapid method for determining the presence of viral infection. The same result was reported by Amer (1999). From the obtained results of biological studies, it is reasonable to conclude that the isolated PVY from open fields in Egypt have some different characters from the other potyvirus strains. Current study indicated that PVY isolate have TIP (66°C) which were differed with Mahfouz (2003) who reported that PVY have TIP 60, 64 and 66°C of PVY-C, PVY-A and PVY-B, respectively and agreed with Nasr El-Din (2007). Also, DEP was (10^{-6}) which agreed with Nasr El- Din, (2007). Meanwhile, LIV was 45 h which disagreed with Mahfouz (2003) who found that PVY have DEP of 10^{-3} and was completely inactivated after 36 h at room temperature (25 to 28°C), however, agreed with Nasr El-Din (2007). The virus stability (TIP, DEP and LIV) as measured by infectivity (often in crude extracts) was an important criterion in attempting to establish group of viruses, control virus distribution and elimination of viruses both *in vitro* and open fields reported by Matthews (1992). The concentration of total RNA extracted from inoculated potato with PVY isolate was 3.2 per 0.5 gm leaves depending on the methods of extraction using guanidine-HCL, sodium phosphate, Tris-HCL and ethanol. The isolated RNA was evaluated before PCR amplification using spectrophotometer at 260 nm which 0.572 running on agarose (1.5%). The total RNA extracted from potato leaves cv. spounta indicating success of total RNA extraction with the high density.

PCR could be used in the diagnosis, detection and identification of viral disease as well as, detection of DNA or RNA pathogens in small samples (Singh & Singh, 1996). The potyvirus genome consists of a positive sense ssRNA molecule of approximately 10 kb which is polyadenylated at 3` end and is translated as single polypeptide encapsidated with one capsid protein (38 KDa).

The coat protein gene of potyviruses is located upstream of the 3` untranslated region and poly (A) tail. The CP gene is the most characterized gene in potyviruses. It is divided into three domains: the amino terminus, the core region, and the carboxy terminus. Both the amino and carboxy termini are variable, however the core region are conserved part in all potyviruses (Urcuqui et al. 2001). Therefore, for successful amplification process, specific DNA primers annealed to core region of coat protein genome was used in the polymerase chain reaction (PCR) as it is the most sensitive and reliable technique until now. So preparation of viral RNA is still a fundamental step in reverse transcription-PCR (RT-PCR) technique especially

when applied in large scale testing as reported by Faggiolli et al. (1998).

The cDNAs were amplified by PCR using the oligonucleotides downstream and upstream primers for PVY- coat protein gene (PVY-CP). The genome of PVY contains partially overlapping open reading frames (OREs) bi-directionally arranged into two transcriptional units that are separated by an intergenic region. Such results indicate that PCR technique as an effective detection tool and greatly facilitate studies of potyvirus epidemiology and etiology. The RT-PCR is an extremely sensitive and specific technique for the detection and determination of genetic diversity. The, size of the PCR products of coat protein gene (CP) amplified from both naturally and artificially infected potato plants was 610 bp of spounta. These results were in agreement with Mahfouz (2003).

Recently, a combination of RT-PCR assays utilizing sequences within the 5′-untranslated region. P1 gene, and known recombination junction sites of the PVY genome has led to the separation of isolates recovered from Europe from those isolated in North America (Piche et al., 2004). A single amino acid substitution within the capsid protein produces a variant PVY strain known as PVY^{o}-O5. This variant does not induce vein necrosis in tobacco or tuber necrosis in susceptible varieties of potato. Furthermore, it is identified by RT-PCR based diagnostics at PVY^{o} and it has a typical PVY^{o} genome sequence **(**Alexander et al., 2010). El-Absawy et al. (2012) collected different potato plants from an experimental station in Giza Governorate, Egypt, and these plants were tested using RT-PCR. PVY was amplified using primers represented portion of the coat protein (CP) gene and 3′untranslated regions (UTR). Phylogenetic tree showed two main strain groups: Group I, regroups PVY^{N} and PVY^{NTN} strains, while group II includes PVY^{o}, PVY^{w} and $PVY^{N:0}$ strains.

The Egyptian PVY isolate was clearly classified within group I and was more closely related to PVY^{NTN} strains. Ten nucleotide substitutions resulted in 3 conserved amino acid substitutions resulted in 3 conserved amino acid substitutions (V_1® I, G_7® E, M or V and S_8® G) and were able to differentiate between both groups. The partial coat protein region was more diverse than that of the 3′UTR (92.6-100% and 97.7-100% identity, respectively). The 3′UTR of the Egyptian isolate showed RNA secondary structures different from those of the 5 PVY strains. Sharma et al. (2013) studied the characterization of PVY on the basis of biological, serological and partial nucleotide sequence properties from different locations of Rajasthan.

A phylogenetic tree of PVY-CP revealed 99% degree of similarity to isolate PVY-1, 98% similarity to PVY-2, 97% similarity to PVY-3. The percentage of nucleotide sequence similarity of PVY-Egyptian isolate and PVY-4, PVY-5 and PVY-6 were 95, 94, and 93%, respectively.

References

Ahmad, A.Y. (2005) Studies on some viral diseases infecting potatoes. *M.Sc. Thesis,* Faculty of Agriculture, Ain Shams University, 120 pp.

Alexander, V., Olga, K. and Nikolaeva, V. (2010) Serological properties of ordinary and necrotic isolates of Potato virusY: a case study of PVYN Misidentification. *American Journal of Potato Research,* **87,** 1-9.

Al-Nagar, A.M. (2007) Studies on some plant virus diseases of some vegetable crops. *M.Sc. Thesis,* Faculty of Agriculture, Al-Azhar University, 165 pp.

Alyokhin, A., Sewell, G. and Groden, E. (2002) Aphid abundance and Potato virus Y transmission in imidacloprid-treated potatoes. *American Journal of Potato Research,* **79**, 255-262.

Amer, A.A. (1999) Comparative studies on the diagnostic methods for Potato virus Y disease. *M.Sc. Thesis,* Department of Plant Pathology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, 153 pp.

Clark, M.F. and Adams, A.N. (1977) Characteristics of the microplate method for enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology,* **34,** 475-783.

Cojocaru, N., Carmen, L. and Mihaela, D. (2009) Potato virus Y. purification and achievement of antisera for identification of infected plants by ELISA. *Analele I.C.P.C. Brasov*, **XX**, 18-25.

Dijkstra, J. and de Jager,C.P. (1998) "*Practical Plant Virology*". Berlin; Heidelberg; New York: Springer. 459 pp.

El-Absawy, E.A., Mahmoud, Amal, Hemeida, A.A. and Helmy, M. (2012) Molecular variation of Potato virus Y Isolated from Egypt. *International Journal of Virology*, **8,** 81-89.

Eraky, A., Abdel-Salam, S., Fahmy, F. G. and Abdel-Haq, O (2014) Molecular identification of viruses responsible for severe symptoms on potato

(*Solanum* sp) growing in Assiut Governorate (Upper Egypt). *Journal of Biotechnology and Biomaterials,* **3**, 5.

Faggiolli, F., Pasquini, G. and Barba, M. (1998) Comparison of different methods of RNA isolation for Plum pox virus detection by reverse transcription, polymearase chain reaction, *Acta-Virologica,* **42**, 219-221.

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. (2005) Virus Taxonomy. *Eighth Report of the International Committee on Taxonomy of Viruses*. Elsevier Academic Press, San Diego, California, USA.

Gawande, S.J., Shukla, A., Chimote, V.P., Kaushal, N., Kaundal, P., Garg, I.D. and Chimote, K.P. (2011) Development of PCR-based techniques for the detection of immobilised Potato virus Y virions. *Journal of Plant Pathology,* **93**(1), 127-132.

Hadidi, A., Montasser, M.S., Levy, L., Goth, R.W., Converse, R.H., Madkour, M.A. and Skrzeckowski (1993) Detection of Potato leaf roll and Strawberry mild yellow edge luteoviruses by reverse transcription polymerase chain reaction. *Plant Disease,* **77,** 595-601.

Hossain, M. and Ali, M.S. (1992) Effect of Potato virus Y severities on virus concentration, dilution end point and potato yield. *Bangladesh Journal of Plant Pathology,* **8**, 27-29.

Jordan, B.M. and Baker, J.R. (1955) A simple pyronin methyl green technique. *Quari. S. Microscope. Sci.* **96,**177.

Kandeel, A.A. (2014) Molecular studies on production of potato minitubers virus-free. *Ph.D. Thesis*. Faculty of Science, Benha University., Egypt, 147 pp.

Mahfouz, S. (2003) Diagnosis of some plant viruses using modern techniques. *M.Sc. Thesis,* Faculty of Agriculture, Ain Shams University, 190 pp.

Mahfouz, S.A. Allam, E.K., Afifi, A.M. and El-Dougdoug, Kh.A. (2004) Isolation of different isolates of PVX, PVY and PVS from naturally infected potato plants. *The Ninth Conference of Agriculture Development Researches,* 22-24 March, Cairo, Egypt.

Mallik, I., Nolan, R., Anderson, Niel, C. and Gudmestad (2012) Detection and differentiation of Potato virus Y strains from potato using immunocapture multiplex RT-PCR. *American Journal of Potato Research,* **89,** 184-191.

Matthews, R.E.F. (1992) "*Fundamentals of Plant Virology*", Academic Press, New York, London, pp.402.

Nasr El-Din, M.A. (2007) Biological and molecular studies on Potato virus Y (PVY) *M.Sc. Thesis,* Faculty of Science, Benha University, 200 pp.

Nerway, Z.A.A. and Kassim, N.A. (2014) Potato Virus Y (PVY) purification and antiserum preparation. *Journal of Agriculture and Veterinary Science*, **7**(4), 9-12.

Noordam, D. (1973) "*Identification of Plant Viruses-Methods and Experiments*". Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands. pp. 207.

Piche, L.M., Singh, R.P., Nie. X. and Gudmestad, N.C. (2004) Diversity among Potato Virus Y isolates obtained from potatoes grown in the united states. *The American Phytopathologial Society*, **94**(12), 1368-1375.

Rupar, M, Ravnikar, M., Tušek-Žnidarič, M., Kramberger, P., Glaisc, L. and Gutiérrez-Aguirre, I. (2013) Fast purification of the filamentous Potato Virus Y using monolithic chromatographic supports. *Journal of Chromatography A,* **1272,** 33– 40.

Rykbost, K.A., Hane, D.C. and Hamm, P.B. (1999) Effects of seed borne Potato Virus Y on Russet Norkotah performance. *American Journal of Potato Research*, **76,** 91-96.

Sabir, J.S.M. (2012) Identification of six Potato Virus Y isolates from Saudi Arabia. *African Journal of Biotechnology,* **11**(41), 9709-9715.

Sharma, P., Sahu, A.K., Verma, R.K., Mishra, R. and Gaur, R.K. (2013) Biological and molecular characterization of Potato VirusY infecting potato (*Solanum tuberosum*) in India. *Asian Journal of Biological Sciences,* **6**(5), 257-264.

Sheikh, M., Ashraf, M. and Mahmood, I. (2012) Biological and molecular detection of a virus infecting *Wedelia trilobata* (Linno) Hitchc (A Medicinal herb). *Journal of Plant Pathology and Microbiology,* **3**(2), 1-4.

Shukla, D.D., Ward, C.W. and Brunt, A.A. (1994) "*The Potyviridae*". CAB. International, Wallingford, England, 516 pp; 102.

Singh, M. and Singh, R.P. (1996) Nucleotide sequence and genome organization of a Canadian isolate of the common strain of Potato virus Y (PVYO). *Canadian Journal of Plant Pathology,* **18,** 209-224.

Sudarsono, S.L.Z., Woloshuk, Z., Xiong, G.M. and Hillmann, E.A. (1993) Protein cistrons from six-Potato virus Y isolates infecting tobacco. *Archives of Virology,* **132,** 161-170.

Urcuqui-Inchima, S. Haenni, A.L. and Bernardi, F. (2001) Potyvirus proteins; A wealth of functions. *Virus Research,* **74,** 157-175.

Visser, J.C., Bellstedt, D.U. and Pirie, M.D. (2012) The Recent Recombinant Evolution of a Major Crop Pathogen, Potato virus Y. *PLoS ONE*. **7,** e50631.

Walkey, D.G.A. (1985) "*Applied Plant Virology*" Wiley-Interscience Publications, New York, USA, pp.93-102.

(Received 25 / 9 /2017; *accepted* 21/ 12 /2017)

الصفات الفسيولوجية والمعدية لفيروس واى البطاطس العزلة المصرية

سهام عبدالشافى، محمد فاروق غالى، خالد الدوجدوج* و محمد طه
قسم النبات والميكروبيولوجى- كلية العلوم- جامعة الزقازيق - الزقازيق و *قسم الميكروبيولوجى- كلية الزراعة- جامعة عين شمس- القاهرة- مصر.

إن فيروس واى البطاطس يعتبر من اهم الفيروسات الممرضة المدمرة التى تنتقل بواسطة المن لنباتات البطاطس على مستوى العالم. عدوى فيروس واى البطاطس تسبب حوالى 80% انخفاض فى انتاج البطاطس العالمى.

أن الهدف من هذه الدراسة هو توصيف فيروس واى البطاطس (العزلة المصرية) المعدى لنباتات البطاطس معتمدا على الصفات البيولوجية و السيرولوجية و الجزيئية. أن نباتات البطاطس المصابة طبيعيا بفيروس واى البطاطس اعطت نتيجة ايجابية مع الإجسام المضادة المتخصصة Polyclonal antibodies لتعريف فيروس واى البطاطس DAS-ELISA لفيروس واى البطاطس

يحتفظ فيروس واى البطاطس بقدرته على احداث اصابة عند حفظه فى العصير الخام على درجة حرارة المعمل لمدة 45 ساعة و 66°م (TIP) ونقطة التخفيف النهائية هى 10^{-6}.

بفحص خلايا البشرة فى اوراق نبات الداتورا ميتيل المصابة بفيروس واى البطاطس تم الكشف عن الاجسام المحتواه الامورفية والاجسام المحتواه البلورية بعد 12 يوم من حقن الفيروس. تم تقييم فيروس واى البطاطس المنقى باستخدام الاشعة فوق البنفسجية عند 235 نـ و 275 نـ . وكان تركيز الفيروس 1.25 مجم/100 جرام انسجة اوراق مصابة. وكانت نسبة 260/280 اعلى من الواحد (1.6). وبالفحص تحت الميكرسكوب الالكترونى النافذ كانت جزيئات الفيروس عصوية طويلة مرنة لها القدرة على احداث اصابة بابعاد 11X570 نانوميتر. مع وجود صفات واضحة تتمثل فى 1024 :1 مقياس للاجسام المضادة.

تم تكبير cDNA باستخدام PCR 1 وباستخدام بادئ متخصص للغطاء البروتينى تم الحصول على 610 زوج من القواعد النيتروجينية.

تطابق التتابع النيوكليتيدى اظهر ان عزلة فيروس واى البطاطس اوضحت نسبة تطابق 99% -93 مع عزلات فيروسات واى البطاطس العالمية الأخرى.