Partial Characterisation of Three Isolates of *Potato virus Y* (PVY)

Mohamed M. Zalat , Maha A. Kawanna* and Elsayed E. Wagih[1]

ABSTRACT

Field observations indicated that the most common symptoms appearing on naturally infected potato plants growing in El-Beheira, Dakahlia, and Kafr El-Sheikh governorates of Egypt were mild to severe mosaic, chlorosis, mottling, vein banding, blisters, and/or stunting. These symptoms were suspected of being caused by *Potato virus Y* (PVY) and detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The tested specimens demonstrated single RT-qPCR amplification product of 480bp long using a specific pair of primers for PVY coat protein gene. Indirect ELISA using antisera specific to PVY, PVX, PVM and PVS gave positive reaction with PVY antiserum. Based on symptomology and disease severity on *Nicotiana glutinosa*, three isolates of PVY were obtained and named PVY-Badr, PVY-Wadi (from El-Beheira) and PVY-Talkha (from Dakahlia) then subjected to this study. Reaction of several diagnostic hosts of seven species belonging to two families; Amaranthaceae and Solanaceae upon inoculation with the three isolates elicited different symptoms distinctive to PVY-infection. PVY-Badr and PVY-Talkha isolates developed, respectively, mild to moderate symptoms, while PVY-Wadi isolate induced sever symptoms. The amplified RT-qPCR product of coat protein gene of isolates PVY-Badr and PVY-Wadi were purified and sequenced. The sequences were edited using Chromas Pro. Version 1.34 software and compared with previously subgrouping of 25 virus isolates retrieved from the GenBank database. Alignment of partial sequence and phylogenetic analysis revealed that PVY-Badr closely related to PVY^{NTN} strain, Ireland isolate with similarity percentage of 99.76%, and similarity percentage of 99.42% was scored between PVY-Wadi and PVY-Wilga strain, China isolate.

Keywords: PVY, RT-qPCR, Indirect ELISA, Diagnostic hosts, Phylogenetic analysis.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the third staple food crop in the world and the world's most widely grown tuber crop ranking fourth after rice, wheat and maize (Abbas *et al*., 2016 and Tussipkan & Manabayeva, 2021). Potato is considered one of the most economically important solanaceous crops cultivated in different regions in Egypt and one of the most important crops grown for local consumption, export and processing. In most recent years, the EU has accounted for about 70% - 90% of Egyptian potato exports (FAO, 2020).

Potato plants are affected with more than 40 different viral diseases, including those caused by *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato leaf roll virus* (PLRV) which are known to cause severe damages to potato (Biswas *et al.*, 2005; Abbas *et al*., 2014 and Deloko *et al*., 2021).

Potato Virus Y (PVY) is the type species of the genus *Potyvirus*, one of the six genera in the family *Potyviridae* (Kerlan & Moury, 2008 and Samarskaya *et al*., 2023). The virus is one of the most important viruses which influences the quantity and quality of potato production causing substantial losses to farmers (Hall *et al*., 1998; Schubert *et al*., 2007 and Samarskaya *et al*., 2023). It is one of the most widespread viruses infecting a large number of important agricultural members of family *Solanaceae* including potato, tobacco, and pepper (De Bokx & Huttinga, 1981; Shukla *et al*., 1994 and Abbas *et al*., 2014).

Al-Kuwaiti *et al.* (2016) reported that PVY is a highly variable plant virus as it includes many strains, three of which, namely PVY^{O} (Ordinary strain), PVY^{N} (Necrosis strain) and PVY^{C} (PVC strain) are identified based on their biological properties (Singh *et al*., 2008). Other strains of PVY, namely PVY^{NTN} (Necrotic Tuber Necrosis strain), $PVY^{N:O}$ (Necrosis: Ordinary strain) (Chikh-Ali *et al*., 2011) and PVY^{NTN-NW}, (Necrotic Tuber Necrosis -Necrosis Wilga strain) do exist.

The objectives of the present research were to isolate and characterise the virus isolates based on molecular (reverse transcription quantitative polymerase chain reaction, RT-qPCR), serological (indirect ELISA with potato viruses' specific antisera), and pathological (the symptoms elicited on diagnostic hosts) techniques, also, alignment of coat protein gene nucleotide sequence and analysis of phylogenetic relationship.

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[1]Department of Plant Pathology, Faculty of Agriculture, El-Shatby, Alexandria University, Alexandria, Egypt.

*Corresponding author: Email: mahakawanna@yahoo.com

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MATERIALS AND METHODS

Virus isolation and detection:

Samples suspected of being infected with *Potato Y virus* (PVY) were collected separately in plastic bags from naturally diseased potato plants grown at different locations, from three governorates of Egypt (Markaz Badr, Modereiat El-Tahrir) and Wadi El-Natrun, El-Beheira; El-Senbellawein 1, El-Senbellawein 2 and Talkha, Dakahlia; Baltim 1, Baltim 2, Biyala and El-Hamoul, Kafr El-Sheikh). *Nicotiana glutinosa* seedlings were used for biological detection of PVY in collected specimens.

Specimens that reacted positively with *Nicotiana glutinosa* were labeled and separated into two plastic bags. The first portion of each specimen was kept frozen at -20° C, while the other portion was later subjected to PVY detection using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), indirect ELISA testing and pathological characterisation on diagnostic hosts.

Inoculation, biological purification, and maintenance:

PVY isolates were maintained on *N. glutinosa* which served as a source of the virus for subsequent studies. Source of inoculum was always renewed by inoculating a new patch of healthy plants. Isolates of PVY used in this study were biologically purified by inoculating *Datura. stramonium*.

Detection by reverse transcription-quantitative polymerase chain reaction (RT-qPCR):

RNA extraction:

Total RNA was extracted from 100mg (fresh weight) of nine samples of PVY-infected *N. glutinosa* leaves as well as an equal quantity of healthy plant as control using IQeasyTM Plus Plant RNA Extraction Mini Kit (iNtRON Biotechnology, Inc). The RNAs pellet were eluted using 50 µl of elution buffer (RE) followed by centrifugation at 10,000 xg for 1 min. The extracted RNA was collected and kept at –20 °C for further studies.

Primer design:

A pair of specific primers for PVY coat protein region of the virus genome capable of generating an amplification fragment with the expected length of 480 bp were used: A forward primer, 5′-ACGTCCAAAATGAGAATGCC-3′ and a reverse primer 5′-TGGTGTTCGTGATGTGACCT-3′ (Verma *et al.*, 2003; Mackenzie *et al.*, 2015 and Khelifa, 2019). Primers were synthesised by Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR):

Total RNA was used for complementary DNA (cDNA) synthesis and DNA amplification using Rotor-Gene Q (QIGEN, Company). The Coat protein (CP) gene of PVY was amplified using one step RT-qPCR kit (TOPreal™ One-step RT-qPCR Kit, SYBER Green with Lo-ROX) Enzynomics co. Ltd, Korea, according to manufacturer instructions.

The reaction mixture for cDNA synthesis was carried out in 20 µL total volume containing: 2 µl of RNA template (about 50 ng), 1 µl TOPreal™ One-step RT-qPCR Enzyme MIX, 10 µl of 2XTOPreal™One-step RT-qPCR Reaction MIX, 1 µl of each primer and the volume was completed with 5 µl of sterile distilled water (*RNase* free). The RT mixture was set up on ice**,** then vortexed for few seconds and transferred to a RT-qPCR machine. The reaction conditions were set up according to the manual of RT-qPCR One-step Kit as following: one cycle of 50°C for 30 min as reverse transcription, one cycle of 95°C for 10 min as polymerase activation, 40 cycles of 95°C for 5 sec (denaturation), 60°C for 45 sec (annealing) and a final extension cycle at 72°C for 45 sec.

Polymerase chain reaction:

The obtained amplification product (amplicon) was reamplified by the conventional PCR to obtain a high yield of cDNA from five samples that reacted positively using RT-qPCR, which was selected based on different locations to be sufficient for completion of the further molecular experiments using Master Mix (OnePCR™ Ultra, GeneDirex®) containing *Taq DNA Polymerase*, *DNA Polymerase* Buffer, gel loading dyes, and dNTPs), using the same primers. PCR reaction was carried out using a thermal cycler (Primus 25 Advanced®, Peqlab Company) in 25 µL mixture containing: 2 µl of cDNA (template), 12.5 µl of Master Mix (OnePCR™ Ultra, GeneDireX®), 0.5 µl of each primer, and 9.5 µl of sterile *nuclease* -free distilled water. The PCR reaction conditions were as follows: one cycle of 90°C for 45 sec as initial denaturation, 40 cycles of 94°C for 45 sec (denaturation), 60°C for 45 sec (annealing), 72°C for 1 min and a final extension cycle at 71°C for 10 min. The PCR products were separated in agarose gel (1.5%), stained with RedSafe™. Electrophoresis was carried out at 130 volts (Cleaver Scientific ltd) for 20 minutes. DNA bands were visualised on a UV-transilluminator.

Serological detection by indirect ELISA:

Naturally infected samples that reacted positively with *N. glutinosa* and tested with the RT-qPCR reaction and suspected of being infected with PVY

were used for serological detection by indirect ELISA using specific polyclonal antisera to each of *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX) and *Potato virus Y* (PVY). Antisera were obtained from the collection of Virology lab. Plant Pathology Dept., Faculty of Agriculture, Alexandria University, Egypt.

Indirect ELISA first reported by Koenig (1981) with some modifications, applied by Zalat (2017) was used for virus detection. ELISA values measured by Universal automated microplate reader ELx 800 and readings were expressed as absorbance at 405 nm. Readings representing tested samples were considered significant if their corresponding absorbance exceeded twice that of the healthy control. In each set of test, wells lacking antigen (i.e. coating buffer only) were included as a blank.

Reaction of diagnostic hosts:

Seven plant species, belonging to two families, namely Amaranthaceae and Solanaceae were inoculated with the test PVY isolates from potato (Markaz Badr and Wadi El-Natrun, El-Beheira and Talkha, El-Dakahlia).

Tissues harboring suspected viruses were inoculated on diagnostic hosts including *Capsicum annum, Chenopodium amaranticolor, C. quinoa, Datura metel, D. stramonium, Nicotiana glutinosa* and *Solanum lycopersicum.* These diagnostic hosts are known to give characteristic symptoms for PVY (d'Aquino *et al*., 1995; Fletcher, 2001; Zaied, 2013 and Abdel-Shafi *et al*., 2017). Five seedlings of each plant species or cultivar to be tested were used for mechanical inoculation with each PVY isolate. Plants to be inoculated were used at the age of 2-4 leaf stage except those of *Solanum tuberosum* which were inoculated at the basal leaf. The inoculation technique was carried out as described elsewhere (Dusi *et al.,* 2001 and Wagih *et al*., 2021). Inoculated plants were kept under glasshouse conditions at 25-28 °C and monitored daily for symptom development.

Molecular studies:

Sequence analysis of PVY (Badr and Wadi El-Natrun) CP gene:

The protein coat gene of the two PVY isolates were PCR amplified, and the PCR product was purified using QIAquick PCR purification kit (Qiagne Inc., Mississauga, Ontario, Canada), subjected to sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit's (Thermo Fisher Scientific, Waltham, Massachusetts) then resolved on an ABI PRISM® 3700 Genetic Analyser at Sigma Scientific Services Company. DNA sequencing was carried out in one direction using the purified PCR product. The sequence was edited using Chromas Pro Version 1.34 software (El-Absawy *et al*., 2012). The isolates were compared with the previously described sequences in the NCBI database using the BLAST program.

Sequence alignment and phylogenetic analysis:

Pairwise and multiple DNA sequence alignment were carried out using Clustal X multiple sequence alignment program version 2 (Edgar, 2004 and Larkin *et al*., 2007). Bootstrap maximum likelihood tree was generated using MEGA program version 11 (Visser *et al*., 2012 and Green *et al*., 2017).

Sequences of Markaz Badr (accession No., MT242381) and Wadi El-Natrun (accession No., MT561046) had been submitted to GenBank Database and then compared with the complete genome sequences of 25 PVY strains which have been classified according to genetic variation as follows: Common strain (PVY^{C}), ordinary strain (PVY^{O}), necrosis strain (PVY^{N}), necrotic tuber necrosis (PVY^{NTN}) and wilga strain (PVY^{W}) (Green *et al*., 2017 and Wani *et al*., 2021). The similarity among strains and our isolates was calculated using the Superimposed Image Analysis System (SIAS) software program by pairwise method (http://imed.med.ucm.es/Tools/sias.html).

RESULTS AND DISCUSSION

Symptomology and virus detection of naturally infected plants:

The naturally infected potato plants showed symptoms including faint mosaic, severe mosaic, mottling, yellowing of intercostal areas, vein banding, blisters, malformation and stunting, suspected of being *potato virus Y* (PVY) symptoms. These symptoms were observed on naturally infected potato plants at nine locations within three governorates (Markaz Badr (Modriat El-Tahrir) and Wadi El-Natrun, El-Beheira; El-Senbellawein 1, El-Senbellawein 2 and Talkha, Dakahlia; Baltim 1, Baltim 2, Biyala, and El-Hamoul, Kafr El-Sheikh). The viral causative agent of the naturally observed symptoms on potato plants and suspected of being PVY was identified based on molecular (reverse transcription-quantitative polymerase chain reaction, RT-qPCR), serological (indirect ELISA with potato viruses-specific antisera), and pathological (the symptoms elicited on diagnostic hosts) techniques.

Detection of PVY by reverse transcription-quantitative polymerase chain reaction (RT-qPCR):

RT-qPCR was performed on the total RNA extracted from *N. glutinosa* leaf samples separately infected with nine PVY isolates using a pair of sequence-specific primers within the coat protein

gene. Analysis of the RT-qPCR results indicated that positive reaction was obtained with seven samples (Baltim2, Biyala, El-Hamol, El-Senbellawein1, Markaz Badr, Talkha, and Wadi El-Natrun), while only two samples (Baltim1 and El-Senbellawein 2) proved to be negative along with healthy mock-inoculated control plants, as evidenced by amplification of the target sequence as compared to mock-inoculated healthy plant (Green-A) and melting curves (Fig. 1). Analysis of the RT-qPCR amplification product was carried out by 1.5% agarose gel electrophoresis (Fig. 2). Using the conventional PCR, the amplicon lengths, approximately 480 bp of PVY isolates, obtained from RT-qPCR were detected (Fig. 3). This finding is supported by a similar result previously obtained by Almasi & Dehabadi, (2013) and Villamil-Garzón *et al.* (2014). Five of the nine isolates suspected of being PVY, were selected based on one isolate for each location to be amplified by conventional PCR and used throughout this study.

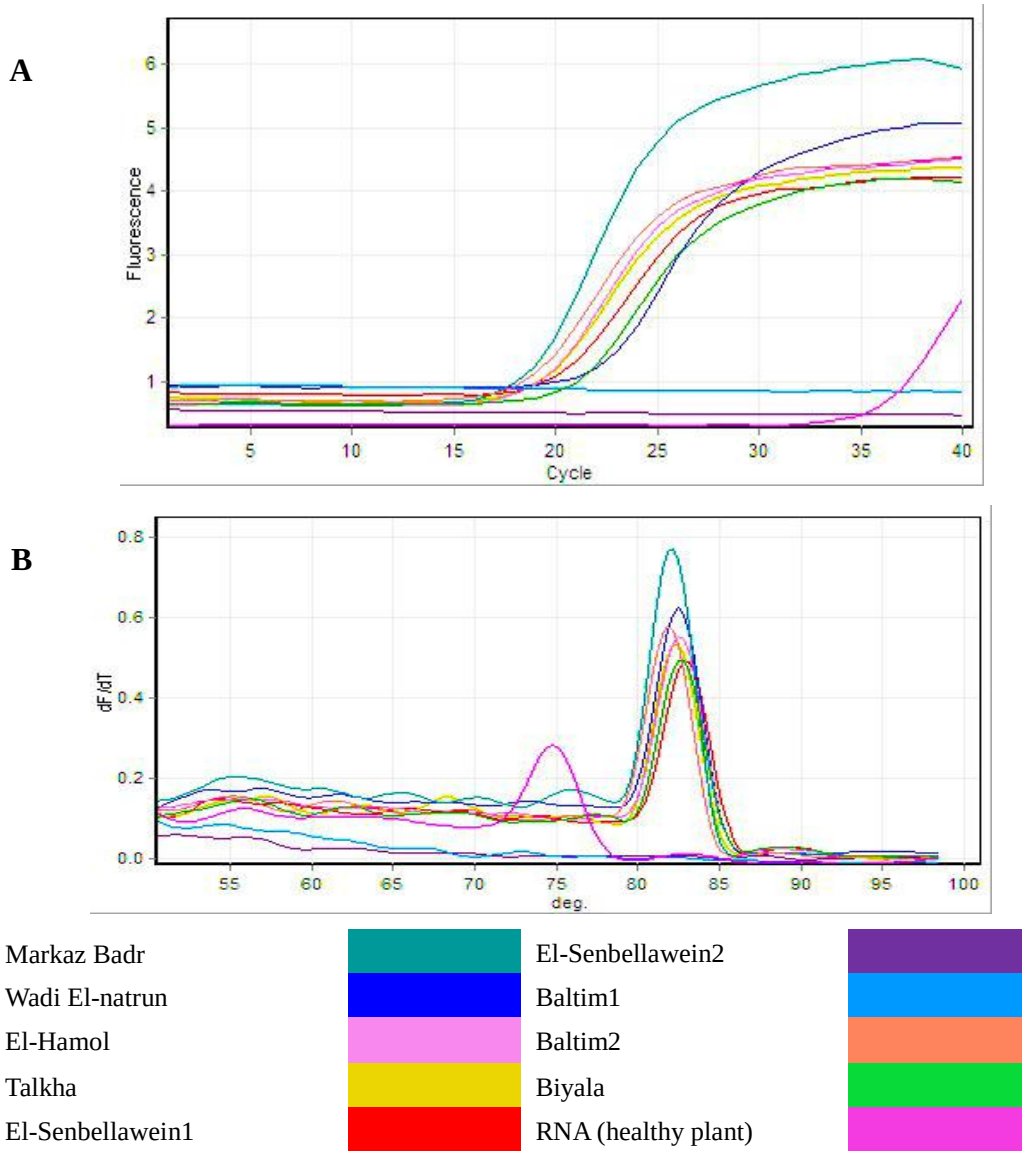

Figure 1. Standardization of the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for the detection of *potato virus y*, showing positive reaction with seven PVY isolates and negative reaction with healthy plant RNA. "A", Amplification (Green A) and "B" Melting curves.

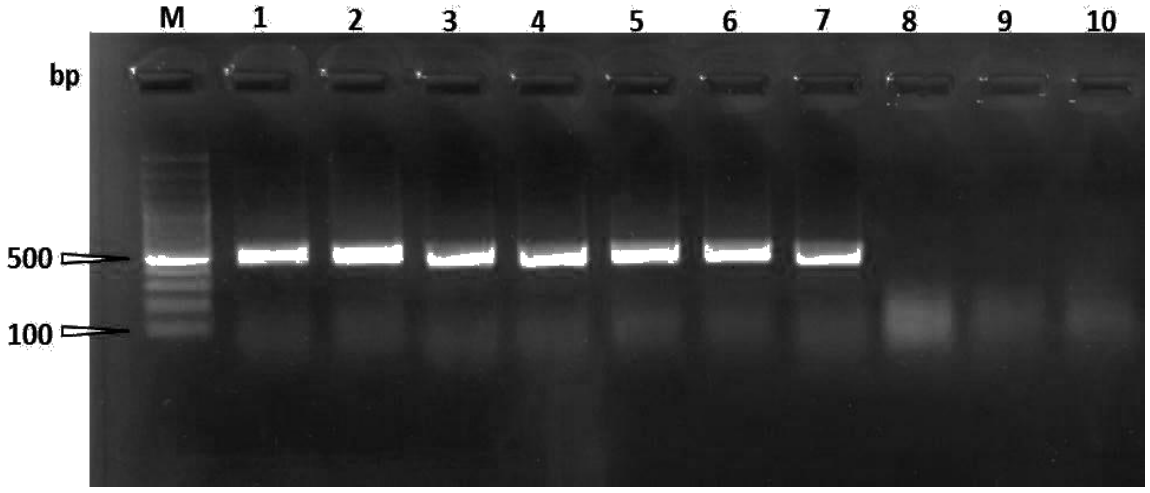

Figure 2. Agarose (1.5%) gel electrophoresis showing amplification products of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) with a molecular length of 480 bp, when the RT-PCR analysis with a pair of coat protein sequence-specific primers was performed on the total RNA extracted from *N. glutinosa* leaf tissues individually infected with PVY isolates collected from Baltim (1, 2), Biyala, El-Hamoul, El-Senbellawein (1, 2), Markaz Badr, Talkha, Wadi El-Natrun) which gave a positive reaction (Lane 1 to 7), whereas healthy plants gave negative reaction (Lane 10). Gel was stained with RedSafe irradiation dye and bands visualised by ultraviolet irradiation. Left Lane (M) is a 3 Kbp DNA marker ladder.

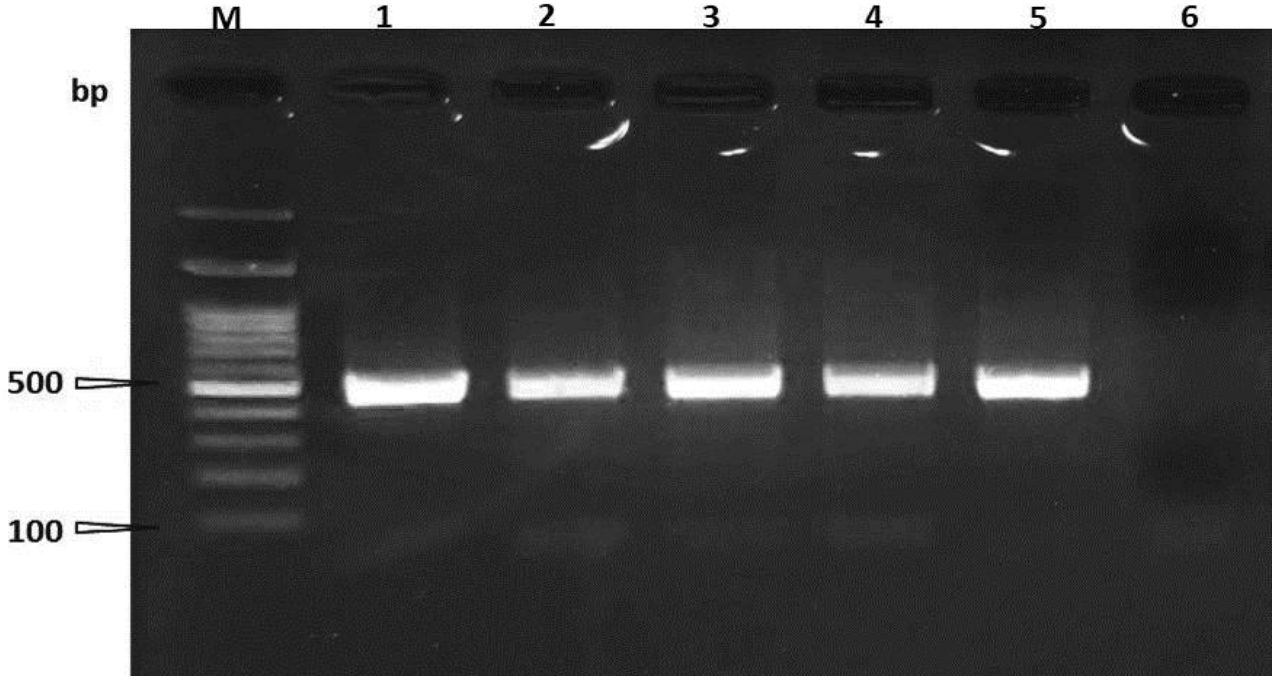

Figure 3. Agarose (1.5%) gel electrophoresis showing the re-amplification product of polymerase chain reaction (PCR) with a molecular length of 480 bp for five different PVY isolates (Baltim 2, El-Hamoul, Markaz Badr, Talkha, and Wadi El-Natrun) which gave a positive reaction (Lane 1 to lane 5), and a negative reaction with healthy control leaves (Lane 6), when the traditional PCR analysis with a pair of coat protein sequence-specific primers was conducted on the cDNA product of the reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Gel was stained with RedSafe dye and bands visualised under ultraviolet radiations. Left Lane (M) is a 3 kbp DNA marker ladder.

Although the detection of the possible variation among PVY isolates was the objective of several other investigations (Kerlan, 2006 and Elbeshehy *et al*., 2023), it was necessary to through further light in this study on the variability of this virus as to disease severity. The results obtained here widen this variability as evidenced by symptomology, reaction of diagnostic hosts, serological reactivity, and molecular relationships.

Serological detection using indirect ELISA:

Serological techniques are useful and accurate assays for identifying PVY (Zaied, 2013 and Wu *et al*., 2021). Serological detection of PVY by indirect ELISA in five potato samples selected from different locations, (Table 1) revealed that the virus associating the previously described symptoms of naturally infected plants is PVY. This was confirmed when positive reaction was obtained when RT-qPCR technique with a pair of PVY -specific primers was obtained when three (Markaz Badr and Wadi El-Natrun from El-Beheira and Talkha from Dakahlia) (Fig. 4) out of the five samples were selected and tested, based on variations of symptoms which appeared on naturally infected potato and distance locations of isolates. The positive reaction of the five isolates suspected of being PVY when tested with indirect ELISA with a PVY-specific polyclonal antiserum confirmed that the obtained isolates were of PVY. Three isolates of these were used throughout this study based on the variability of symptoms they induce on potato plants (source of isolates) and *N. glutinosa* (propagation host). ELISA can differentially detect even closely related strains of the same virus (Lin *et al.*, 1990 and Badarau *et al.*, 2009). It was the most common serological test used to diagnose viral diseases in potato (Folwarczna *et al*., 2008; Kostiw, 2011 and Wang *et al*., 2020).

Table 1. Serological assay of *Nicotiana glutinosa* leaves infected with different *potato virus y* (PVY) isolates collected from different locations in several governorates (Markaz Badr, El-Beheira; Talkha, Dakahlia); Wadi El-Natrun (El-Beheira) using indirect ELISA technique and potato viruses-specific antisera.

Test sample	Governorate	Location	Indirect ELISA reading* (E 405nm)			
			PVM	PVS	PVX	PVY
Potato	El-Beheira	Markaz Badr	0.0745	0.0904	0.0814	**0.3447**
		Wadi El-Natrun	0.0772	0.0804	0.0738	**0.4069**
	Dakahlia	Talkha	0.0722	0.0882	0.0857	**0.3560**
	Kafr El-Sheikh	Baltim	0.0815	0.1532	0.1392	**0.4187**
		El-Hamoul	0.0691	0.0950	0.0781	**0.3901**
Healthy			0.1304	0.1550	0.1088	0.1028

*, ELISA readings in terms of extinction at 405nm (E 405nm) are the mean value of three replicates each. Extinction values of at least double that of the healthy control were considered positive; Bold figures= Positive reaction

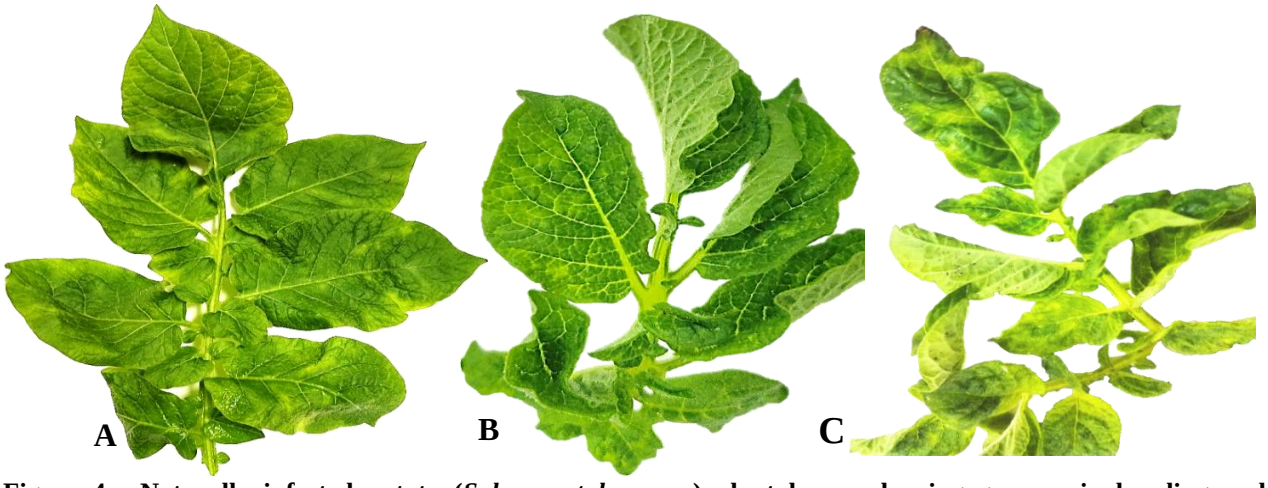

Figure 4. Naturally infected potato (*Solanum tuberosum*) plant leaves showing green vein banding and mottling (A), mosaic and vein yellowing (B), sever mosaic, green vein banding, yellowing of intercoastal areas, blisters, and malformation (C) from which the three *potato virus y* (PVY) isolates, Markaz Badr (El-Beheira), Talkha (Dakahlia) and Wadi El-Natrun (El-Beheira), respectively, were isolated.

Reaction of diagnostic hosts:

The three isolates of PVY, namely, PVY-Badr, PVY-Talkha and PVY-Wadi, investigated here were found to be infectious to potato and many other hosts. PVY-Badr and PVY-Talkha isolates developed, respectively, mild to moderate symptoms on test plants, while PVY-Wadi Natrun isolate induced sever symptoms (Fig. 4). Reactions of diagnostic hosts to the three isolates are presented in Table (2). These isolates caused faint to severe mosaic and blisters on plants of *N. glutinosa* (Fig. 5). Tested isolates produced vein clearing followed by systemic mottling, raised green blisters and leaf deformation on *Datura metel* (Fig. 6) and vein banding, mild mosaic, and severe mosaic on *Capsicum annum* (Fig. 7).

In *Solanum lycopersicum* L. cv. Super strain B, PVY-Badr and PVY-Talkha isolates showed mild mosaic and green vein banding symptoms whereas PVY-Wadi isolate produced sever mosaic, vein yellowing and green vein banding (Fig. 8). Reaction of mechanical inoculation of localised symptoms was observed on *Chenopodium amaranticolor* as necrotic local lesions with PVY-Badr and not reacting with PVY-Talkha and PVY-Wadi (Fig. 9).

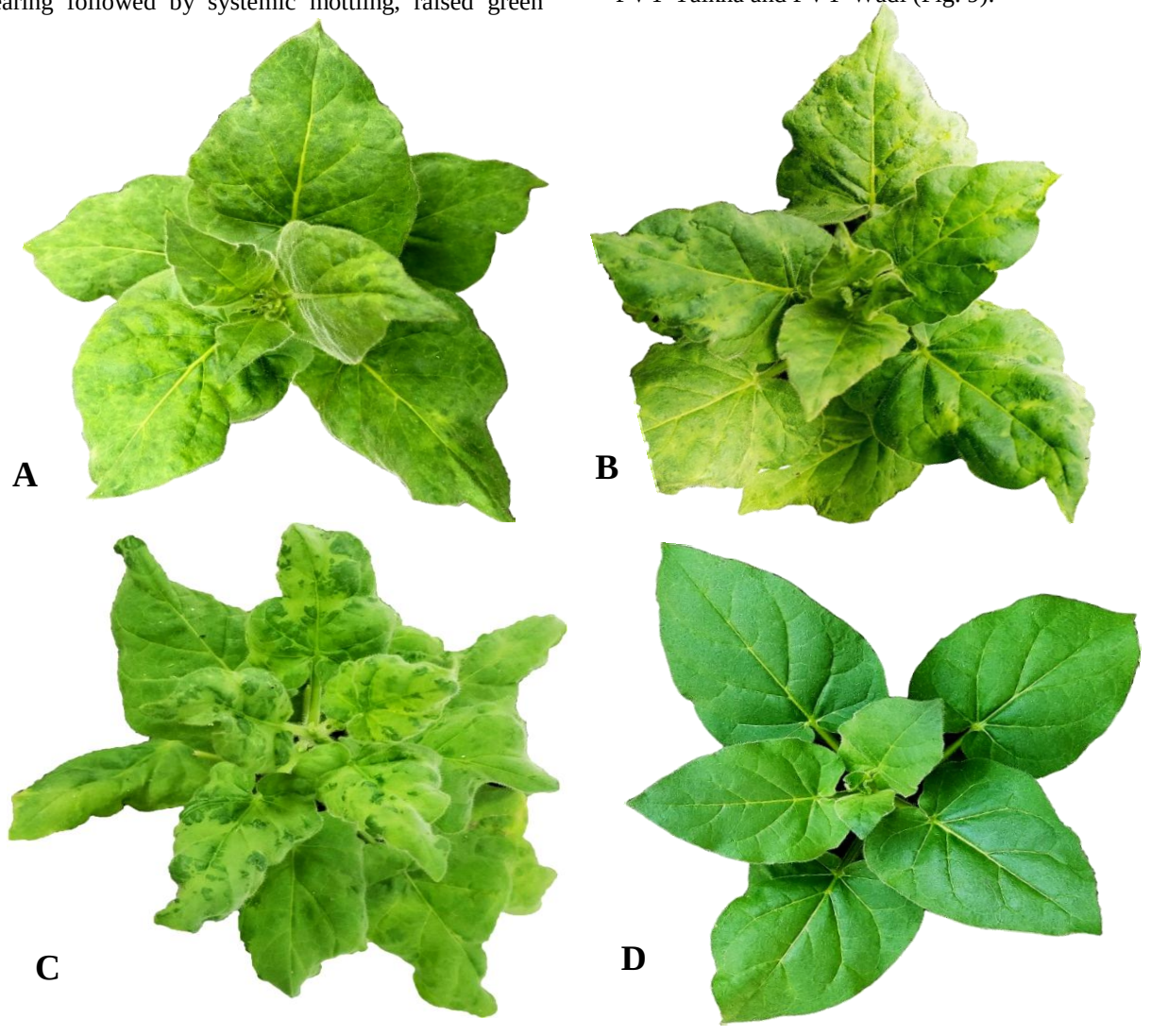

Figure 5. Tobacco (*Nicotiana glutinosa)* plants showing mosaic and green vein banding induced by the *potato virus y* (PVY)-Badr isolate (A), severe mosaic, green vein banding induced by PVY- Talkha isolate (B), severe mosaic, green vein banding, blistering and malformation elicited by the PVY-Wadi isolate (C), 25 days post-inoculation as compared to phosphate buffer mock-inoculated healthy plants (D).

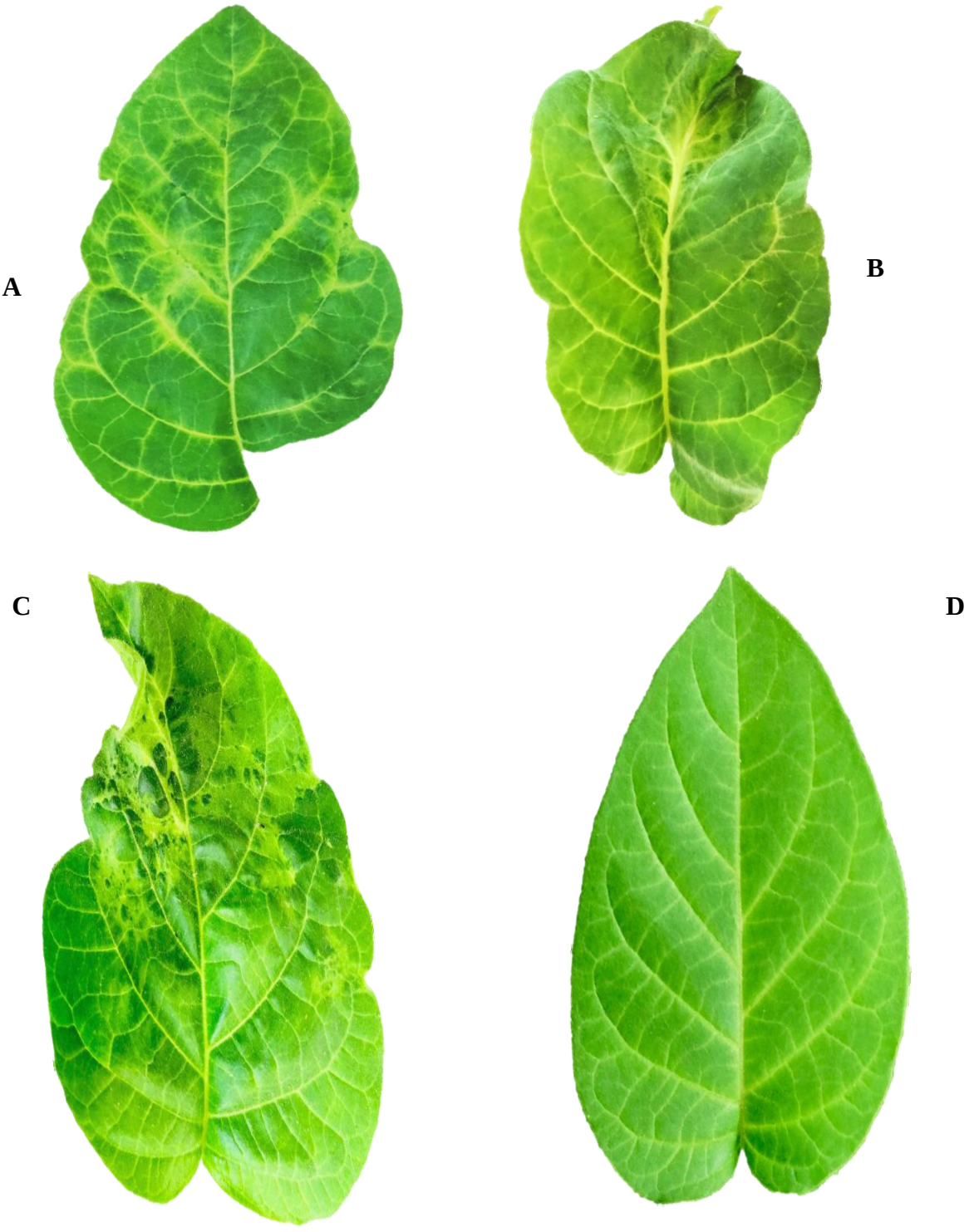

Figure 6. ***Datura metel*** **showing line pattern and vein yellowing induced by the** ***potato virus y*** **(PVY)-Badr isolate (A), vein yellowing and malformation when inoculated with the PVY-Talkha isolate (B), yellowing intercostal veins, blistering and malformation elicited by PVY-Wadi isolate (C), 25 days post-inoculation as compared to phosphate buffer mock-inoculated healthy leaf (D).**

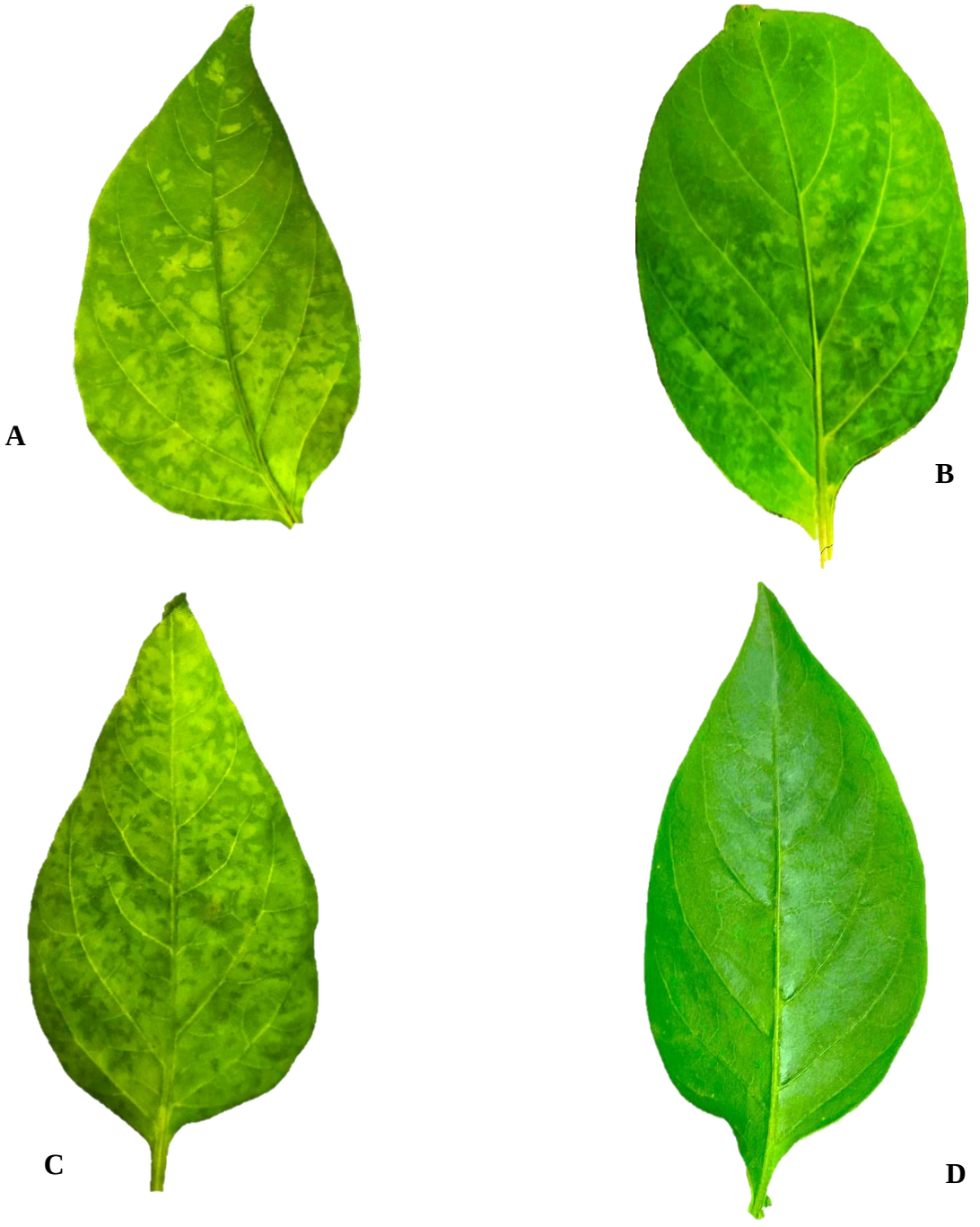

Figure 7. Pepper (*Capsicum annum*) plant leaves expressing mosaic and green vein banding and yellowing of intercoastal areas evolved by *potato virus y* (PVY)-Badr (A), mild mosaic induced by PVY-Talkha isolate (B), severe mosaic, green vein banding and yellowing intercostal veins elicited by PVY-Wadi isolate (C), 25 days post-inoculation as compared to phosphate buffer mock-inoculated healthy plant leaf (D).

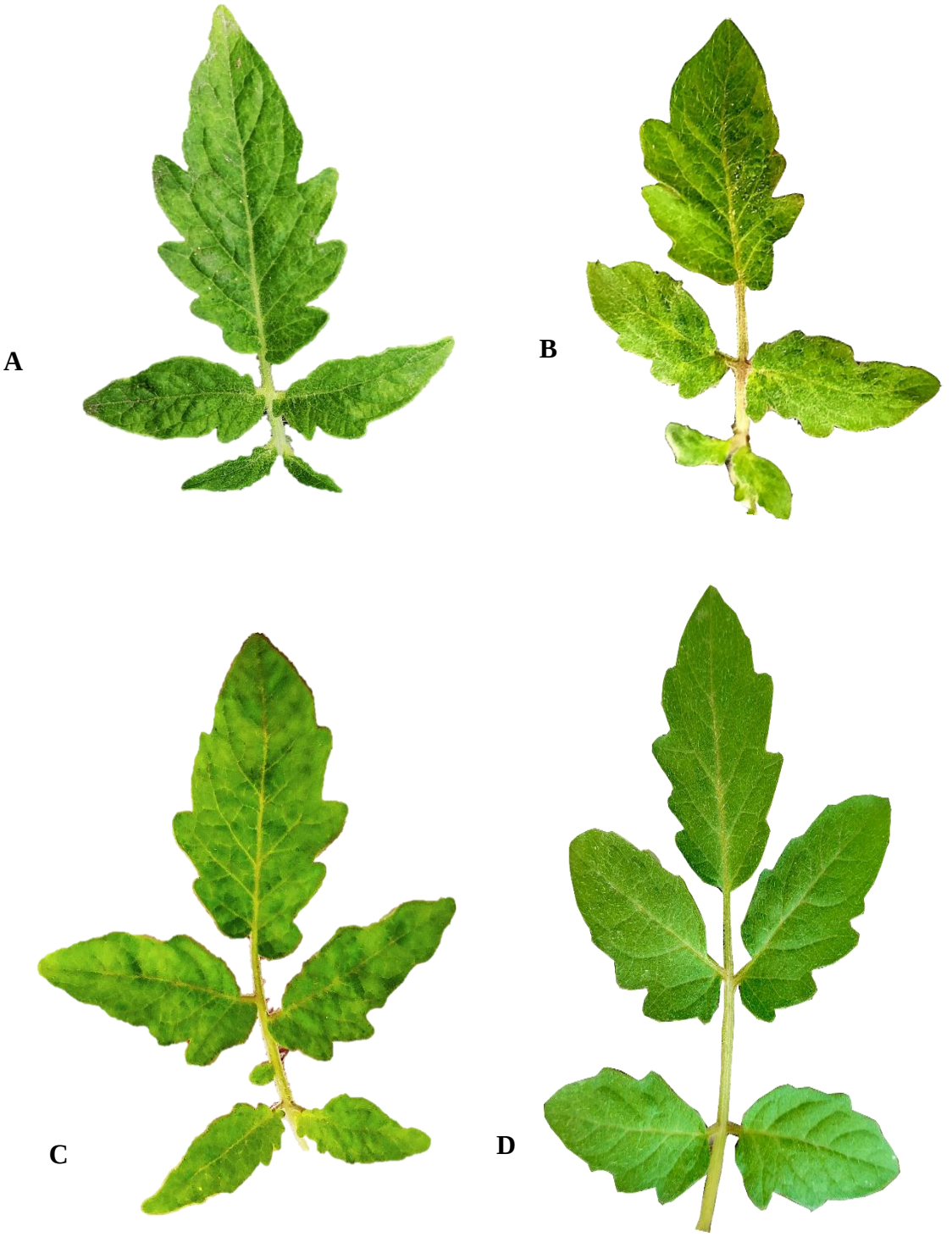

Figure 8. Tomato (*Solanum lycopersicum* cv. Super strain B) leaves expressing symptoms of vein clearing, mild mosaic and green vein banding induced by the *potato virus y* (PVY)-Badr isolate (A), mild mosaic and vein yellowing caused by a PVY-Talkha isolate (B) and severe mosaic, vein yellowing and green vein banding elicited by PVY-Wadi isolate (C), 25 days post-inoculation as compared to phosphate buffer mock-inoculated healthy control leaf (D).

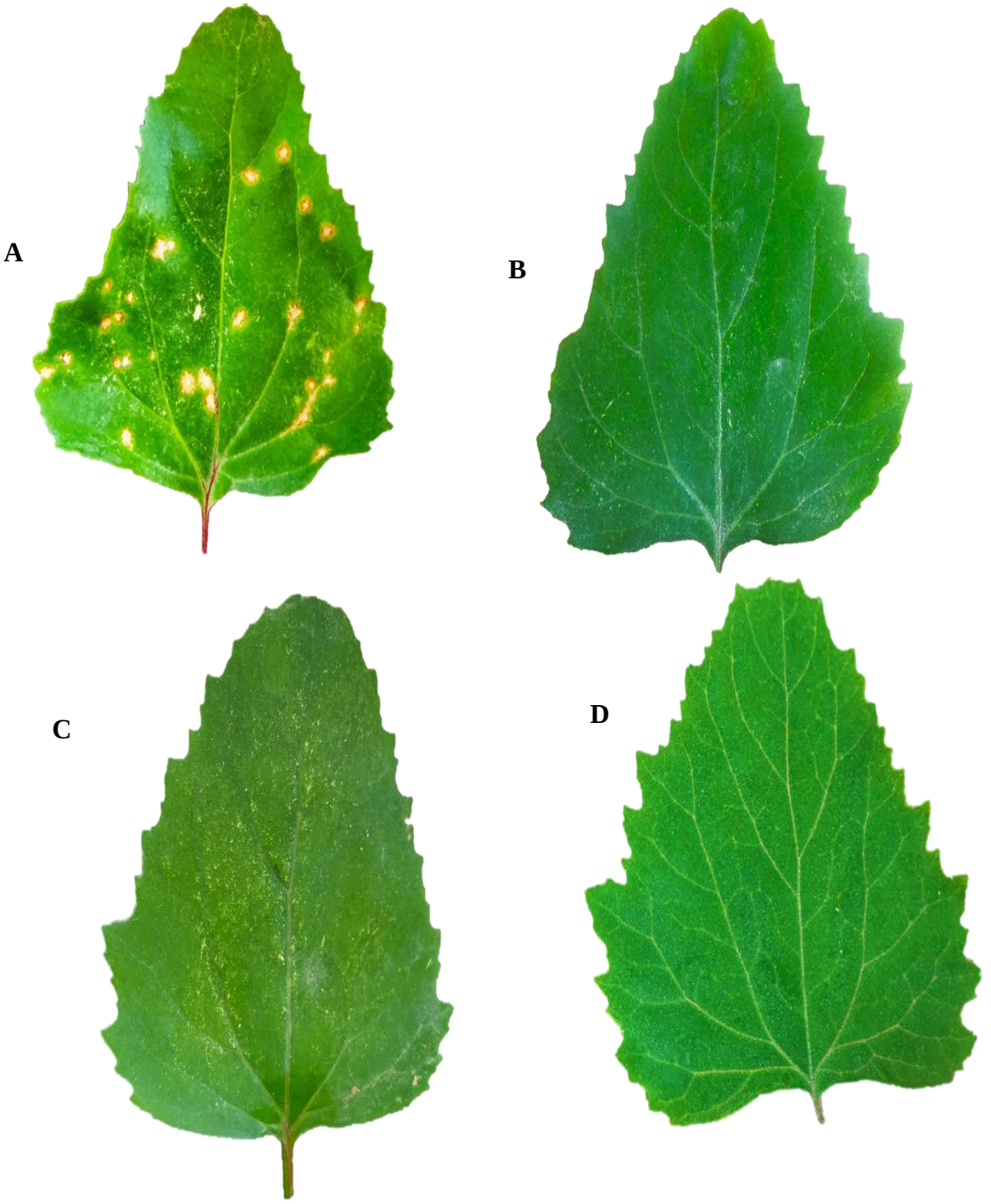

Figure 9. Goosefoot (*Chenopodium amaranticolor*) plant leaves showing necrotic local lesions surrounded by reddish halos induced by the *potato virus y* (PVY)-Badr isolate (A) and no reaction to PVY-Talkha and PVY-Wadi isolates (B and C), as compared to phosphate buffer mock-inoculated healthy leaves (D), 5 days post-inoculation.

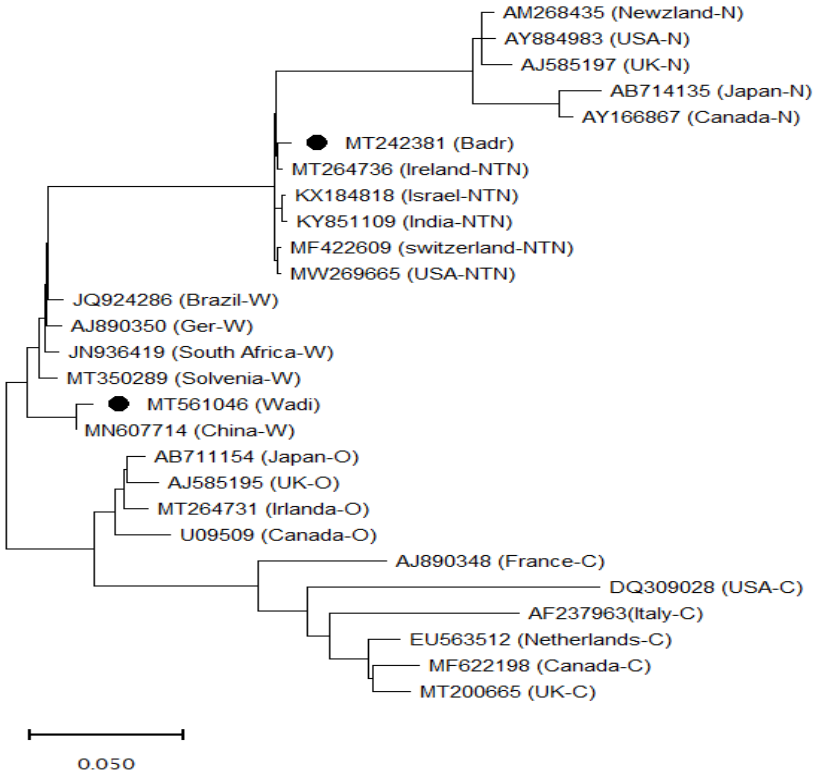

Figure 10. Maximum likelihood phylogenetic tree demonstrating the evolutionary relationship among nucleotide sequence of coat protein gene of two *potato virus y* (PVY) isolates, MT242381 (PVY-Badr) and MT561046 (PVY-Wadi), as compared with twenty-five genomic sequences of PVY isolates deposited in the GenBank database. The tree was produced by MEGA program, version 11 software with bootstrap test (1000 replicates). Scale indicates the percentage of divergence. Accession number, strain abbreviation and country are given for each sequence.

Table 2. Differential reaction of some diagnostic hosts to inoculation with three isolates of *potato virus Y* (PVY), namely PVY-Badr, PVY-Talkha, and PVY-Wadi.

	Isolate*/Host reaction**		
Diagnostic host	**PVY-Badr**	**PVY-Talkha**	**PVY-Wadi**
Fam.: Amaranthaceae			
Chenopodium amaranticolor	NLL	-	-
Chenopodium quinoa	-	-	-
Fam.: Solanaceae			
Capsicum annum	M/ GVB/YIA	MM	SM/ GVB/ YIV
Datura metel	LP/VY	VY/ Mf	YIV/B/Mf
Datura stramonium	-	-	-
Nicotiana glutinosa	M/GVB	SM/GVB	SM/GVB/B/Mf
Solanum lycopersicum	VC/MM/GVB	MM/VY	SM/VY/GVB

*, PVY-Badr, *Potato virus Y* isolated from Markaz Badr; PVY-Talkha, isolated from Talkha; PVY-Wadi, isolated from Wadi El-Natrun.

**, B, Blister; LP, Line pattern; M, Mosaic; Mf, Malformation; MM, Mild mosaic; NLL, Necrotic local lesions, -, No Symptoms; SM, Severe mosaic; VC, Vein clearing; GVB, Green vein banding; VY, Vein yellowing; YIA, Yellowing intercoastal areas; YIV, Yellowing intercostal veins.

The finding that PVY-Badr, produced mosaic and green vein banding on *N. glutinosa* and that PVY-Talkha isolate elicited severe mosaic, green vein banding on infected plants, while PVY-Wadi, induced severe mosaic, green vein banding, blistering and malformation, a group of symptoms like those reported by Mostafae *et al.* (2006), Zaied (2013) and Elbeshehy *et al*. (2023).

The symptoms appearing on *Datura metel* in the form of line pattern and vein yellowing when infected with the PVY-Badr isolate and vein yellowing and malformation when inoculated with the PVY-Talkha isolate, as well as, yellowing of intercostal veins, blistering and malformation elicited by PVY-Wadi isolate agreed with those reported by Zaied (2013) and Hamza *et al*. (2018).

The mosaic, green vein banding and yellowing of intercoastal areas induced by PVY-Badr, the mild mosaic elicited by PVY-Talkha isolate, while PVY-Wadi isolate produced sever mosaic, green vein banding and yellowing intercostal veins on *C. annum* leaves which were similar to those reported by Moodley *et al*. (2019), but different from those published by Stace-smith & Mellor (1968) and El Banna *et al*. (2015).

On tomato (*S. lycopersicum* L.) cv. Super strain B, vein clearing, mild mosaic, green vein banding and yellowing were induced by the three PVY isolates matching those
reported by Quenouille *et al*. (2013) and Muimba-Kankolongo (2018), but different from those of Crescenzi *et al*. (2005) and Wei *et al*. (2021).

The necrotic local lesions surrounded with reddish halos induced by PVY-Badr isolate without systemic spread on *C. amaranticolor*, as reported before by Nisbet *et al*. (2006) and Karasev *et al*. (2011), contrasted with those of Crescenzi *et al*. (2005), while PVY-Talkha and PVY-Wadi isolates didn't show symptoms that were in agreement with those of Crescenzi *et al*. (2005).

The variability observed in the foregoing part of the discussion between the three
isolates, PVY-Badr, PVY-Talkha and PVY-Wadi and between them and those reported in the literature could be attributed to several factors including plant cultivars, geographical location, virus reservoir, alternative hosts, virus strain and environmental conditions.

Molecular studies:

Nucleotide sequence

Coat protein gene primers (forward and reverse sequences) were designed for the PVY-Badr and PVY-Wadi isolate genome. The results obtained confirmed the specificity of the primers used under the conditions embraced in this study. The PCR product with the expected amplicon length amounting to 480bp long was obtained.

When nucleotide sequencing of the coat protein gene of PVY-Badr and PVY-Wadi isolates was carried out on the corresponding purified PCR product using BigDye® Direct Sanger Sequencing Kit and purified the product of sequencing using PureLink™ PCR Purification Kit and resolved on an ABI PRISM® 3100 Genetic Analyser at Sigma Scientific Services Company. The nucleotide sequence of PVY-Badr and PVY-Wadi coat protein gene generated were accepted in the GenBank nucleotide sequence database and given an accession number of MT242381 (PVY-Badr) and MT561046 (PVY-Wadi).

Coat protein nucleotide sequence alignment and phylogenetic relationship analysis among PVY-Badr and PVY-Wadi and several isolates of the same virus kept in GenBank.

After partial sequence of PVY coat protein gene of both PVY-Badr (420 nucleotide long) and PVY-Wadi (345 nucleotide long) isolates was separately determined, and sequences edited and analyzed using Chromas Pro Version 1.34 software. Multiple alignment of the cDNA sequences of the two PVY isolates, PVY-Badr and PVY-Wadi, with those of 25 PVY isolates representing the common strain (PVY^{C}), ordinary strain (PVY^{O}), necrosis strain (PVY^{N}), necrotic tuber necrosis (PVY^{NTN}) and wilga strain (PVY^{W}) retrieved from the GenBank made available through the NCBI was conducted using ClustalW software program version 2 (http://www.ebi.ac.uk./clustalw/), (Larkin *et al*., 2007). A phylogenetic tree showing the nucleotide sequence distance among the sequences of PVY strains (Fig. 10) using MEGA, version 11 software by applying the Maximum likelihood phylogenetic analysis (Visser *et al*., 2012 and Green *et al*., 2017) was generated. Bootstrap resampling value set to 1000 was used to measure the reliability of individual nodes.

The percentage of similarity was calculated using SIAS software program by the pairwise method and found PVY-Badr and PVY- Wadi isolates among 25 PVY isolates investigated here being PVY-Badr (Accession number, MT242381) closely related to PVY^{NTN} strain, Ireland isolate (Accession number, MT264736) with a similarity percentage of 99.76%, and similarity percentage of 99.42% was scored between PVY-Wadi (Accession number, MT561046) with PVY-Wilga strain, China isolate (Accession number, MN607714).

CONCLUSION

Three isolates of *Potato Virus Y* (PVY), PVY-Badr, PVY-Wadi (from El-Beheira) and PVY-Talkha (from Dakahlia) have been recognized as members of the PVY community in Egypt when partially characterised based on the reaction of diagnostic hosts, symptomology, serological reactivity, molecular characteristics using reverse transcription quantitative polymerase chain reaction (RT-qPCR). Alignment of CP gene nucleotide sequence and phylogenetic relationship analysis revealed the high similarity between PVY-Badr isolate and PVY^{NTN} strain, (Ireland isolate) also, between PVY-Wadi isolate and PVY-Wilga strain, China isolate.

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الملخص العربي

توصيف جزئى لثلاث عزلات من فيروس واى البطاطس *Potato virus Y*

محمد محسن زلط، مها عادل كونه، السيد السيد وجيه

أثناء الفحص الميدانى لبعض حقول البطاطس فى محافظات البحيرة والدقهلية وكفر الشيخ بجمهورية مصر العربية وجد أن الأعراض الأكثر انتشاراً على النباتات المصابة طبيعياً هى: الموزايك المعتدل الى الشديد، الأصفرار، البرقشة، تحزم العروق، ارتفاعات موضعية لسطح الورقة و/أو التقزم. هذه الأعراض يتوقع ارتباطها بالإصابة بفيروس واى فى البطاطس (PVY) والتى تم اختبارها باستخدام تفاعل النسخ العكسى وتفاعل البوليميريز المتسلسل الكمى(RT-qPCR) حيث أعطت العينات المختبرة ناتج مضاعفة (PCR-product) طوله ٤٨٠ زوج من القواعد وذلك باستخدام زوج من البادئات المتخصصة لجين الغطاء البروتينى. وقد أعطى الكشف بتفاعل الإليسا غير المباشرة باستخدام المصل المضاد المتخصص لفيروسات PVY, PVX, PVM, PVS نتيجة ايجابية للإصابة بفيروس PVY. وبناءاً أيضاً على الأعراض وشدة المرض على نباتات دخان من نوع جلوتينوزا (*Nicotiana glutinosa*) تم اختيار ثلاث عزلات وتسميتهم "عزلة بدر" و"عزلة وادى" (من محافظة البحيرة) وعزلة طلخا (من محافظة الدقهلية) للاستخدام فى هذه الدراسة. حيث أعطت عدوى سبعة أنواع من العوائل المشخصة التابعة للعائلة الباذنجانية والعائلة القطيفية بالثلاث عزلات مجموعة متباينة من الأعراض المميزة للإصابة بفيروس PVY وكانت الأعراض خفيفة إلى معتدلة ،على التوالى مع العزلتين "بدر" و"طلخا"، بينما كانت الأعراض شديدة فى حالة العزلة "وادى". تم تنقية ناتج RT-qPCR للعزلة "بدر" والعزلة "وادى" وتحديد تتابع جين الغطاء البروتينى لهما باستخدام برنامج .Chromas Pro الإصدار ١,٣٤. أيضا أجريت مقارنة التتابعات لهذه العزلات مع ٢٥ عزلة فيروس PVY معزولة ومسجلة ببنك الجينات مسبقاً وتحليل درجة القرابة (Phylogenetic analysis) بينها، حيث اتضح أن نسبة القرابة للعزلة "بدر" تصل إلى ٩٩,٧٦% مع السلالة PVY ^{NTN} (العزلة الايرلندية) بينما وصلت إلى ٩٩,٤٢% بين العزلة "وادى" والسلالة PVY-Wilga (العزلة الصينية).

الكلمات المفتاحية: فيروس واى البطاطس (PVY)، النسخ العكسى والتفاعل المتسلسل للبوليميريز الكمى، الإليسا غير المباشرة، العوائل المشخصة، تحليل درجة القرابة.