Nucleotide and Amino Acid Sequencing of a Coat Protein of an Egyptian Isolate of Potato Virus Y (PVY)

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ABSTRACT--- ***We are reporting here a molecular tool for detection and identification of a PVY isolate by Nucleotide and amino acid sequencing of coat protein gene. The total RNA was isolated from PVY infected Solanum tuberosum L. leaves and then used as a template for RT-PCR to amplify the cDNA followed by amplification of cp gene (801bp). This followed by sequencing of PVY cp gene and comparing it with (29) PVY overseas strains or isolates. The similarity between our PVY Egyptian isolate and the (29) overseas strains or isolates ranged from 24.4 to 99.4% and from 98.1 to 99.3% based on the level of DNA and deduced amino acids sequence, respectively. In conclusion, our Egyptian virus isolate is PVY strain N.***

Keywords---- PVY, Detection, Polymerase chain reaction, *cp* gene, sequence.

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important crop by production in metric tonnes worldwide, after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.), and the United States is the fifth largest producer by metric tonnes **(FAO 2012).** Potatoes are the number one vegetable crop in the U.S. **(USDA 2012).** Potatoes are cultivated mainly for fresh consumption, processed foods, production of starch, and for seed tubers.

PVY^N is important because a variant of PVY^N, PVY^{NTN}, is associated with tuber symptoms **(Le Romancer *et al.*, 1994)**. Since the 1990s, strains associated with mild foliage symptoms and/or tuber symptoms have increased and are displacing PVY^O **(Gray *et al*,. 2010).**

The PVY genome consists of a single stranded positive sense RNA of about 10 Kb with a VPg protein covalently linked to its 5'-end and a poly-A tail at its 3'-end. It is translated into a single, large polyprotein which is subsequently processed by three virus encoded proteinase into nine gene products: P1, Helper component-proteinase (HC-Pro), P3, 6K1, cytoplasmic inclusion (CI), 6K2, nuclear inclusion a (NIa), nuclear inclusion b and RNA-dependant RNA polymerase (NIb-Pol) and the capsid protein (CP) **(Urcuqui-Inchima *et al.*, 2001)**.

Molecular detection is achieved through reverse transcription polymerase chain reaction (RT-PCR) using primers specific to a strain or a group of strains of the virus **(Massumi *et al.*, 2009)**.

Phylogenic studies are essential in the characterization of plant viruses. They are sources of valuable information on their biological characteristics and possible pathways of evolution. Molecular and phylogenic studies of PVY isolates have been carried out on the coding and noncoding regions of the genome containing useful information **(Margaritopoulos *et al.*, 2009)**. PVY^O, PVY^C and PVY^N isolates have been reported to produce similar phylogenic patterns with any region of the virus genome studied **(Margaritopoulos *et al.*, 2009).**

Phylogenic analyses of African isolates of PVY are not well documented. Therefore the aim of this study was to sequence and establish the phylogenic relation of selected PVY isolates occurring in Egypt with isolates from other parts of the world.

The objectives of the present study are:

I. Molecular characterization of PVY isolate.

1- Isolation of viral RNA.

2- RT-PCR isolation of the coat protein (*CP*) gene of the viral isolate.

3- Determination of the nucleotide sequences of coat protein (*CP*) gene.

4- Sequencing analysis compared with some overseas strains of PVY.

2. REVIEW OF LITERATURE

1. Molecular characterization of PVY isolates.

PVY particles are non-enveloped flexuous filaments (730 x 11 nm) containing a single positive single-strand positive sense ribonucleic acid (RNA) of about 9.7 Kb in length, polyadenylated at the 3'end, and covalently linked via a tyrosine residue to a genome linked protein at its 5' end. PVY encodes a single, large polyprotein which is later processed by three virus-encoded proteinase into nine polypeptides **(Figure 1)** which include the following: P1, Helper component-proteinase (HC-Pro), P3, 6K1, cytoplasmic inclusion (CI), 6K2, nuclear inclusion a (NIa), nuclear inclusion b and RNA-dependant RNA polymerase (NIb-Pol) and the capsid protein (CP) **(Urcuqui-Inchima *et al.* 2001; Hu *et al.* 2009)**. The functions of these proteins are summarized in **Table(1)**.

Table 1 : Function of PVY proteins.

Proteins	Size (KDa)	Functions
P1	32-64	Trypsin-like serine proteinase involved in C terminal autocleavage and in symptomatology.
HC-Pro	50	Multifunctional protein involved in C terminal autocleavage, local and systemic movement, gene silencing suppression, aphid transmission, synergism and symptom development.
P3	37	Involved in plant pathogenecity.
6k1	6	Function still unknown.
CI	70	The protein displays an ATPase and RNA helicase that are involved in local movement of the virus.
6k2	6	Attaches viral replication complex to endoplasmic reticulum-like membranes.
NIa	49	Trypsin-like serine proteinase that processes the polyprotein in *cis* and *trans* to produce functional proteins. It is involved in genome replication (VPg) and protein-protein interaction.
NIb-Pol	58	RNA-dependent RNA polymerase involved in genome replication.
CP	30	Multifunctional protein involved in virus assembly, local and systemic movement and aphid transmission.

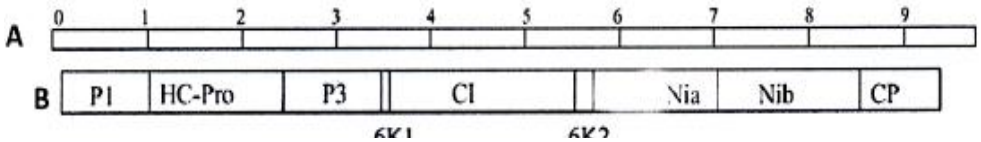

Figure 1: PVY genome organisation. A: Genome length in kb; B: Polypetides .

Mousavi, L. *et al*. (2014) mentioned that the RT-PCR detection of PVY strains by using specific primers resulted in the amplification of DNA fragments specific to the PVY strains NTN, C, O, and N at 725, 1553, 352, and 616 bp, respectively. The highest strain diversity of PVY was detected in the district of Shirehjin in Sarab city and the lowest in the district of Ghaleh Jugh in Bostan-Abad city. Both single and multiple infection types of the PVY stains were observed in the region. Of the 79 PVY-infected samples, 77.21% were infected with strain O, 62.02% with strain C, 39.24% with strain N, and 8.86% with strain NTN. The highest level of multiple infections was observed in the combinations of the strains C+O (27.84%) and the triple strains O+N+C (15.18%). This paper is the first to report the detection of the PVY strain NTN in Iran.

Al-Saikhan, M. S. *et al*. (2014) designed specific primer pairs to amplify the coat protein gene of each virus (627 bp for PLRV, 801 bp for PVY and 714 bp for PVX) were successfully applied. A multiplex RT-PCR (mRT-PCR) was developed for the simultaneous detection of the three viruses in potato leaves.

2. Sequencing studies of PVY strains.

Were, H. K. *et al*. (2013) showed the sequencing of polymerase chain reaction products from PVY-infected plants revealed the presence of recombinant strains of PVY (NTN and Wilga). Four aphid species, Macrosiphum euphorbiae, Aphis gossypii, Myzus persicae, and Aphis fabae, colonized potato in all districts, occurring in greater numbers west of the Great Rift Valley than to the east. There was a positive correlation between virus incidence and aphid numbers in the long rains (main) potato-growing season. PLRV, PVM, PVS, PVX, and PVY were detected in solanaceous weeds. Ralstonia solanacearum was detected in soils from 13 farms in 8 of the 18 districts surveyed. Approximately 38% of soil samples were infested with Meloidogyne spp. Phytophthora infestans isolates belonging to the US 1 and 2-A1 genotypes were identified. Although many economically important diseases are present in Kenya, the lower aphid incidence in districts east of the Great Rift Valley may indicate that these districts are more suitable for seed potato production.

Gao FangLuan *et al*. (2014) studied the complete sequence of GF_YL20, a potato virus Y (PVY) isolate from China, encodes a polyprotein of 3,061 amino acids. Sequence analysis indicates that GF_YL20 has a genomic structure different from previously reported PVY strains. It shares 99% nucleotide sequence identity with PB209 (PVY^{N:O}) except in VPg, but more than 97% nucleotide sequence identity with the VPg of Mont (PVY^N), PB312 (PVY^{NTN}) and HN2 (SYR-I). Phylogenetic analysis indicates that GF_YL20 is a novel N:O recombinant with three recombination breakpoints.

Al-Saikhan, M. S. *et al*. (2014) detected the nucleotide sequences of the coat protein genes of the Saudi isolate of PLRV (PLRV SA3), the Saudi isolate of PVY (PVY SA2) and the Saudi isolate of PVX (PVX SA1) were submitted in the GenBank under accession numbers: KC875235, KC875237 and KC875236, respectively. The nucleotide sequences PLRV SA3, PVY SA2 and PVX SA1 were compared to the sequences of the coat protein genes of other PLRV, PVY and PVX isolates. The similarity of the nucleotide sequences suggested that the architecture of the polerovirus (PLRV), potyvirus (PVY) and potexvirus (PVX) are highly conserved. This study describes an assay where three common potato-infecting viruses, Potato leafroll virus, Potato virus Y and Potato virus X, were detected simultaneously from total RNA potato leaves in a multiplex RT-PCR.

3. MATERIALS AND METHODS

This investigation was carried out at AGERI, ARC, Giza, Egypt.

Potato plants (*Solanun tuberosum* L.) (exhibiting faint mosaic and infected with PVY) were transplanted at AGERI greenhouse under a controlled temp. (24^{o}-30^{o}) and the following tests were carried out:

1. Molecular identification of the PVY isolate.

1.1. Isolation of the PVY *CP* gene.

1.1.1. RNA extraction.

Fresh PVY infected leaves isolate were collected and kept on ice over all the time of work.

- The Spin or Vacuum (SV) Total RNA Isolation System (Promega, USA) was used for isolating total RNA according to the manufacturer's instructions. All equipments used during the isolation procedure was made RNase-free. Glass wares were baked at 200oC overnight. Plastic ware and solutions were made RNase-free by adding diethyl pyrocarbonate (DEPC) to 0.1% (v/v) and then incubated overnight at room temp. Traces of DEPC were removed by autoclaving for 30min. 0.05 g of frozen ground tissue or fresh tissue were added to a grinder tube on ice and then 3 ml cell lysis solution were added.

- The frozen tissues were finely grounded in liquid nitrogen using porcelain mortar and pestle.

- Homogenization was done using 10 strokes with a tube pestle. Sample was transferred to an Oak Ridge centrifuge tube. One ml protein-DNA precipitation solution was added to the cell lysate.

- The tube was inverted gently 10 times and placed into an ice bath for 10 min. After 5 min. centrifugation at 15000 rpm, the precipitated proteins and DNA formed a tight pellet.

- The supernatant containing the RNA was poured into a clean Oak Ridge centrifuge tube containing 3 ml 99% isopropanol.

- The tube was gently inverted 50 times, centrifuged at 15000 rpm for 5 min. and the RNA was visible as a translucent pellet.

- The supernatant was poured off and the tube was dried on clean absorbent paper.

- Three ml of 70% ethanol were then added followed by inver- ting the tube several times to wash the RNA pellet.

- The sample was centrifuged at 15000 rpm for 2 min. and the ethanol was carefully poured off.

- The tube was inverted and drained on a clean absorbent paper and sample was allowed to air drying for 15 min., 300 µl of RNA hydration buffer were added to the RNA pellet and left for 30 min. on ice, vortexed vigorously for 5 sec. and carefully transferred to a 1.5 ml microcentrifuge tube and then stored at –70oC until use.

1.1.2. RT-PCR analysis.

All oligonucleotides used through this study were designed according to the nucleotide sequence of the *cp* gene of PVY as shown in **Table (2).** They were designed by DNASTAR lasergene (DNASTAR Inc., MD) based on PVY sequences in GenBank Accession # (D00441, X97895, U09509) and synthesized at AGERI, ARC, Giza, Egypt. The oligonucleotides were ethanol precipitated, dissolved in d.H2O and quantitated by determining the OD at 260 nm **(Sambrook et al., 1989)**.

Table 2 : The nucleotide sequences of the primers used for RT-PCR analysis.

Primer	Size	Sequences (5'----------3')
cp F	24	ATGGSAAATGACACAATYGATGCA
cp R	25	TCACATGTTYTTSACTCCAAGYAG

1.1.3. Protocol using QIAGEN one step RT-PCR kit.

RNA template was thawed, then primer solution, dNTPs mix., 5 X QIAGEN one step RT-PCR buffer and RNase free water were added and placed on ice. A master mix. was prepared as described in **Table (3)**. The master mix. was mixed and dispensed as appropriate volume into PCR tubes. Template RNA was added to the individual PCR tubes. The thermal cycle was programed according to the profile as following:

40 Cycle	94oC	for 1 min. (denaturation)
	50oC	for 2 min. (annealing)
	72oC	for 2 min. (extension)
1 Cycle	72oC	for 5min. (final extension)
1 Cycle	4oC (Stored)	

Table 3: Master mix for RT-PCR analysis.

Components	Volume/reaction
5X QIGEN one step RT-PCR buffer	10.0 µl
dNTPs mix. (containing 10 mM of each dNTP)	2.0 µl
Primer *cp*F (10 Picomol/µl)	1 µl
Primer *cp*R (10 Picomol/µl)	1 µl
RNA Template (10 µg/µl)	1.5 µl
Rnase-free water	Variable
Rnase inhibitor	5-10 units
QIAGEN one step RT-PCR Enzyme Mix	2.0 µl
Total volume	50.0 µl

1.1.4. Agarose gel electrophoresis.

The PCR amplified product was detected by electro- hporesis as described by Sambrook et al. (1989) using 1% agarose gel in 1 X TAE buffer composed of: (40mM tris acetate, 1mM EDTA, PH 8.0) at 80 volts for one h. The DNA was visualized by staining gel in ethidium bromide (0.5 µg/ml) and photographed under UV transilluminator using a polaroid camera.

2.1. Nucleotide sequencing of PVY *Cp* gene.

2.1.1. Sequence reaction.

The PCR product,801bp was sent to the Sequencing Laboratory for studying the nucleotide sequence of the PCR product. In this experiment, the ABI PRISM Model 310, Version 3.4, SemiAdaptive Version 3.2, Genetic Analyzer was applied for automated DNA sequencing.

2.1.2. Sequences data collection.

Using the NUC directory, European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany and site http:// www.ncbi.nlm.nih.gov, DNA sequences related to PVY *CP* gene of different strains or isolates were collected. These strains or isolates are:

AF255659	Potato virus Y isolate PVY-OBR polyprotein gene, partial cds.
AJ390292	Potato virus Y partial genomic RNA for polyprotein gene, isolate v951204
EF027888	Potato virus Y isolate v983805 coat protein gene, partial cds.
AF525081	Potato virus Y polyprotein mRNA, partial cds.
GQ853652	Potato virus Y isolate CC55-8-146 coat protein gene, partial cds.
GQ853648	Potato virus Y isolate WW154A-62-86 coat protein gene, partial cds.
GU074000	Potato virus Y isolate Dalian coat protein gene, partial cds.
EU073859	Potato virus Y isolate aL-Ramtha coat protein gene, partial cds.
GQ853653	Potato virus Y isolate CC66-91-47 coat protein gene, partial cds.
GQ853634	Potato virus Y isolate N484-1 coat protein gene, partial cds.
HM036200	Potato virus Y isolate Ur2-PVYCP1 coat protein gene, partial cds.
JF804784	Potato virus Y isolate 08-sz polyprotein gene, partial cds.
JN034575	Potato virus Y isolate BL coat protein gene, partial cds.
JN635310	Potato virus Y isolate Ningxia-2011GYY.1 coat protein gene, partial cds.
JQ954297	Potato virus Y strain German-34*2004 isolate 34 capsid protein gene, partial cds.
JQ954298	Potato virus Y strain German-35*2004 isolate 35 capsid protein gene, partial cds.
JQ954299	Potato virus Y strain German-37*2004 isolate 37 capsid protein gene, partial cds.
JQ954301	Potato virus Y strain German-39*2004 isolate 39 capsid protein gene, partial cds
JQ954308	Potato virus Y strain German-52*2004 isolate 52 capsid protein gene, partial cds.
JQ954311	Potato virus Y strain German-57*2004 isolate 57 capsid protein gene, partial cds.
JQ954312	Potato virus Y strain German-58*2004 isolate 58 capsid protein gene, partial cds.
JQ954327	Potato virus Y strain PB-702*1957 isolate 702 capsid protein gene, partial cds.
JQ954344	Potato virus Y strain TC-2-196*2006 isolate 2-196 capsid protein gene, partial cds.
JQ954345	Potato virus Y strain TC-2-197*2006 isolate 2-197 capsid protein gene, partial cds.
JQ954354	Potato virus Y strain US05-3*2005 isolate 3 capsid protein gene, partial cds
JQ954328	Potato virus Y strain PB-707*1958 isolate 707 capsid protein gene, partial cds.
JQ954369	Potato virus Y strain US06-52*2006 isolate 52 capsid protein gene, partial cds.
JQ954376	Potato virus Y strain SLO4*2009 isolate SLO4 capsid protein gene, partial cds.
JQ954387	Potato virus Y strain German-20*2004 isolate 20 capsid protein gene, partial cds.

2.1.3. Sequences alignment.

The previous sequences of PVY isolates or strains were aligned with the nucleotide sequence of the PCR product using the ClustalW Sequence Analysis Software **(Al-Saikhan, M. S. *et al*. 2014)**. DNA sequence was translated to protein using EditSeq program, DNA and protein sequences of our viral isolate and overseas PVY strains were alignment using ClustalW program.

4. RESULTS

1. Molecular characterization of PVY isolate.

1.1. Isolation of viral RNA.

The total RNA was isolated from PVY infected potato samples; that gave positive ELISA value with PVY specific antiserum; using SV total RNA isolation system with high quality and substantially free of genomic DNA contamination (**Figure2**).

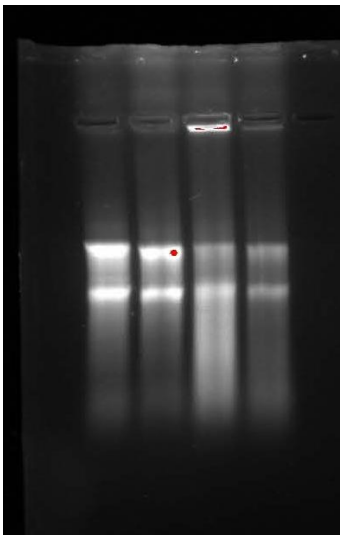

Figure 2: 1% agarose gel electrophoresis stained with ethidium bromide showing the total RNA extracted from PVY infected potato samples.

1.2. RT-PCR isolation of coat protein (*cp*) gene of the virus isolate.

The RNA was then used as a template for RT-PCR to amplify the cDNA *via* the QIAGEN one step RT-PCR system by use of an oligo (dT), as a common primer.

Nearly full length cDNA could be synthesized and the (*cp*) gene (a size of about 801 bp) was amplified as shown in **Figure 3** using a specific antisense primer (*cp*R) and forward primer (*cp*F) designed by DNASTAR lasergene (DNASTAR Inc., MD) based on PVY sequences in GenBank Accession # (D00441, X97895, U09509) to amplify the *cp* gene of our PVY Egyptian isolate as shown in **Table (6)**.

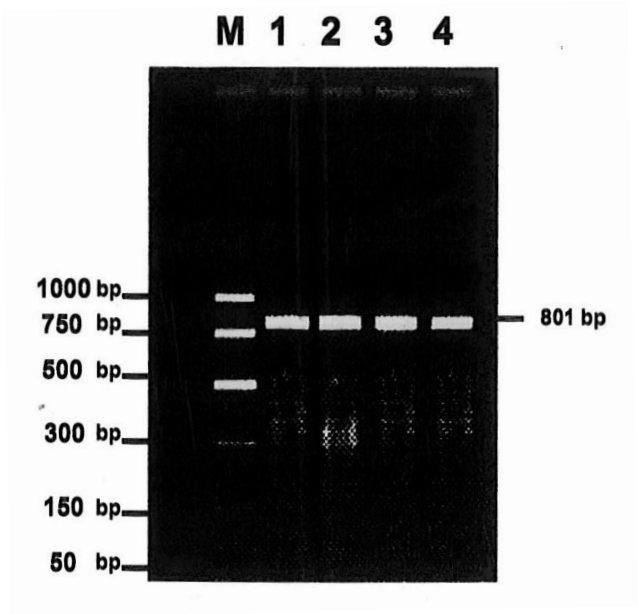

Figure 3: 1% agarose gel electrophoresis stained with ethidium bromide showing the amplification of PVY *cp* gene (801 bp) *via* RT-PCR using the extracted total RNA from PVY infected potato samples. M: DNA molecular marker (50 – 1000 bp).

2.1. Determination of nucleotide sequence of the coat protein gene.

Coat protein (*cp*) gene sequencing was found to be composed 801 nucleotides **(Figure 4)**; depending on the comparison *cp* gene of PVY under study was found to be composed of : 268 (A, 33.4%), 201 (G, 25.09%), 168 (T, 20.9%) and 164 (C, 20.47%); and the deduced amino acid, 267.

All of determined using computer software **(Figure 4 and 5)**.

1	GCAAATGACA	CAATTGATGC	AGGAGGAAGC	AACAGGAAAG	ATGCAAAACC	AGAGCAGGGC
61	AGCAACCAGC	CAAACCCGAA	CAAAGGAAAG	GATAAGGATG	TTAATGCAGG	CACATCTGGG
121	ACACATACTG	TGCCGAGAAT	CAAGGCTATC	ACGTCCAAAA	TGAGAATGCC	CACAAGCAAG
181	GGAGCAACCG	TGCTAAACTT	AGAACATTTG	CTTGAGTATG	CTCCACAACA	AATTGATATT
241	TCAAATACTC	GGGCAACTCA	ATCACAGTTT	GATACGTGGT	ATGAGGCAGT	GCGGATGGCA
301	TACGACATAG	GAGAAACTGA	GATGCCAACT	GTGATGAATG	GGCTTATGGT	TTGGTGCATT
361	GAAAATGGAA	CCTCGCCAAA	TGTCAACGGA	GTTTGGGTTA	TGATGGATGG	GAATGAACAA
421	GTTGAGTACC	CGTTGAAACC	AATCGTTGAG	AATGCAAAAC	CAACCCTTAG	GCAAATCATG
481	GCACATTTCT	CAGATGTTGC	AGAAGCGTAT	ATAGAAATGC	GCAACAAAAA	GGAACCATAT
541	ATGCCACGAT	ATGGTTTAAT	TCGAAATCTG	CGGGATATGG	GTTTAGCGCG	TTATGCCTTT
601	GACTTTTATG	AGGTCACATC	ACGAACACCA	GTGAGGGCTA	GGGAAGCGCA	CATTCAAATG
661	AAGGCCGCAG	CATTGAAATC	AGCCCAACCT	CGACTTTTCG	GGTTGGACGG	TGGCATCAGT
721	ACACAAGAGG	AGAACACAGA	GAGGCACACC	ACCGAGGATG	TCTCTCCAAG	TATGCATACT
781	CTACTTGGAG	TCAAGAACAT	G			

Figure 4: showing DNA sequences of 801 bp nucleotides of *cp* gene of PVY Egyptian isolate.

A N D T I *D A G G S	N K K D A K P E Q G	20
S I Q P N P N K G K	D K D V N A G T S G	40
T H T V P R I K A I	T S K M R M P T S K	60
G A T V L N L E H L	L E Y A P Q Q I D I	80
S N T R A T Q S Q F	D T W Y E A V R M A	100
Y D I G E T E M P T	V M N G L M V W C I	120
E N G T S P N V N G	V W V M M D G N E Q	140
V E Y P L K P I V E	N A K P T L R Q I M	160
A H F S D V A E A Y	I E M R N K K E P Y	180
M P R Y G L I R N L	R D M G L A R Y A F	200
D F Y E V T S R T P	V R A R E A H I Q M	220
K A A A L K S A Q P	R L F G L D G G I S	240
T Q E E N T E R H T	T E D V S P S M H T	260
L L G V K N M		

Figure 5: showing deduced sequences of amino acids 267 of *cp* gene of PVY Egyptian isolate.

2.2. sequencing analysis compared with some other strains of the virus isolate.

The sequences of the *cp* gene of PVY isolate under study was compared and aligned with the sequence of 29 isolates or strains as follow:

AF255659	Potato virus Y isolate PVY-OBR polyprotein gene, partial cds.
AJ390292	Potato virus Y partial genomic RNA for polyprotein gene, isolate v951204
EF027888	Potato virus Y isolate v983805 coat protein gene, partial cds.
AF525081	Potato virus Y polyprotein mRNA, partial cds.
GQ853652	Potato virus Y isolate CC55-8-146 coat protein gene, partial cds.
GQ853648	Potato virus Y isolate WW154A-62-86 coat protein gene, partial cds.
GU074000	Potato virus Y isolate Dalian coat protein gene, partial cds.
EU073859	Potato virus Y isolate aL-Ramtha coat protein gene, partial cds.
GQ853653	Potato virus Y isolate CC66-91-47 coat protein gene, partial cds.
GQ853634	Potato virus Y isolate N484-1 coat protein gene, partial cds.
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JQ954376	Potato virus Y strain SLO4*2009 isolate SLO4 capsid protein gene, partial cds.
JQ954387	Potato virus Y strain German-20*2004 isolate 20 capsid protein gene, partial cds.

The aligment was carried out using the ClustalW sequences Analysis software for the nucleotide, sequences and it's deduced amino acids sequences of our PVY Egyptian isolate and the previous mentioned twenty nine overseas PVY strains or isolates.

On the basis of the nucleotide sequences, our PVY Egyptian isolate was confirmed to be PVY strain N and their similarity was: (99.3, 99.4, 99.3, 27.3, 99.4, 99.1, 27.5, 98.5, 98.5, 24.2, 26.1, 98.4, 24.2, 24.3, 98.8, 98.4, 98.4, 98.4, 98.4, 98.4, 98.4, 98.4, 98.4, 98.9, 24.5, 24.3, 98.4 and 98.9%) **(Table 4)** with the previous PVY strains or isolates respectively.

On other hand, the similarity of deduced amino acids sequences between our PVY Egyptian isolate and previous PVY strains or isolates was: (99.3, 98.9, 98.9, 98.9, 98.9, 98.9, 98.5, 98.5, 98.5, 98.5, 98.9, 98.5, 98.5, 98.5, 98.5, 98.5, 98.5, 99.2, 98.5, 98.5, 98.1, 98.1, 98.5, 98.1, 98.1, 98.9, 98.1, 98.1 and 98.1%) **(Table 5)** respectively.

Based on the phylogenetic analysis for the nucleotide sequence of *cp* gene (801 bp), two main groups were observed **(Figure 6)**.

First group include: (JQ 954345.I, JQ 954344.I, JQ954312.I, JQ954308.I, GU074000.I, JQ954298, JQ954299.I, JQ954301.I, JQ954311.I, JQ954387.I, JQ954297, JN635310.I, JU954376.I, JQ954354.I, EU073889.I, HM036200.I, JN034575.I, EF0227888.I, JQ954328.I, JQ954327.I, JQ954369.I and our untitled PVY isolate).

Second group include: (GQ 853652.I, GQ853648.I, GQ853634.I, GQ853653.I, AF525081.I, JF 804784.I, AJ390292.I and AF255659.I).

On other direction the alignment of amino acids of the previous strains or isolates showed the presence of two groups as in **(Figure 7)**.

First group include: (AB015902.I, AB015931.I, ACZ26409.I, AF084370.I, AF084322.I, AF084334.I, AE254790.I, ACZ26414.I, AB015918.I, ACZ26393.I, AF084381.I, AB015923.I, ACZ26420.I, AAW28830.I, AAW28827.I, AF084353.I, ADP21130.I, CAJ87129.I, AAW28824.I, CAJ87128.I, ACD99638.I, ADP21119.I, AAW28834.I, AAW28831.I, AAT41429.IM, AAW28826.I, AAW28828.I and ACD99636.I).

The second group include only our untitled PVY isolate.

Sequence pair distances of Untitled ClustalW (Weighted) Page 1
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Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
1		99.3	99.4	99.3	27.3	99.4	99.1	27.5	98.5	98.5	24.2	26.1	98.4	24.2	24.3	98.8	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.9	24.5	24.3	98.4	98.9	1	Untitled
2	0.8		99.4	99.3	27.2	98.9	99.1	27.5	98.5	98.5	24.6	25.8	98.4	24.1	24.2	98.9	98.6	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.4	98.9	24.3	24.2	98.4	98.9	2	JQ954328.1
3	0.6	0.6		99.9	27.1	99.0	99.8	27.3	98.4	98.4	24.3	25.8	98.3	23.8	24.0	98.8	98.3	98.4	98.3	98.3	98.3	98.3	98.3	98.3	98.5	98.8	24.1	24.0	98.3	98.8	3	EF027888.1
4	0.8	0.8	0.1		27.2	98.9	99.6	27.2	98.3	98.3	24.2	26.0	98.1	23.8	24.0	98.6	98.1	98.3	98.1	98.1	98.1	98.1	98.1	98.1	98.4	98.6	24.1	24.0	98.1	98.6	4	JQ954327.1
5	295.5	309.9	330.2	318.2		27.3	27.0	26.5	26.8	27.1	24.7	27.2	27.0	25.4	25.0	27.1	27.0	27.0	26.8	26.7	27.0	26.8	26.8	26.7	26.8	27.2	25.2	25.4	26.7	27.2	5	AF255659.1
6	0.6	1.1	1.0	1.1	295.5		98.8	27.2	98.1	98.1	24.0	26.0	98.0	24.1	24.2	98.4	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.5	24.3	24.2	98.0	98.5	6	JQ954369.1
7	0.9	0.9	0.3	0.4	364.8	1.3		27.5	98.1	98.1	24.2	25.8	98.0	24.0	24.1	98.5	98.0	98.1	98.0	98.0	98.0	98.0	98.0	98.0	98.3	98.5	24.2	24.1	98.0	98.5	7	JN034575.1
8	350.0	350.0	350.0	350.0	284.2	350.0	350.0		27.1	27.1	27.8	26.7	27.0	27.4	27.4	27.2	27.2	27.1	27.1	27.1	27.1	27.0	27.2	27.0	27.1	27.5	27.3	27.3	27.1	27.2	8	AF525081.1
9	1.5	1.5	1.6	1.8	350.0	1.9	1.9	350.0		99.8	24.1	25.6	99.6	24.2	24.3	99.5	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.1	24.5	24.3	99.9	99.4	9	JQ954387.1
10	1.5	1.5	1.6	1.8	330.2	1.9	1.9	350.0	0.3		24.0	25.6	99.6	24.2	24.3	99.5	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.1	24.5	24.3	99.6	99.4	10	JQ954376.1
11	350.0	350.0	350.0	350.0	350.0	350.0	350.0	223.0	350.0	350.0		26.8	24.1	26.8	26.6	24.3	24.2	24.2	24.1	24.1	24.2	24.2	24.2	24.0	24.1	24.5	26.5	26.5	24.0	24.2	11	JF804784.1
12	335.3	349.7	349.7	333.0	321.7	342.0	349.7	307.4	363.2	363.2	303.9		25.7	25.9	25.9	25.6	25.6	25.5	25.5	25.6	25.7	25.6	25.6	25.6	25.7	25.6	25.8	25.9	25.6	25.8	12	AJ390292.1
13	1.6	1.6	1.8	1.9	398.7	2.0	2.0	350.0	0.4	0.4	350.0	349.2		24.2	24.3	99.4	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.0	24.5	24.3	99.5	99.3	13	JN635310.1
14	350.0	350.0	350.0	350.0	350.0	350.0	350.0	259.1	350.0	350.0	291.3	350.0	350.0		99.3	24.1	24.2	24.1	24.2	24.2	24.1	24.3	24.2	24.2	24.2	24.1	99.3	99.5	24.2	24.3	14	GQ853653.1
15	350.0	350.0	350.0	350.0	350.0	350.0	350.0	260.7	350.0	350.0	304.2	350.0	350.0	0.8		24.2	24.3	24.2	24.3	24.3	24.2	24.5	24.3	24.3	24.3	24.2	99.5	99.0	24.3	24.5	15	GQ853634.1
16	1.3	1.1	1.3	1.4	330.2	1.6	1.5	350.0	0.5	0.5	350.0	363.2	0.6	350.0	350.0		99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	24.3	24.2	99.4	99.6	16	JQ954354.1
17	1.6	1.4	1.8	1.9	364.8	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.0	24.5	24.3	99.8	99.3	17	JQ954345.1
18	1.6	1.6	1.6	1.8	398.7	2.0	1.9	350.0	0.1	0.4	350.0	380.7	0.5	350.0	350.0	0.6	0.3		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.0	24.3	24.2	99.8	99.3	18	JQ954344.1
19	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	383.5	0.5	350.0	350.0	0.6	0.3	0.3		99.8	99.8	99.8	99.8	99.8	99.8	99.0	24.5	24.3	99.8	99.3	19	JQ954312.1
20	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6	0.3	0.3	0.3		99.8	99.8	99.8	99.8	99.8	99.0	24.5	24.3	99.8	99.3	20	JQ954311.1
21	1.6	1.6	1.8	1.9	364.8	2.0	2.0	350.0	0.1	0.4	350.0	348.8	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3		99.8	99.8	99.8	99.8	99.0	24.3	24.2	99.8	99.3	21	JQ954308.1
22	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3	0.3		99.8	99.8	99.8	99.0	24.6	24.5	99.8	99.3	22	JQ954301.1
23	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3	0.3	0.3		99.8	99.8	99.0	24.5	24.3	99.8	99.3	23	JQ954299.1
24	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3		99.8	99.0	24.5	24.3	99.8	99.3	24	JQ954298.1
25	1.6	1.6	1.5	1.6	350.0	2.0	1.8	350.0	0.1	0.4	350.0	348.8	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3		99.0	24.5	24.3	99.8	99.3	25	JQ954297.1
26	1.1	1.1	1.3	1.4	318.2	1.5	1.5	350.0	0.9	0.9	350.0	363.9	1.0	350.0	350.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		24.3	24.2	99.0	99.8	26	HM036200.1
27	350.0	350.0	350.0	350.0	350.0	350.0	350.0	268.7	350.0	350.0	311.6	350.0	350.0	0.8	0.5	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0		99.3	24.5	24.6	27	GQ853652.1
28	350.0	350.0	350.0	350.0	350.0	350.0	350.0	266.6	350.0	350.0	307.4	350.0	350.0	0.5	1.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	350.0	0.8		24.3	24.5	28	GQ853648.1
29	1.6	1.6	1.8	1.9	350.0	2.0	2.0	350.0	0.1	0.4	350.0	363.2	0.5	350.0	350.0	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.0	350.0	350.0		99.3	29	GU074000.1
30	1.1	1.1	1.3	1.4	318.2	1.5	1.5	350.0	0.6	0.6	350.0	351.3	0.8	350.0	350.0	0.4	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.3	350.0	350.0	0.8		30	EU073859.1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		

Table 4: Percents of similarity between the DNA sequences of cp gene of PVY Egyptian isolate and the other (29) overseas isolates or strains.

Sequence pair distances of Untitled ClustalW (Slow/Accurate, Gonnet) Page 1
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Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
1		99.3	98.9	98.9	98.9	98.9	98.5	98.5	98.5	98.5	98.9	98.5	98.5	98.5	98.5	98.5	98.5	99.2	98.5	98.5	98.1	98.1	98.5	98.1	98.1	98.9	98.1	98.1	98.1	1	Untitled
2	0.8		98.1	98.1	99.6	99.6	99.3	97.8	97.8	97.8	99.6	99.3	97.8	97.8	99.3	97.8	99.3	100.0	99.3	99.3	98.9	98.9	99.3	98.9	98.9	99.6	98.9	98.9	98.9	2	AAW28826.1
3	1.1	1.9		100.0	98.5	97.8	98.1	99.6	99.6	99.6	97.8	98.1	99.6	99.6	97.4	99.6	98.1	98.1	98.1	98.1	97.8	98.5	97.4	97.8	99.3	97.7	99.3	97.0	97.8	3	ABO15902.1
4	1.1	1.9	0.0		98.5	97.8	98.1	99.6	99.6	99.6	97.8	98.1	99.6	99.6	97.4	99.6	98.1	98.1	98.1	98.1	97.8	98.5	97.4	97.8	99.3	97.7	99.3	97.0	97.8	4	ACZ26409.1
5	1.1	0.4	1.5	1.5		99.3	99.6	98.1	98.1	98.1	99.3	98.9	98.1	98.1	98.9	98.1	98.9	99.6	98.9	98.9	99.3	98.5	98.9	98.5	99.3	99.2	99.3	98.5	98.5	5	AAT81429.1
6	1.1	0.4	2.3	2.3	0.8		98.9	97.4	97.4	97.4	99.3	98.9	97.4	97.4	99.6	97.4	98.9	99.6	98.9	98.9	99.3	98.5	99.6	98.5	98.5	100.0	98.5	99.3	98.5	6	CAJ87129.1
7	1.5	0.8	1.9	1.9	0.4	1.1		97.8	97.8	97.8	98.9	98.5	97.8	97.8	98.5	97.8	98.5	99.2	98.5	98.5	99.6	98.1	98.5	98.9	98.9	98.9	98.9	98.1	98.1	7	AAW28834.1
8	1.5	2.3	0.4	0.4	1.9	2.7	2.3		99.3	99.3	97.4	97.8	99.3	99.3	97.0	99.3	97.8	97.7	97.8	98.5	97.4	98.1	97.0	97.4	98.9	97.4	98.9	96.6	97.4	8	AFO84370.1
9	1.5	2.3	0.4	0.4	1.9	2.7	2.3	0.8		99.3	97.4	97.8	99.3	99.3	97.0	99.3	97.8	97.7	98.5	97.8	97.4	98.1	97.0	98.1	98.9	97.4	98.9	96.6	97.4	9	AFO84334.1
10	1.5	2.3	0.4	0.4	1.9	2.7	2.3	0.8	0.8		97.4	97.8	99.3	100.0	97.8	99.3	97.8	98.1	97.8	97.8	97.4	98.1	97.0	97.4	98.9	97.7	98.9	96.6	97.4	10	AEZ54790.1
11	1.1	0.4	2.3	2.3	0.8	0.8	1.1	2.7	2.7	2.7		98.9	97.4	97.4	98.9	97.4	98.9	99.6	98.9	98.9	98.5	98.5	98.9	98.5	98.5	99.2	98.5	98.5	98.5	11	AAW28828.1
12	1.5	0.8	1.9	1.9	1.1	1.1	1.5	2.3	2.3	2.3	1.1		97.8	97.8	98.5	97.8	99.3	99.2	99.3	99.3	98.1	99.6	98.5	98.9	98.9	98.9	98.9	98.1	99.6	12	AFO84381.1
13	1.5	2.3	0.4	0.4	1.9	2.7	2.3	0.8	0.8	0.8	2.7	2.3		99.3	97.0	99.3	97.8	97.7	97.8	97.8	97.4	98.1	97.0	97.4	98.9	97.4	98.9	96.6	98.1	13	AFO84322.1
14	1.5	2.3	0.4	0.4	1.9	2.7	2.3	0.8	0.8	0.0	2.7	2.3	0.8		97.8	99.3	97.8	98.1	97.8	97.8	97.4	98.1	97.0	97.4	98.9	97.7	98.9	96.6	97.4	14	ACZ26414.1
15	1.5	0.8	2.7	2.7	1.1	0.4	1.5	3.1	3.1	2.3	1.1	1.5	3.1	2.3		97.0	98.5	99.6	98.5	98.5	98.9	98.1	99.3	98.1	98.1	100.0	98.1	98.9	98.1	15	CAJ87128.1
16	1.5	2.3	0.4	0.4	1.9	2.7	2.3	0.8	0.8	0.8	2.7	2.3	0.8	0.8	3.1		97.8	97.7	97.8	97.8	97.4	98.1	97.0	97.4	98.9	97.4	98.9	96.6	97.4	16	ABO15931.1
17	1.5	0.8	1.9	1.9	1.1	1.1	1.5	2.3	2.3	2.3	1.1	0.8	2.3	2.3	1.5	2.3		99.2	99.3	99.3	98.1	98.9	98.5	98.9	98.9	98.9	98.9	98.9	98.9	17	ADP21130.1
18	0.8	0.0	1.9	1.9	0.4	0.4	0.8	2.3	2.3	1.9	0.4	0.8	2.3	1.9	0.4	2.3	0.8		99.2	99.2	98.9	98.9	99.2	98.9	98.9	99.6	98.9	98.9	98.9	18	ACD99636.1
19	1.5	0.8	1.9	1.9	1.1	1.1	1.5	2.3	1.5	2.3	1.1	0.8	2.3	2.3	1.5	2.3	0.8	0.8		99.3	98.1	98.9	98.5	99.6	98.9	98.9	98.9	98.1	98.9	19	AAW28830.1
20	1.5	0.8	1.9	1.9	1.1	1.1	1.5	1.5	2.3	2.3	1.1	0.8	2.3	2.3	1.5	2.3	0.8	0.8	0.8		98.1	98.9	98.5	98.9	98.9	98.9	98.9	98.1	98.9	20	AFO84353.1
21	1.9	1.1	2.3	2.3	0.8	0.8	0.4	2.7	2.7	2.7	1.5	1.9	2.7	2.7	1.1	2.7	1.9	1.1	1.9	1.9		97.8	98.9	98.5	98.5	99.2	98.5	98.5	97.8	21	AAW28831.1
22	1.9	1.1	1.5	1.5	1.5	1.5	1.9	1.9	1.9	1.9	1.5	0.4	1.9	1.9	1.9	1.9	1.1	1.1	1.1	1.1	2.3		98.1	98.5	99.3	98.5	99.3	97.8	99.3	22	ACZ26420.1
23	1.5	0.8	2.7	2.7	1.1	0.4	1.5	3.1	3.1	3.1	1.1	1.5	3.1	3.1	0.8	3.1	1.5	0.8	1.5	1.5	1.1	1.9		98.1	98.1	99.6	98.1	98.9	98.1	23	AAW28824.1
24	1.9	1.1	2.3	2.3	1.5	1.5	1.1	2.7	1.9	2.7	1.5	1.1	2.7	2.7	1.9	2.7	1.1	1.1	0.4	1.1	1.5	1.5	1.9		98.5	98.5	98.5	97.8	98.5	24	AAW28827.1
25	1.9	1.1	0.8	0.8	0.8	1.5	1.1	1.1	1.1	1.1	1.5	1.1	1.1	1.1	1.9	1.1	1.1	1.1	1.1	1.1	1.5	0.8	1.9	1.5		98.5	100.0	97.8	98.5	25	ABO15918.1
26	1.1	0.4	2.3	2.3	0.8	0.0	1.1	2.7	2.7	2.3	0.8	1.1	2.7	2.3	0.0	2.7	1.1	0.4	1.1	1.1	0.8	1.5	0.4	1.5	1.5		98.5	99.2	98.5	26	ACD99638.1
27	1.9	1.1	0.8	0.8	0.8	1.5	1.1	1.1	1.1	1.1	1.5	1.1	1.1	1.1	1.9	1.1	1.1	1.1	1.1	1.1	1.5	0.8	1.9	1.5	0.0	1.5		97.8	98.5	27	ACZ26393.1
28	1.9	1.1	3.1	3.1	1.5	0.8	1.9	3.5	3.5	3.5	1.5	1.9	3.5	3.5	1.1	3.5	1.1	1.1	1.9	1.9	1.5	2.3	1.1	2.3	2.3	0.8	2.3		97.8	28	ADP21119.1
29	1.9	1.1	2.3	2.3	1.5	1.5	1.9	2.7	2.7	2.7	1.5	0.4	1.9	2.7	1.9	2.7	1.1	1.1	1.1	1.1	2.3	0.8	1.9	1.5	1.5	1.5	1.5	2.3		29	ABO15923.1
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		

Table 5: Percents of similarity between the amino acids sequences of cp gene of PVY Egyptian isolate and the other (29) overseas isolates or strains.

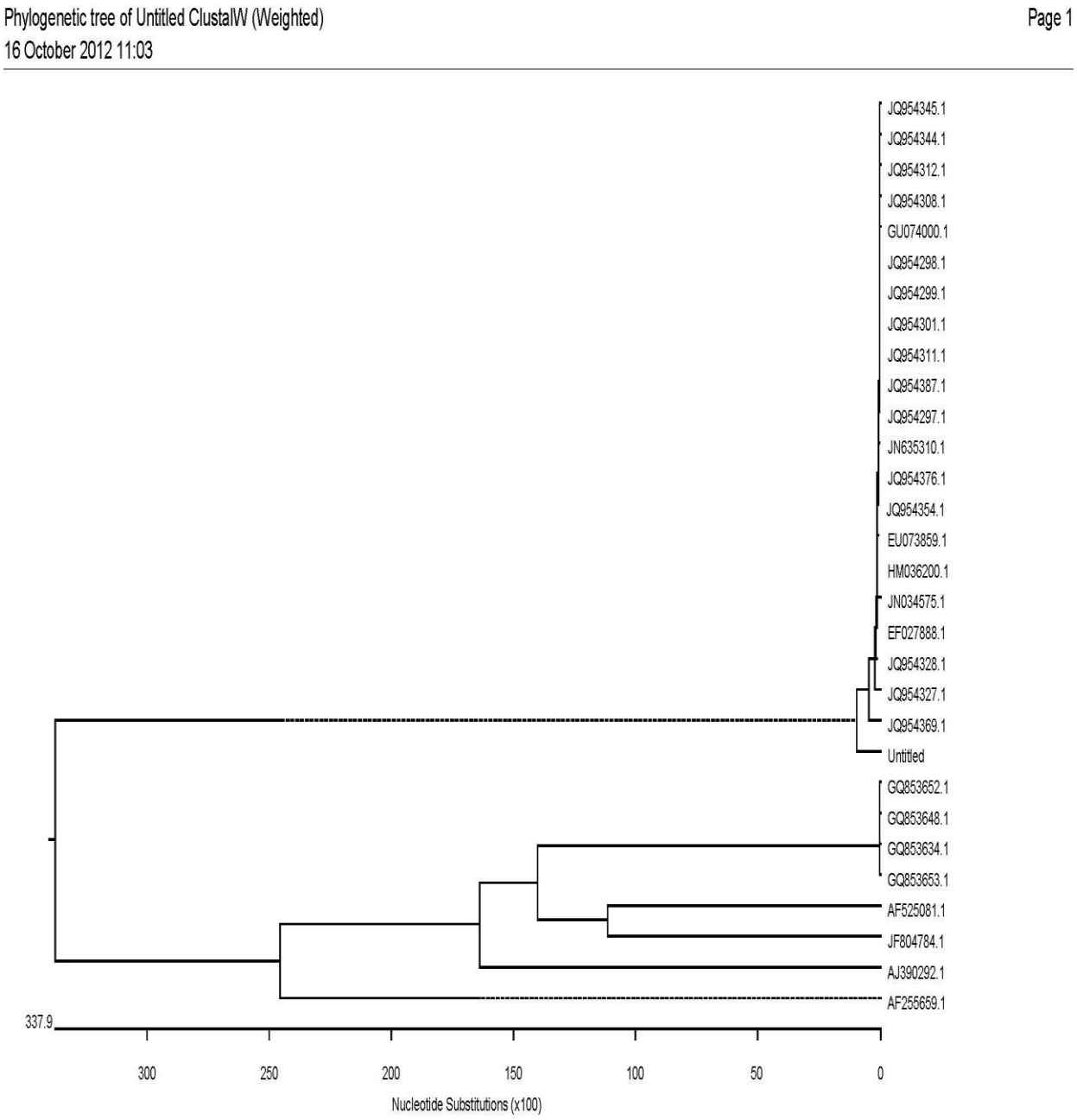

Figure 6: Phylogenetic tree of aligned sequences of different PVY strains, built with ClustalW programme. A tree based on the DNA sequences of *cp* gene (801)bp of PVY Egyptian isolate compared to (29) overseas isolates of strains.

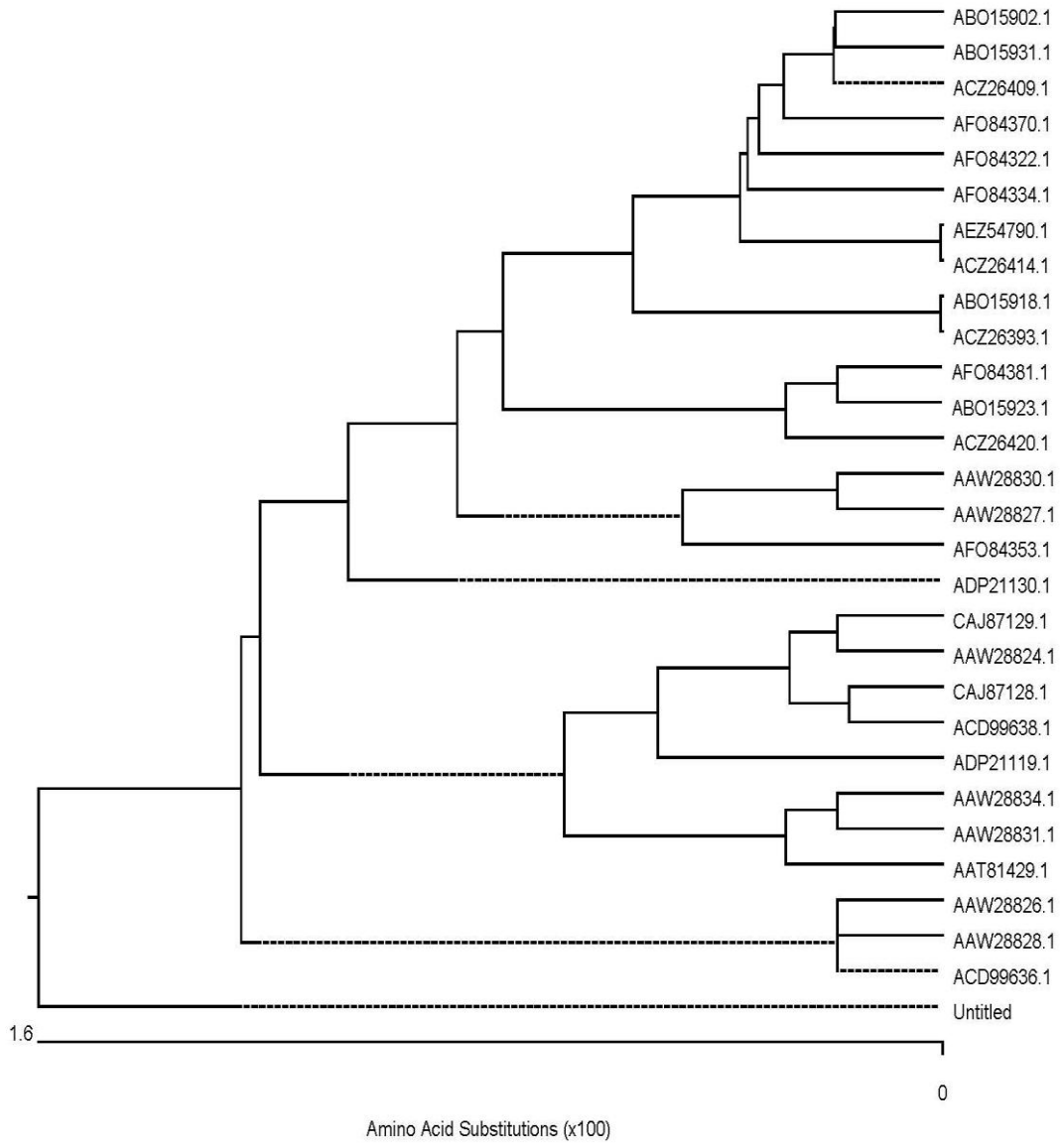

Figure 7: Phylogenetic tree of aligned sequences of different PVY strains, built with ClustalW programme. A tree based on the sequences of amino acids (267) of *cp* gene of PVY Egyptian isolate compared to (29) overseas strains or isolates.

5. DISCUSSION

Potato virus Y (PVY, Potyvirus) is the fifth most important plant virus worldwide in terms of economic and scientific impact. It infects members of the family Solanaceae and causes losses in potato, tomato, tobacco, pepper and petunia production **Tomczynska, I. *et al.*, (2014)**. PVY transmitted by a variety of aphid species in a non-persistent manner and through plant sap. **Kamangar, S. B. *et al.*, (2014)**. **Takas (1999)** reported that PVY is a member of the potyvirus group in the potyviridae family.

PVY is the most known species of the group potyvirus. Most isolates of PVY are somewhat closely related, so it can be distinguished by gene sequence comparison. Moreover, the molecular characterization of a plant virus clearly established that identity of this virus **Van Regenmortel (1986)**. The full length of PVY-RNA genome of the virus under study showed that its size fall within the range of other known potyviruses **Van Regenmortel (2000) and Moodley, V. *et al.*, (2014).**

Members of potyvirus group have a single molecule of positive sense, ssRNA genome approximately 9.7 Kbp in size. The *cp* gene is the most characteristic gene product of potyviruses, which it is multifunction protein involved in virus assembly, local and systemic movement and aphid transmission **Hu *et al.*, (2009)**.

RT-PCR positive some of the samples were then used in biological studies as mentioned by **Yardmci, N. *et al.*, (2014)**.

In this study, the viral RNA was isolated and directly used as a template for RT-PCR amplification of the *cp* gene (its size is about 801 bp) using specific primer that designed basing on the nucleotide sequence of the *cp* gene of PVY. This is in agreement with **Pooja Sharma *et al.*, (2013) and Al-Saikhan *et al.*, (2014).** Also, **Abdel-Salam, A. M. *et al.*, (2014)** amplified the CP gene of PVY was amplified with the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using primers, designed from a recombinant CP sequence for PVY strain O (common strain) and PVY strain N (the necrotic strain).

Budzanivska, I. G. *et al.*, (2014) Found that the identification of the widespread Ukrainian isolate(s) of PVY (Potato virus Y) in different potato cultivars and subsequent phylogenetic analysis of detected PVY isolates based on sequences of coat protein.

Sun and Chun Qing (2005) detected PVY^N and PVY^O through RT-PCR and nucleotide sequence comparisons of PVY^N and PVY^O.

Therefore, a number of twenty nine overseas PVY isolates or strains were selected and compared with our PVY Egyptian isolate.

Data of nucleic acid sequences and deduced amino acids alignment showed average of similarity reach to 99.4% and 99.3%, respectively, between our PVY Egyptian isolate; that was proved to be PVY strain N; and AF255659; AJ390292; EF027888; AF525081; GQ853652; GQ853648; GU074000; EU073859; GQ853653; GQ853634; HM036200; JF804784; JN034575; JN635310; JQ954297; JQ954298; JQ954299; JQ954301; JQ954308; JQ954311; JQ954312; JQ954327; JQ954344; JQ954345; JQ954354; JQ954328; JQ954369; JQ954376 and JQ954387.

This is in agreement with **Budzanivska, I. G. *et al.*, (2014)** which demonstrated that the Ukrainian isolate of Potato virus Y (*cp* gene) has a higher percentage of homology with the recombinant isolates (strains) of this pathogen (approx. 98.8-99.8% of homology for both nucleotide and translated amino acid sequences of the *cp* gene). Also, **Sadik *et al.*, (2003)** who showed that the similarity between the strain PVY-Egy111 and the overseas strains ranged from 91.9 to 95.7% and from 94.3 to 99.1% based on the levels of the DNA and deduced amino acid sequences of the viral coat protein *cp* gene (870 bp), respectively. In which his conclusion that the PVY-Egy111 isolate could be considered as a new Egyptian PVY^N strain .

The dendogram analysis of the nucleotide sequence of the PCR fragments showed that our PVY Egyptian isolate belonging to group I which includes: (JQ 954345.I, JQ 954344.I, JQ954312.I, JQ954308.I, GU074000.I, JQ954298, JQ954299.I, JQ954301.I, JQ954311.I, JQ954387.I, JQ954297, JN635310.I, JU954376.I, JQ954354.I, EU073889.I, HM036200.I, JN034575.I, EF0227888.I, JQ954328.I, JQ954327.I, JQ954369.I and our untitled PVY isolate.

On the other hand, the phylogentic tree of the amino acids alignment showed the presence of two groups (as recommended by the nucleic acid sequence dendogram) with a slight difference, as our untitled isolate only belonging to group II.

From the previously mentioned findings of molecular studies it was deduced that our Egyptian isolate from naturally infected potato plants from open fields near Giza city, is an Egyptian isolate of PVY strain N.

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