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The relationship and relativity between three isolates of *Potato virus Y Potyvirus* infecting potato (*Solanum tuberosum* L.) at Alexandria and El-Beheira governorates, northern Egypt

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Abstract

Potato virus Y (PVY) is a highly pathogenic virus, causing enormous economic losses in potato (*S. tuberosum*) crop. Three isolates of PVY were obtained from naturally infected potato plants showing mosaic; yellowing and vein necrosis symptoms, during 2017-2018 growing seasons at certain locations of El-Beheira and Alexandria governorates, Egypt. PVY could be easily transmitted mechanically by aphids. Detection of the PVY-3 in different organs of infected *Nicotiana glutinosa* plants by Indirect-ELISA; Dot blot immunoassay (DBIA) and Tissue blot immunoassay (TBIA), indicated the possibility of using these methods for viral detection. Egyptian PVY (MK376452) isolate was of close homology to PVY isolated from South Africa. The other Egyptian isolates were found to be close to a French PVY (KJ741115) isolate. There were variations on comparing nucleotide and amino acid sequences; however, nucleotide sequencing could be more reliable. Application of sequence inspection allowed us to identify the PVY isolates by phylogenetic analysis. Phylogenetic analysis of the genomic coat protein (CP) from 24 PVY isolates registered in GenBank indicated the presence of relationships between each other's. This reflected the high degree of genetic variability among our local Egyptian isolates. The aims of the current work were to; isolate and detect PVY from naturally infected potato plants in northern Egypt, characterize the PVY isolates using different assays, detect PVY in different organs of infected potato plants, study the CP gene of the PVY isolates using Reverse transcription-Polymerase Chain Reaction (RT-PCR), and register these isolates in GenBank.

Keywords: *Potato virus Y potyvirus*, Potato, Indirect ELISA, TBIA, DBIA, RT-PCR

1. Introduction

PVY is one of the most destructive plant pathogens all over the world. It caused a lot of damage in many economically important crops such as; potato, tomato, tobacco and pepper. In addition; PVY is a

member of the Genus *Potyvirus* that had been classified within members of the potyviridae family, which is the second largest plant virus family (Ivanov *et al*., 2014). PVY represented one of the highest threats to potato production worldwide, and it reduced crop yields up to 90% (Ivanov *et al*., 2014). Likewise, it has long been recognized as a threat to potato cultivation in Egypt (El-Borollosy, 2015; Abdalla *et al*., 2018). PVY diagnosis was based on serological testing such as indirect ELISA, which used specific polyclonal antiserum to this virus (Hamza *et al*., 2018). Serological diagnosis was confirmed by RT-PCR, using specific primers for coat protein gene of the virus. Analysis of PCR products on agarose gel electrophoresis revealed amplification of specific bands detected in the concerned virus (Shalaby *et al*., 2002). Viruses as causal agents of plant diseases can have significant and devastating impacts on many cultivated crops worldwide. Potato belongs to the Solanaceae family. Haase, (2007) reported that potato is the most important species of this family for the global diet, and is one of the most consumed crops worldwide. According to the last estimates from Food and Agriculture Organization of the United Nations (FAO. 2014); the area cultivated with potato in Egypt was about 409,535 feddans (172,005 hectares), and total production of this crop was 4,611,065 tons with an average of 11.26 tons/ feddan (26.81 tons/ hectare). Currently, Egypt is ranked among the world's top potato exporters. In 2014, potato exports were about 679 thousand tons for different international markets such as; Russia, England, Western European countries, and some Arab countries (Samy *et al*., 2016). The most cited virus which could affect potato crop all over the world is *potato virus Y* (PVY). The objectives of the current study were to detect and isolate PVY from infected potato plants in Alexandria and El-Beheira governorates, to characterize these PVY isolates using host range; symptomology, mode of transmission, and serological studies including; Indirect ELISA, TBIA, DBIA, to detect PVY in different organs and at different periods of infection of potato plants, to study these PVY isolates using a molecular techniques as RT-PCR, and to register these isolates in the GenBank.

2. Material and methods

2.1. Isolation of PVY from infected potato leaves

Leaf samples from infected potato plants exhibiting mosaic and vein necrosis symptoms were separately collected in plastic bags from plants grown at certain locations of Alexandria and El-Beheira governorates, during the growing seasons 2017 - 2018. Inoculum was prepared by grinding infected potato leaf tissues in a mortar and pestle with a small amount of 0.1 M phosphate buffer (pH 7). Leaves of healthy *N. glutinosa* plants in seedling stage were first dusted with carborundum (600 meshes), and then inoculated with a freshly prepared viral inoculum using forefinger method as described by Abd El-Aziz and Younes, (2019). Inoculated plants were shortly rinsed with tap, and then kept in an insect proof greenhouse for symptoms development. The isolated virus was maintained in *N. glutinosa* plant leaves for virus propagation, and served as a source of the virus for subsequent studies.

2.2. Characterization of the prevalent PVY-3 isolate

Characterization of the prevalent PVY-3 isolate was based mainly on; Diagnostic host and symptomology, Modes of virus transmission, Serological assays, and Reverse transcription-Polymerase Chain Reaction (RT-PCR).

2.2.1. Diagnostic hosts and symptomology

Several diagnostic hosts were tested including; *N. glutinosa*, *N. repanda*, *N. rustica*, *Solanum nigrum*, *Datura metal*, *Gomphrena globosa*, and *Chenopodium amaranticolor*. Five seedlings of each tested plant species were mechanically inoculated with PVY-3, and then kept under greenhouse conditions. Plants were observed daily for 4 weeks for symptoms expression. Inoculated plants which did not show any

symptoms were checked for latent infection, by back-inoculation to the indicator host *N. glutinosa*.

2.2.2. Modes of transmission

PVY-3 isolate was studied for its transmissibility by different methods such as;

2.2.2.1. Mechanical transmission

N. glutinosa plants were used both as a virus source and as an assay host. *N. glutinosa* leaves showing typical symptoms of infection by PVY-3 were ground in 0.1 M phosphate buffer 1:10 (w/v) pH 7.0., using a mortar and pestle. Healthy leaves of *N. glutinosa* plants were first lightly dusted with carborandum (600 mesh), and then rubbed with forefinger previously soaked in the freshly prepared viral inoculum.

2.2.2.2. Aphid transmission

According to Hamza *et al*., (2018), two species of aphids namely; *Aphis nerii* (Boyer) and *Aphis faba* were tested for their ability to transmit PVY-3. Apterous forms of aphids were starved for one hour, and then allowed to feed on PVY-3 infected *N. glutinosa* leaves for 3-5 min. before being transferred to 12 healthy *N. glutinosa* seedlings. They were applied at the rate of 10 aphids/ plant, and then left for 5 min. as a feeding period. These aphids were finally killed with an aphidicide, Malathion (0.1%). Plants were kept under insect proof cages, and observed carefully for symptoms development.

2.3. Serological Diagnosis

2.3.1. Source of antisera

Antisera to Alfalfa Mosaic *Alfamovirus* (AMV), *Potato Virus Y Potyvirus* (PVY), Potato Leaf Roll Virus (PLRV) *Polerovirus*, and Potato virus X *Potexvirus* (PVX) were supplied by Bioreba (Switzerland) prepared according to Hamza *et al*., (2018). Serial dilutions of plant sap extracted from leaves, stems and roots of infected potato plants with PVY-3 were made to determine the serological sensitivity to; indirect ELISA, Dot blot immunoassay (DBIA), and Tissue blot immunoassay (TBIA).

2.3.2. Indirect ELISA

Extracts from infected and healthy plants were used. The ELISA values measured by Sunrise ELISA plat reader; were expressed as absorbance at 405 nm. Absorbance values (Optical Density) of at least double that of healthy control, were considered positive. In each set of test, wells lacking antigen (coating buffer only) were included as blanks (Hamza *et al*., 2018).

2.3.3. Dot blot immunoassay (DBIA)

DBIA was carried out according to Abd El-Aziz and Younes, (2019). A grid consisting of 1 cm^2 was drawn on nitrocellulose membrane sheet (NCM of 0.45 nm, BIO-Rod Laboratories, Richmond, CA) with a pencil. This sheet was then cut to a size that would accommodate all the number of samples in each test. Dilutions of the extracted cell sap of healthy and infected stem and root of *N. glutinosa* plants were prepared as; 1:10, 1:100, 1:1000, 1:10000 and 1:100000, in carbonate buffer. The NCM was dipped in 0.05 carbonate buffer (pH 9.6), and then placed on a filter paper for 5 min. to dry. Four µl of each sample was spotted on the NCM in the center of each grid square and then left to dry for 5 min. The membrane was placed in a Petri dish containing 10 ml blocking solution (2% bovine serum albumin in carbonate buffer, pH 9.6), and then gently agitated for 1 h (40 rpm). The membrane was removed from the blocking solution with forceps, dipped in dist. water and then transferred to another glass Petri dish containing 10 ml of virus antiserum diluted to 1:500 in phosphate buffer saline tween 20 (PBST). NCM was removed from the first antibody solution, dipped in dist. water, and then washed twice by agitation for 10 min. in phosphate buffer + tween 20. It was transferred to 1:1000 dilution of goat anti-rabbit IgG conjugate to alkaline phosphatase in PBST, and then gently agitated for 1 h. Finally; the membrane was removed from the second antibody dilution, dipped in dist. water and then washed twice by agitation for 10 min. each in PBST.

The 5-bromo-4- chloro- 3- indolyl 1 phosphate (BCIP) and nitro blue tetrazolium (NBT) substrate solutions were made during the final washing in which membrane was incubated for color development. After color development, the reaction was stopped by washing the treated membrane in 0.01 M of phosphate buffer containing 0.05 M EDTA (pH 7.0). The positive reaction of DBIA was indicated by the development of purple color on the blots; whereas, the negative reaction did not develop a color.

2.3.4. Tissue blot immunoassay (TBIA)

Tissues of rolled leaves, stems and roots of healthy and infected *N. glutinosa* plants were cut with a razor blades in a steady motion to obtain a single plane cut surface. Exposed cut edges were pressed onto NCM (0.45 nm, BIO-Rod Laboratories, Richmond, CA), that were cut to a size that would accommodate the number of samples in each individual test as described by Lin *et al*. (1990); Makkouk and Kumari, (1996); Abd El-Aziz and Younes, (2019). Treated membranes were then placed in a glass Petri dish containing 10 ml blocking buffer (2% Bovine serum albumin (BSA), in phosphate buffer saline (PBS)(pH 7.0), and then the previous methodology of DBIA was followed till color development.

2.4. Molecular studies of PVY isolates

Viral isolates PVY 1, 2 and 3 were coded in this section as; PVY-K1, K2 and K3, respectively.

2.4.1. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from fresh leaves of *N. glutinosa* samples, where H_2O was used as a negative control. In RT-PCR assay, a sample which yielded positive results in previous ELISA test was used as positive control. The Quick RNA Mini-Prep kit (Enzomyics, Korea) was used to extract the total RNA from the infected plants. Extraction was carried out as described by the manufacturer.

A total of three PVY-inoculated *N. glutinosa* plants; one plant from each sampling site, were randomly selected. The RT-PCR was carried out using two steps RT-PCR Kit (Enzomyics, Korea, Inc.). Reverse transcription was carried out in a 50 µl reaction mixture containing, 21µl H_2O, 25 µl 2 × 1 Prime Script RT-PCR buffer, 2 µl Prime Script 1 step enzyme mix, and 2 µl of 20 ml mol primers. Oligonucleotide primer sequences reported by Shalaby *et al*., (2002) from the conserved region of the coding sequences of CP of PVY, were used to detect the presence of the PVY isolates. Primer I: 5' TCAAGGATCCGCAAATGACACAATTGATGCAG G 3'; Primer II: 5' AGAGAGAATTCATCACATGTTCTTGACTCC 3'. The amplified fragment length was of 801 bp. The primer set were synthesized by Macrogen, Korea, Inc. Thermocycling was carried out as follows; 50ºC for 30 min., 94ºC for 2 min., then 30 cycles at 94ºC for 30 sec, 55ºC for 30 sec, and at 72ºC for 1 min., followed by 72ºC for 3 min. PCR products were separated on 1% agarose gel in Tris-acetate EDTA (TAE) buffer by electrophoresis, pre-stained with Red Safe solution (Intrbion, Korea). An image was captured after exposing the red safe stained gel on a transilluminator with a digital camera. DNA markers (100 bp DNA ladder, Fermentas) were used in each electrophoretic run.

2.4.2. DNA sequencing for the amplified gene

The purified PCR product was subjected to DNA sequencing using a forward primer in the sequence reaction. Sequencing was performed using Big Dye® Terminator v3.1 Cycle Sequencing kit (Macrogen, Seoul, Korea) in reference to Thompson *et al*., (1994). Bootstrap neighbor joining tree was generated using MEGA version 3.1 from CLUSTALW alignment (Kumar *et al*., 2004), and compared with sequences in the GenBank. Database was achieved in BLASTN searches at the National Centre for Biotechnology Information site (http: //ncbi.nlm.nih.gov).

2.5. Phylogentic studies

The obtained DNA nucleotide sequences were analyzed using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), for confirming the identity of the recovered sequences. Multiple sequences alignment of the current sequences and the other published ones were carried out using ClustalW (1.83), according to Thompson *et al*., (1994). The amino acid sequences were used for comparison using MEGA 6 according to Tamura *et al*., (2013), whereas phylogeny was tested using bootstrap method with 2,000 replications. The phylogenetic tree was analyzed and generated based on UPGMA statistical method.

3. Results

3.1. Detection of some viruses infecting potato

Three PVY isolates were recovered from leaf samples of infected potato plants exhibiting mosaic and vein necrosis symptoms. Two isolates of PVY were isolated from El-Behaira governorate (Abo Homos reigion (PVY-1) and El Nobaria regions (PVY-2); whereas, one isolate was isolated from the farm of Faculty of Agriculture-Saba Basha, Abis, Alexandria governorate (PVY-3).

3.2. Biological studies

3.2.1. Diagnostic hosts and symptomology

Leaf samples from *S. tuberosum* exhibiting mosaic and vein necrosis symptoms were observed in Al Nubaria region and El-Behaira governorate (Fig.1a). Diagnostic hosts showed symptoms similar to those produced by PVY. The PVY virus induced mild mosaic symptoms on *S. nigrum* (Fig. 1b), on *N. glutinosa* (Fig. 2a, b, and c), mosaic and leaf deformation on *Datura metal* (Fig. 2d), chlorotic mosaic and blasters on *N. repanada* (Fig. 2e), and chlorotic local lesions without systemic spread on *C. amaranticolor* (Fig. 2f). No symptoms were observed and no virus was recovered from *Datura stramonium* and *N. rustica* plants.

Fig. 1a: Mosaic symptoms on potato plants naturally infected with PVY; **1b**): Mild mosaic symptoms caused by PVY on *S. nigrum*

Fig. 2a: Mosaic symptoms induced on *N. glutinosa* leaves with PVY isolate 1; **2b:** Mosaic symptoms induced on *N. glutinosa* leaves with PVY isolate 2; **2c:** Mosaic symptoms induced on *N. glutinosa* leaves with PVY isolate 3; **2d:** PVY caused mosaic and leaf deformations on *Darura metal*; **2e:** Chlorotic mosaic and blasters on *N. repanada* leaves infested by PVY; **2f:** Chlorotic local lesions without systemic spread on *C. amaranticolor* induced by PVY isolates.

3.2.2. Modes of transmission

3.2.2.1. Mechanical transmission

Potato virus Y (PVY) was easily transmitted mechanically (100%) using 0.1 M phosphate buffer (pH 7.0) on *N. glutinosa* plants.

3.2.2.2. Aphid transmission

PVY-3 was transmitted non-persistently by two species of aphids namely; *Aphis faba* and *Aphis nerii*, with average transmission rate of 66.6% and 33.3%; respectively, when 10 viruliferous aphids were used in each test. Plant data is clear in Table (1).

Table 1: Percentage and transmission rate of PVY by Aphid spp.

***Aphis* spp.**	**Transmission**	
	Rate*	**%**
A. faba	8/12	**66.6**
A. nerii	**4/12**	**33.3**

*Number of infected plants/ no. of tested plants, 10 aphids were used per plant

3.3. Serological detection

3.3.1. Indirect ELISA

Serological detection by indirect ELISA using antisera of four viruses namely; Alfalfa mosaic *Alfamovirus* (AMV), *Potato virus Y Potyvirus* (PVY), Potato leaf roll virus (PLRV) *Polerovirus*, and *Potato virus X Potexvirus* (PVX), revealed that PVY was the only detected virus by this technique in the tested *N. glutinosa* leaf samples, as no reaction was observed with AMV, PLRV, and PVX antisera. Highest absorbance values (OD) were observed by PVY-3 at 405 nm with indirect ELISA (Table 2). Results showed that at antiserum dilution of 1: 500; PVY-3 could be detected by indirect ELISA in extracted plant sap diluted up to 1: 10 in roots; 1: 103 in stems; and 1:104 in leaves of infested *N. glutinosa* plants as shown in Table (3).

Table 2: Reaction of infected potato leaf extracts (containing the three PVY isolates) against antisera of PVY, AMV, PLRV and PVX, determined through measuring the OD (at 405 nm) by indirect ELISA

Potato isolates	**Absorbance (OD) at 405 nm**			
	PVY	**AMV**	**PLRV**	**PVX**
Isolate 1	1.087	0.063	197	0.094
Isolate 2	1.178	0.071	235	0.102
Isolate 3	1.569	0.053	0.204	0.083
Healthy	0.195	0.069	0.174	0.087

Table 3: Absorbance values (OD) at 405 nm for serial dilutions of PVY-3, detected from different organs of infested *N. glutinosa* plant sap by indirect ELISA

Sap extract dilution	**Absorbance (OD) at 405 nm**					
	Leaves		**Stem**		**Root**	
	Infected	Healthy	Infected	Healthy	Infected	Healthy
1:10	1.531	439	893	423	592	271
1:100	893	380	642	310	413	245
1:1000	621	267	464	221	290	233
1:10000	398	177	333	189	289	208
1:100000	330	169	294	165	270	163

3.3.2. Dot blot immunoassay (DBIA)

DIBA could detect PVY-3 in infected sap diluted up to 1: 105 in leaves; 1:103 in stems; and 1: 102 in roots, using antiserum dilution of 1: 500.

3.3.3. Tissue blot immunoassay (TBIA)

PVY-3 was easily detected by TBIA from infected leaves, stems and roots of *N. glutinosa* (Fig. 3). The presence of PVY-3 on NCM of infected tissue was detected by the development of purple color. Conversely; the control healthy leaves, stems and roots did not develop such color change.

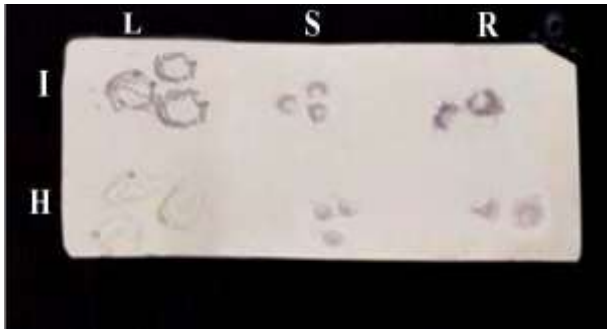

Fig. 3: TBIA assay for detection of PVY-3 in infected (I) and healthy (H); leaves (L), stems (S) and roots (R), of *N. glutinosa* plants on NCM

3.4. Molecular characteristics

3.4.1. RT-PCR studies

In order to determine the presence of PVY in infested plant samples, RT-PCR studies were carried out. In three potato leaf samples which caused systemic infections of *N. glutinosa* plants, expected bands of approximate size about 801 bp were observed (Fig. 4).

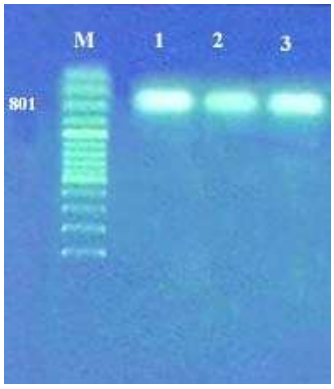

Fig. 4: Agarose gel electrophoresis (1.5%) in TAE buffer pre-stained with Red Safe. RT-PCR amplification of PVY isolates CP gene showed characteristic bands at 801 bp. Where; Lane M: DNA marker 100 bp ladder; Lanes (1, 2 and 3) represent PVY-K1, K2 and K3 isolates, showing characteristic bands at 801 bp.

3.4.2. Nucleotide sequence analysis of CP gene

The CP gene of PVY isolates amplified from potato leaves was evaluated and sequenced. The annotated sequences were deposited in GenBank under accession numbers; MK376452, MK376453, 52MK376454 for isolates: PVY-K1, PVY-K2 and PVY-K3, respectively.

3.4.3. Alignment and phylogenetic analysis of CP gene

Multiple alignment of CP gene sequence of the Egyptian PVY revealed that it had significant alignment with the same gene of the other PVY isolates, and had also conserved regions as clear in Fig. (5). In addition, the multiple alignments of Egyptian PVY-2 and PVY-3 CP gene DNA sequences were highly similar to the French isolate PVY (KJ741115) CP gene (Fig. 5). Similarly, multiple alignment of Egyptian PVY-CP gene deduced amino acid sequences had high similarity with the Colombian

CP gene of PVY isolates (KY711359, MF176821 and KT336551), as shown in Fig. (6).

The neighbor-joining distance analysis with maximum sequence difference of 1 and the topology, yielded two distinct lineages based on the DNA sequence of the CP gene of the Egyptian PVY, and the CP genes selected from different PVY viral isolates available in the GenBank Fig. (6). The neighbor-joining distance analysis based on the deduced amino acid sequences for the Egyptian CP gene and the 21 CP genes of PVY isolates available in the GenBank, was depicted in Fig. (6). Phylogenetic analysis indicated that the current PVY isolates were closely related to the French isolate (KJ741115), with identity of 100%. Likewise, comparison of amino acid sequences revealed that the CP gene of the Egyptian PVY shared 93-100% sequence identity with other PVY isolates recorded in GenBank.

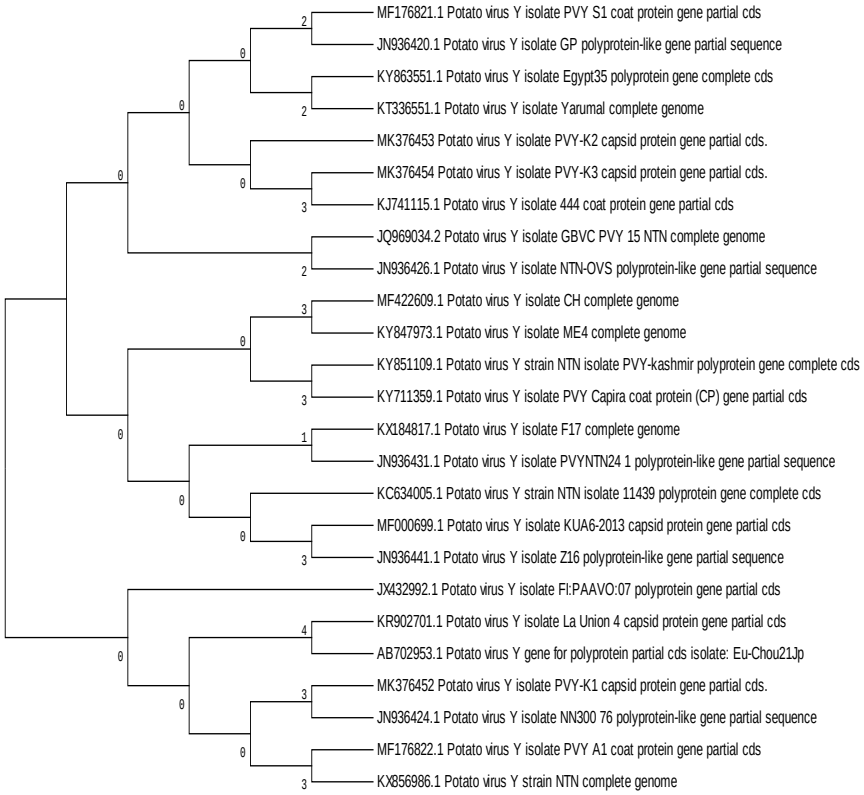

Fig. 5: Cluster dendrogram based on the DNA nucleotide sequence of the partial viral CP gene of the Egyptian PVY isolates, and the CP genes selected from different PVY viral isolates available in GenBank. The phylogeny was tested using bootstrap method with 2,000 replications, and generated based on UPGMA statistical method

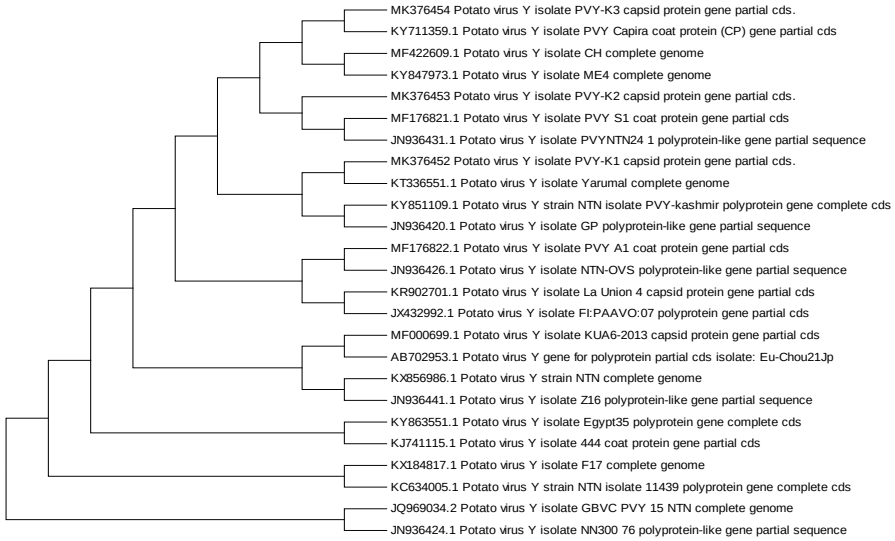

Fig. 6: Cluster dendrogram based on the deduced amino acid sequence of the partial viral CP gene of the Egyptian PVY isolates, and the CP genes available in the GenBank. The phylogeny was tested using bootstrap method with 2,000 replications, and generated based on UPGMA statistical method

4. Discussion

Changes in the agricultural landscape; crop management, crop intensification and climatic changes favor the emergence of infectious plant diseases (Fargette *et al*. 2006). Potato is considered as one of the most economically important solanaceous crops cultivated in different regions of Egypt. Under field conditions, potato plants are subjected to attack by many viruses. Karasev and Gray, (2013) reported that the most cited virus which could affect potato production in the world is *Potato virus Y*. In Egypt, PVY has long been recognized as a threat to potato cultivation as pointed by El-Borollosy, (2015); Abdalla *et al*., (2018).

Current results showed that PVY is one of the most frequently detected viruses in Alexandria and El-Beheira governorates, Egypt. Results of serological diagnosis using indirect ELISA revealed the presence of *Potato virus Y* with different frequencies during the growing seasons 2017, 2018 in naturally infected potato leaf samples, collected from different regions of Alexandria and El-Beheira governorates. The three isolates of PVY recovered in this study induced similar symptoms on several diagnostic hosts such as; *N. glutinosa*, *N. repanda*, *Datura metal*, *S. nigrum* and *C. amarenticolor*. Symptoms appeared on such hosts were in agreement with those reported by Chikh-Ali *et al*., (2008). PVY-3 was easily transmitted mechanically, in complete agreement with results reported by Singh and Boiteau, (1984); Kamenfkova, (1987). PVY-3 was also successfully transmitted to test plants via two species of Aphids namely; *A. fabae*, and *A. nerii*. However, the most efficient vector was *A. fabae*, in agreement with results of Sigvald, (1984).

Although Indirect DBIA used by Powell, (1987) and optimized by Fegla *et al*. (2000) who increased its sensitivity in virus detection 10 times was used in this study; however, this method had sensitivity nearly similar to that of TBIA. The present study reported the

advantages of indirect DBIA, as it could detect much lower amounts of virus due to the smaller sample volume about 4 µl, compared with 100 µl of ELISA. The comparisons between different serological assays were demonstrated by many investigators (Fegla *et al*., 2001a; Younes *et al*., 2018). Indirect TBIA has been used by many investigators for surveys; diagnosis and detection of viruses in different parts of the plants. This was attributed to being cheap; could be completed in less than four hours without sacrificing sensitivity, did not require sophisticated facilities, and was sensitive enough to detect the virus in all parts of infected plants as reported by Fegla *et al*., (2001a); Abd El-Aziz and Younes, (2019). According to Fegla *et al*., (2001b); both of DBIA and TBIA were promising because they were very rapid, and can be carried out without the use of specialized equipment, and they could be done in few hours.

RT-PCR was a universally used tool for sensitive detection of PVY in infected host leaf tissues in routine surveys, as well as in phyto-sanitary programs (Gray *et al*., 2010). It has been recorded to be more specific and informative than Indirect ELISA; and was therefore used for testing symptomatic samples, by using specific CP primers. RT-PCR allowed the identification of the more dominant strains used in this study. This was achieved through the use of sequence technology in differentiating between PVY strains (named; N, NTN, O and C). Our results have indicated the prevalence of PVY^{NTN} in the three samples and no PVY^{O} or PVY^{C} strains were detected .It was reported by Kerlan *et al*., (2011) that symptoms on potato indication showed that PVY^{NTN} and PVY^{Z} isolates appeared very close and clearly destructive from the PVY^{N} and PVY^{O} strain groups. Similarly, in Tunisia a 67% of PVY^{NTN} variants were reported by Tayahi *et al*., (2016). Conversely; Kamangar *et al*., (2014) study from Belgium indicated that strains belonging to the N group were reported to be the most prevalent. Molecular characterization of some Egyptian isolates attempted through sequencing of CP gene followed by phylogenetic analysis, shed light on possible sources of PVY infection in a certain location or governorate. This is crucial especially when certification program for seed transfer and seed potato growth is not properly implemented. The unmonitored trafficking of potato seeds could subject such economically important crop to yield loss, and extensive crop quality damage. Application of the sequence of inspection, allowed us to identify the isolates by phylogenetic analysis based on the amplified CP of the 3 PVY isolates.

Egyptian PVY (MK376452) was of close homology to PVY isolate from South Africa. PVY (MK376453 and MK376454) isolates resemble and distinct from each other, and occupied a separate clade on their own. Currently; there were variations among comparison of nucleotide and amino acid sequences; however, the nucleotide sequencing was more reliable. Based on nucleotide and amino acid sequences, the degree of homology between the PVY isolates were clearly distinct. Results showed that isolates PVY-K2 and PVY-K3 were clustered in one clade with high degree of homology. Isolate PVY-K1 from El-Beheira governorate occupied a separate clade on its own, as it was very different in homology from the other Egyptian isolates (Kamangar *et al*., 2014). The phylogenetic analysis of the genomic CP segment reflected the high degree of genetic variability among current Egyptian isolates, in accordance with similar studies of Gray *et al*., (2010); Aseel *et al*., (2015).

The remarkable resemblance between PVY recombinants found in potato crops in several countries of Africa and Europe; suggested a common source of infection, most likely seed potatoes transported through international trade. The closest relatives for the current three Egyptian PVY isolates partial genomes and amino acids sequences were found among the Colombian PVY sequences, which might suggest that the source of these recombinants was resided somewhere in South America.

Conclusion

PVY distribution in Egypt is high at least in the sampling areas. The three PVY isolates recovered

from infected potato plants did not share the same place of origin, thus we suggested they might have been introduced into the Egyptian fields through the imported potato seed tubers. Moreover, potato plant could avoid the secondary infection with PVY by managing the aphids. We could detect the incidence of PVY in infected tubers by serological studies such as; Indirect ELISA, DBIA, and TBIA as a one day assays, or by molecular techniques such as RT-PCR.

Conflict of interests

The authors declare that there are no conflicts of interest.

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5. References

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