Egyptian J. Virol. 1, 81-92 (2004)

Biological and Molecular Characterization of Different Isolates of *Potato Virus Y* (N Group)

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Ten isolates of *Potato virus Y* (PVY) were collected from diseased potatoes grown at different locations in Egypt and some of their biological and molecular characters were studied using several techniques. Many different symptotypes were appeared on *Chenopodium amaranticolar, Ch. quinoa, Datura metal* and *Nicotiana tabaccum* cvs. Samsun and White Barley as a result of mechanical inoculation. All isolates reacted positively with monoclonal antibody specific to *Potato virus Y* group^{N}. Reverse transcription-polymerase chain reaction (RT-PCR), hemi-nested and nested-RT-PCR were used to study their molecular characters. Three pairs of primers were selected to react with 5'-non-translation region (5'-NTR) and PI gene from the PVY genome. PCR products yielded by one primers pair reached 835 bp, while reached about 1kb (974, 926 bp) by the other primers. Partial sequencing of fragment 835 bp was performed. Comparing with standard PVY strains showed highest similarity with PVY^{NTN}-H followed by PVY^{NTN}-Tu660, PVY^{N}-Jg, PVY Wi and PVY^{N}-Fre in rate of 98.8, 90.0, 89.9, 83.9 and 70.1 % respectively using DNASTAR software.

INTRODUCTION

Potato virus Y (PVY) is the type member species of the genus *Potyvirus* , family *Potyviridae* (Walsh *et al.*, 2001). PVY is recorded as the most economically important virus infecting potato and other plants as well. On the basis of local and systemic symptoms in different potato cultivars and indicator plant species, the PVY isolates have classically been divided into three main strains (De Bokx and Huttinga, 1981 and Kerlan *et al.*, 1999). The ordinary or common strain (PVY^{O}) induces mild to severe mosaic and leaf drop streaks in potato, and systemic mottle in tobacco. The tobacco veinal necrosis strain (PVY^{N}) induces very mild mottling in most potato cultivars with occasional necrotic leaves in some cultivars (Chachulska *et al.*, 1997 and Kerlan *et al.*, 1999), but induces severe systemic necrosis of leaf veins and petioles in tobacco (De Bokx and Huttinga, 1981). *Stipple streak strain* (PVY^{C}) isolate evoke stipple streak symptoms in several cultivars and induce a hypersensitive response in potato cultivars bearing the Nc resistance gene (Cockerham, 1970 and Blanco-Urgoiti *et al.*, 1998).

A mergence of new particular PVY isolates were detected, inducing new diseases in potato crop, such as: PVY^{NTN} (Le Romancer *et al.*,1994 and Van den Heuvel *et al.*,1994) constitute a sub-group of PVY^{N} responsible for potato tuber necrosis ringspot disease (PTNRD), which firstly mentioned in Hungary at the beginning of the eighties (Beczner *et al.*,1984). PVY^{N}-Wi is found to be more infectious than the standard PVY^{N} isolate. This PVY^{N}-Wi induced a typical vein necrosis on tobacco and

failed to react with PVY^N-specific monoclonal antibodies (MAbs) (Chrzanowska, 1994). PVY^Z was proposed by Jones (1990) to classify some isolates found in Great Britain, which overcome the hypersensitive resistance to PVY^C and PVY^O conferred by the genes Nc and *Ny*tbr, respectively.

Serologically, polyclonal antibodies are unable to differentiate different PVY strains, therefore, monoclonal antibodies specific to O and N strains have been used to characterize selected PVY isolates, allowing determining possible localization of strain-specific epitopes in the N-terminal part of the coat protein (Hataya *et al.*, 1994 and Cerovska, 1998). Monoclonal antibodies (MAbs) specific to most of PVY^N strain isolates have been obtained (Gugerli and Fries, 1983 and Rose *et al.*,1987). However, currently monoclonals are unable to discriminate the more serologically diverse O and N strains (Boonham and Barker, 1998), in some cases this is probably due to the presence of recombination events (Kerlan *et al.*, 1999).

At the genomic level, comparison of coat protein, 5'-NTR and PI gene nucleotide sequence of a large number of PVY isolates confirmed the diversity between the two major groups, PVY^O and PVY^N, based on biological and serological properties (Van der Vlugt *et al.*, 1993; McDonald *et al.*, 1997; Glais *et al.*, 1996, 2002; Nie and Singh, 2002 and Moravec *et al.*, 2003). On the other hand, Marie-Jeanne Tordo *et al* (1995) found that, on the basis of the sequence of the 5' non-translated region and the adjacent PI gene, potato isolates of PVY could be grouped into three phylogenetic clusters. Croup I comprised potato isolates causing veinal necrosis on tobacco, Group II contained isolates causing rather mosaic or veinal necrosis symptoms, and Group III contained isolates causing only mosaic symptoms.

The present study aims to detect and differentiate between Egyptian PVY isolates basing on biological and molecular characterization.

MATERIALS AND METHODS

Virus isolates

Naturally diseased potato (*Solanum tuberosum* L.) showing variable symptoms including a necrosis and vinal necrosis (isolates No. 1, 2, 3), chlorosis (isolates No. 4, 6), crinkle and dwarf (isolate No. 5), mottling (isolates No. 7, 8) and severe and mild mosaic (isolates No. 9, 10) suspected due to viral infection(s) were obtained from different locations in certain Governorates (Gharbia, Ismaillia)

Detection and biological isolation

Obtained samples are firstly checked for PVY infection by enzyme-linked immunosorbent assay (ELISA) which allowed samples infected by other viruses to be discarded. Anti-PVY, PVX, PVA, PVM, PLRV polyclonal antibodies from Serology Laboratory, Crop Protection Department, International Potato Center (CIP), Lima, Peru, were used in DAS-ELISA (Clark and Adams, 1977). Secondly, the samples, which only reacted positively with anti-PVY, were mechanically inoculated onto different plant species for isolation and propagation of PVY and to differentiate biologically between PVY isolates. Five seedlings of each host were inoculated and observed daily for symptom development. A number of healthy seedlings of the same species and age were left without inoculation to serve as a control. Two to three weeks after inoculation, plants were

examined for symptom expression and assayed using ELISA test. Those plants were tested again by TAS-ELISA using monoclonal antibody (MAb), specific to PVY^N group, from, Institute of Plant Virology, Microbiology and Biosafety, Federal Biological Research Center for Agriculture and Forestry, Germany, to recognize the presence PVY^N isolates. *Datura metal, Chenopodium amaranticolar, Ch. quinoa* and *Nicotiana tabaccum* cvs. White Barley and Samsun were used as differentiated hosts for biological confirmation of PVY^N (Thomas *et al.*, 1986 and Ellis *et al.*, 1996).

RNA extraction

Total RNA was extracted from infected and uninfected plant using SV-Total RNA Isolation System as recommended by the manufacture instructions (Promega Corporation, Madison, WI, USA).

cDNA synthesis

Viral cDNA was synthesized and amplified as the following procedure according to Hadidi *et al.* (1993). For each sample, 1 µg of RNA, 3 µ l of the primer d (Table, 1) complementary to PVY nucleotides 955-974 , 6 µl of 5X first strand cDNA buffer (250 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM MgCl2), 3 µl of 0.1 M dithiothreitol (DTT), were added to a final volume of 30 µl by deionized water. The mixture was heated for 5 min at 100 °C ,and directly chilled in ice for 2 min , then incubated at room temperature for 1 hour to allow primer annealing to the RNA template. Twenty µl of reaction solution (4 µl of 5X first strand cDNA buffer, 5 µl of 0.3 M 2-β mercaptoethanol, 2.5 µl of 10 mM each deoxynucleotide triphosphate (dNTPs), 1 µl of RNasin (40 units/µl), 2 µl of 0.1 M dithiothreitol (DTT), 4.5 µL deionized water, and 1 µl of Moloney Murine Leukamia Virus reverse transcriptase (M - MuLV –RT) , (Bioron corporation, Germeny) were mixed with annealing reaction mixture , and incubated for 1 hour at 42 °C.

Polymerase chain reaction (PCR)

The oligonucleotide primers were selected depending on the PVY 5' region sequences (Marie-Jeanne Tordo et at., 1995 and Glais *et al.*, 1996). The degeneracy of the primer d+c allows recognizing all PVY isolates (Glais *et al.*, 1996). For detection of PVY , PCR test with d+c primers carried out as a following: 5 µl of 10X PCR buffer (160 mM (NH4)2 So4 ; 670 mM Tris-Hcl pH 8.8; 0.1% Tween-20; 25mM MgCl2), 1 µl of 10 mM dNTPs , 1 µl each of primers d and c , and 2.5 units of Taq DNA polymerase. Sterile water was added to raise the volume to 45 µl. Then 5 µl from the cDNA was added to the PCR mixture and amplified with the following cycling parameters: denaturation at 94 °C For 1 min, primer annealing at 57 °C for 1 min. and extension at 72 °C for 1 min for 35 cycles, with a final extension at 72 °C for 10 min.

In case of hime-nested amplification, primer d with primer b were used as the above. Also primer P103 was used with primer P919 in case of nested RT-PCR to make amplification, with the following cycling parameters: (denaturation at 94 °C for 45 sec, primer annealing at 51 °C for 1 min. and extension at 72 °C for 1 min) for 30 cycles, with a final extension at 72 °C for 5 min and cooling to 4 °C (Weidemann *et al.*, 1995).

PCR amplified DNA products were separated by agarose gel electrophoresis. Aliquots of 10 µl of PCR protects were analyzed on 1% agarose gel in TBE buffer (1X = 89 mM Tris, 89 mM borate and 2.0 mM EDTA pH 8.3) at 100 volt for 1 hour. The gel was stained with ethedium bromide at a concentration of 0.5 µl/ml. DNA molecular weight marker (50, 150, 300, 500, 750, 1000 bp) 1 kb DNA marker was used to determine the size of RT-PCR amplified cDNA products of PVY isolates. Bands of DNA were visualized on a UV transilluminator and photographed using gel documentation system [Bio-Doc Analyze (Biometra)].

Partial nucleotide sequencing

The PCR products (835 bp) of NTR region and P1 gene from PVY 5' terminus, which were amplified with P103 and P919 primers, was purified from the low melting point agarose gel using QIAquick gel extracted kit Cat. No. 28704 (as recommended by the manufacture instructions for QiAGene Kit. The nucleotide sequencing was carried out at VACERA by used ABI PRISM Sequencer model 310, version 3.4. The sequence data, multiple alignment, phylogenetic relationship and antigenic index were translated and analyzed by DNASTAR Laser gene (DNA STAR Inc. MD, USA).

RESULTS AND DISCUSSION

Several samples of naturally infected potato (*Solanum tuberosum*) showing variable symptoms including necrosis and vinal necrosis (isolates No. 1, 2, 3), chlorosis (isolates No. 4, 6), crinkle and dwarf (isolate No. 5), mottling (isolates No. 7, 8) and severe and mild mosaic (isolates No. 9, 10), that suspected to be due to single infection with PVY as detected by ELISA (Fig.1) were used and propagated on *Datura metal* and *Nicotiana tabaccum* cv. White Barley. *Datura metal* showed veinal clearing (isolates No. 7, 8), mottling (isolate No.3), sever and mild mosaic (isolates No. 2, 4, 5, 9, 10), leaf deformation and leaf cup shape (isolates No. 4, 6) (Fig. 2). *Chenopodium amaranticolar, Ch. quinoa* showed large and irregular chlorotic local lesion and small and circular chlorotic local lesion on the inoculated leaves. *Nicotiana tabaccum* cvs. White Barley and Samsun showed veinal necrosis and leaf falling (isolates No. 3, 7, 8), mosaic (isolates No. 1, 2, 9, 10), crinkle and leaf deformation (isolates No. 4, 5, 6) (Table,2 and Fig. 3). DAS- ELISA was used to confirm the PVY infection. These results are generally agreed with those of De Bokx and Huttinga (1981); Le Romancer *et al.* (1994); Blanco-Urgoiti *et al.* (1998); Weidemann and Maiss (1996) and Kerlan *et al.* (1999). These differences between symptoms may be due to differences between virus isolates and the symptom differences are prime importance in the recognition of mutant virus strains (Matthews, 1992).

Present isolates were well reacted with anti-PVY polyclonal antibodies without any serological relationships with the other common potato viruses (PVA, PVX, PVS PVM and PLRV) used in DAS-ELISA. In case of TAS-ELISA these isolates were also reacted with the MAb specific to *potato virus Y* strain ^{N}.

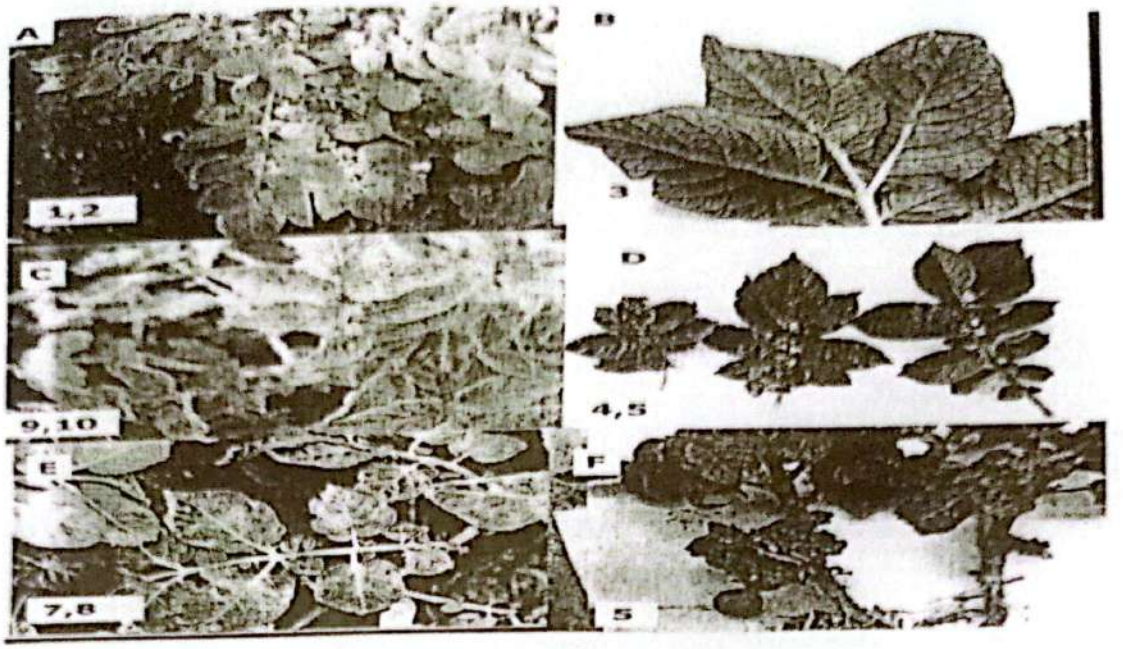

Fig (1): Naturally infected potato plants showing different PVY symptoms. A: chlorosis and necrosis (isolates No. 1, 2, 4); B: vienal necrosis (isolate No. 3); C: mild mosaic (isolates No.9, 10); D: crinkle and dwarfing (isolate No. 5); E: mottled mosaic (isolates No. 7, 8); F: crinkle and dwarfing (isolates No. 4, 5)

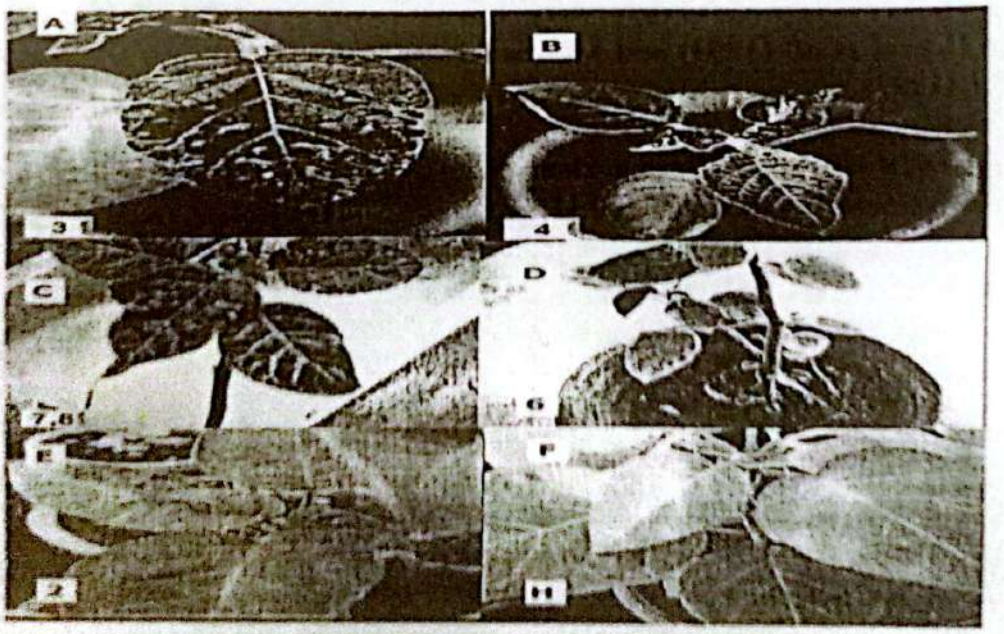

Fig (2): *Datura metel* leaves infected with PVY isolates showing different symptoms due to differences between the isolates. A: mottling (isolate No. 3); B: mosaic and leaf deformation (isolate No. 4); C: vein clearing and mottling (isolates No. 7, 8); D: mild mosaic and leaf cup shape (isolate No. 6); E: typical mosaic (isolate No. 2); F: healthy

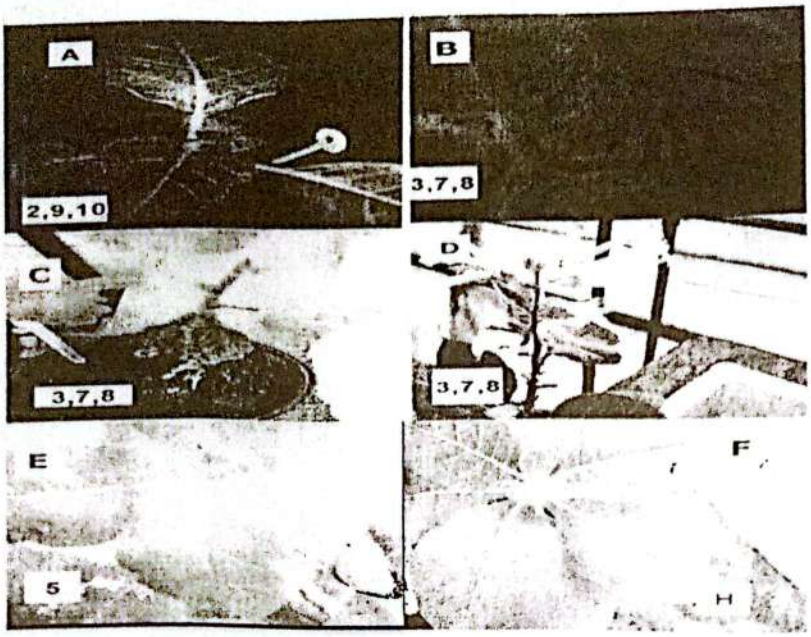

Fig (3): Nicotiana tabacum leaves infected with PVY isolates showing different symptoms. A: mosaic (isolates No. 2, 9, 10); B: veinal necrosis (isolates No. 3, 7, 8); C: leaf falling (isolates No. 3, 7, 8); D: veinal necrosis on flowers (isolates No. 3, 7, 8); E: crinkle and mosaic (isolate No. 5); F: healthy.

Obtained resultes indicated that, the present isolates of PVY are belonging to group N, and these results are in agreement with the findings of Gugerli and Fries (1983); Rose *et al.* (1987); Van den Heuvel *et al.* (1994) and McDonald *et al.* (1997).

To study the molecular characters of the present isolates, infected D. metal leaves were used in RT-PCR, nested PCR and hemi-nested PCR using certain primers (Table. 1). Obtained data (Fig. 4: A, B, C) revealed that the fragment 974 bp long was detected with primer d+c, fragment 926 bp long with primers d+b and fragment 835 bp long by primers P103 +P919 . In general, pair of primers d+c reacted with all selected isolates and it used for detection of these isolates. And pair of primers d+b reacted positively only with isolates number 1 and 5. On the other hand, pair of primers P103 +P919 was reacted with all isolates, depending on this data isolates No. 1 and 5 may be containing mixed infection between PVY isolates. In hemi-nested and PCR, amplification reaction was performed with the internal primers b, P103 and P919 corresponding to known PVY sequences (Glias *et al.*, 1996; Marie-Jeanne Tordo *et al.*, 1995 and Weidemann *et al.*, 1995) these primers (d, c, b, P103 and P919) were reported also by (Glias *et al.*, 1996; Weidemann *et al.*, 1995) reacted positively with different strains of PVY. However, despite of RT-PCR with those primers, NTN isolate could not be separate from the others isolates, and the reason may due to that NTN consider as a subgroup from N group, and primer a reacted only with PVYO (O group), also primers c, P103 and P919 reacted with PVYN (N group which contain NTN).

The partial sequence of 5'-NTR and P1 of the studied selected isolate (PVY-3) was predicted to be 835 nucleotides in length, encoding 249 amino acid only in P1 and no translation to amino acid was found in 5'-NTR region. The codon start (ATG) was found at position 88-90. The nucleotide composition of cDNA revealed the highest contents for Adenine (A) 265 (32%) followed by Thymine (T)

202 (24%), Guanine (G) 191 (23%) and Cytosine (C) 177 (21%), (Fig, 5).

Similarities between present isolate (PVY-3) and some of others recorded in GeneBank (9 PVY isolates or strains and 6 potyviruses) were investigated on the 5'-NTR and P1 nucleotide and amino acid sequences. Highest similarity was proved with PVYNTN-H then PVYNTN-Tu660. phylogenetic tree illustrating phylogenetic relationships based on multiple alignment of 5'-NTR and P1 nucleotide sequences of 10 PVY isolates and 6 potyviruses indicated that the studied isolate (PVY-3) tended to cluster with PVYNTN isolates (Table, 3 and Fig. 6). Singh and Singh (1996) found that the homology was only 67 % - 71.9 % between PVYO and PVYNTN basic on sequences of 5'-NTR and P1. Also Thole *et al.* (1993) reported that the homology between PVYNTN and PVYN basis on sequences of 5'NTR and P1 was 70.3 % - 72.6 %. And basis on amino acid (aa) sequence of P1 protein was 70 % - 95 %, and those were agreed with the present results. Nucleotide sequence analysis of isolates PVY-Fr (Robaglia *et al.*, 1989) and PVY-H (Thole *et al.*, 1993), which have been completely sequenced, shows that the degree of sequence similarity differs across the genome. The overall nucleotide identity between these two isolates was 88.5 % while the 5' non-translated region (5'-NTR) and the adjacent P1 coding region have only 70.3 % and 72.6 % identity, respectively.

The multiple 5'-NTR and P1 sequence alignment obtained with DNASTAR software indicated that the position of start codon (ATG) of different PVY isolates showed little variation, it was at 88-90 in present isolate (PVY-3) and PVYNTN-H, but at position 87-89 in PVYNTN-Tu660 and PVYN-Jg, while it was at position 83-85 in other PVY isolates (PVYN-Fre, PVYN-Egy, PVY-Pvn, PVY-Wi, PVYN and PVYO-139). Three insertions, one nucleotide at positions 2 and 60 and six nucleotides at positions 9, 10, 11, 12, 13 and 14, were observed with PVY-3 and PVYNTN isolates. In addition, one insertion at the position 775 was found only with the studied isolates (PVY-3). On the other hand, two absences were found, one of them was three nucleotides at positions 20, 21 and 22, which observed in PVY-3 and PVYNTN isolates. And the other absence was ne nucleotide at position 762, which found only in the studied isolates (PVY-3). The variations are not uniformly distributed throughout the 5'-NTR and P1, being more abundant in the 5'-NTR.

The classification of PVY isolates into the internal groups may be different based on the part of genome, which studied in sequencing. For example: PVY-Fre isolate has been classified by Van der Vluget *et al* (1993) in the PVYO group according to its capsid protein gene sequence, and in the PVYN group according to its 5'-NTR and P1 or 3'-NTR sequence. Therefore, the partial nucleotide sequences or the symptoms in plants are not sufficient to classify PVY strains and there is a need for additional complete nucleotide sequences of PVY.

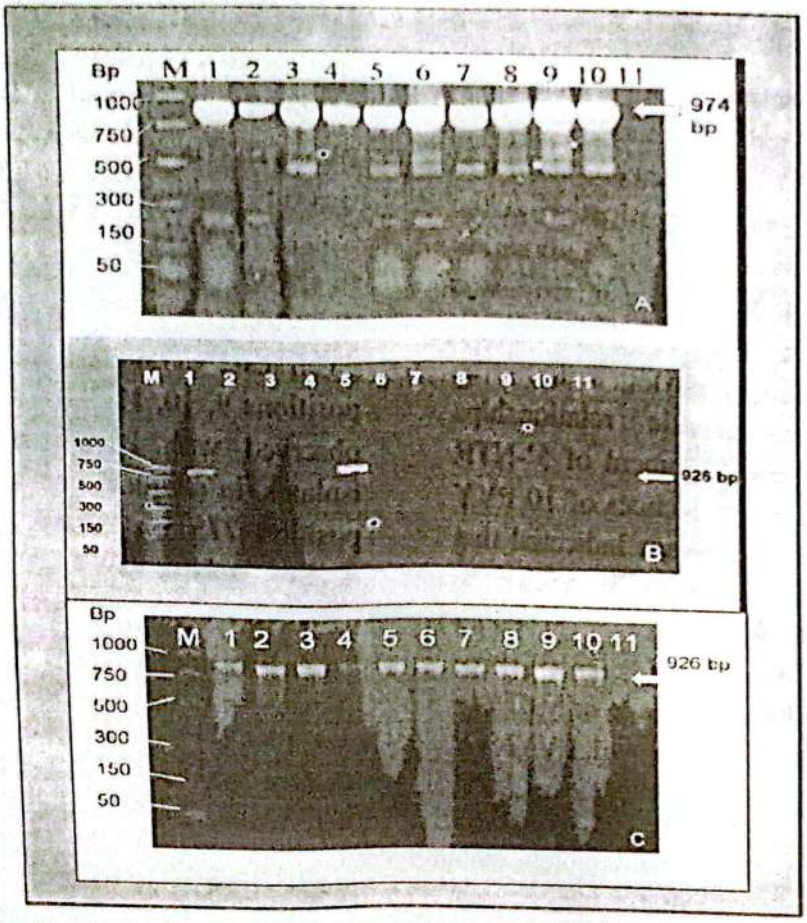

Fig (4): Electrophoretic agarose gel (1%) stained by ethedium bromide for RT-PCR amplification of the total RNA obtained by SV-total RNA isolation system from PVY infected and uninfected plant materials using (A): d and c primers; (B): d and b primers; (C): P103 and P919 primers. Infected N. tabaccum leaves (lanes 1. 2, 3, 10); infected potato leaves (lanes 4, 5, 6); infected D. metel leaves (lanes 7, 8, 9); healthy potato (lane 11). Lane M: PCR marker 50,150, 300, 500, 750, 1000 bp.

Table (1): The oligonucleotide primer pairs used for RT-PCR, PCR and Hemi-nested- PCR for cDNA amplification of PVY

Primer code	Sequence	Genomic location	References
B	[5'- t (ct) a(ct) aaa c(ag) ct (ct) att (ct) tca c – 3']	48-70	Glais *et al.* (1996)
C	5'- aat taa aac aac tca ata ca – 3'	2-21	
D	[5'- tg(ct) ga (cta) cca cgc act atg aa – 3']	955-974	
P103	5' – tca tca aac aaa ctc ttt c -3'	103-121	Weidemann *et al.* (1995)
P919	5'- ttc caa agt gtc ctt tga g – 3	919-937	

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1   tca tca aac aaa ctc ttt caa ttt cag tgt aag cta tcg taa ttc
46  agt aag tta ttt caa act ctc gta aat tgc aga aga tca tcc atg
                                                      M   1

91  gca act tac aca tca aca atc cag ttt ggt tcc att gaa tgc aaa
      A   T   Y   T   S   T   I   Q   F   G   S   I   E   C   K  16
136 ctt cca tac tca ccc gct cct ttt ggg cta gtt gcg ggg aaa cga
      L   P   Y   S   P   A   P   F   G   L   V   A   G   K   R  31
181 gaa gtt tca acc acc act gac ccc ttc gca agt ttg gag atg cag
      E   V   S   T   T   T   D   P   F   A   S   L   E   M   Q  46
226 ctt agt gcg cga tta cga agg caa gag ttt gca act att cga aca
      L   S   A   R   L   R   R   Q   E   F   A   T   I   R   T  61
271 tcc aag aat ggt act tgc atg tat cga tac aag act gat gtc cag
      S   K   N   G   T   C   M   Y   R   Y   K   T   D   V   Q  76
316 att gcg cgc att caa aag aag cgc gag gac aga gaa aga gag gaa
      I   A   R   I   Q   K   K   R   E   D   R   E   R   E   E  91
361 tat aat ttc caa atg gct gcg tca agt gtt gtg tcg aag atc act
      Y   N   F   Q   M   A   A   S   S   V   V   S   K   I   T  106
406 att gct ggt gga gag cca cct tca aaa ctt gaa tca caa gtg cgg
      I   A   G   G   E   P   P   S   K   L   E   S   Q   V   R  121
451 agg ggt gtt atc cac aca act cca agg atg cgc aca gca aaa aca
      R   G   V   I   H   T   T   P   R   M   R   T   A   K   T  136
496 tat cac acg cca aag ttg aca gag gga caa atg gac cac ctt atc
      Y   H   T   P   K   L   T   E   G   Q   M   D   H   L   I  151
541 aag cag gtg aag caa att atg tca acc aaa gga ggg tct gtt caa
      K   Q   V   K   Q   I   M   S   T   K   G   G   S   V   Q  166
586 ctg att agc aag aaa agt acc cat gtt cac tat aaa gaa gtt ttg
      L   I   S   K   K   S   T   H   V   H   Y   K   E   V   L  181
631 gga tca cat cgc gca gtt gtt tgc act gca cat atg aga ggt tta
      G   S   H   R   A   V   V   C   T   A   H   M   R   G   L  196
676 cga aag aga gtg gac ttt cgg tgt gat aaa tgg acc gtt gtg cgc
      R   K   R   V   D   F   R   C   D   K   W   T   V   V   R  211
721 cta cag cat ctc gcc agg acg gac aag tgg act aac caa gtc gtg
      L   Q   H   L   A   R   T   D   K   W   T   N   Q   V   V  226
766 cta ctg atc ata cgc aag ggc gat agt gga gtt ata ttg agt aat
      L   L   I   I   R   K   G   D   S   G   V   I   L   S   N  241
811 act aat ctc aaa gga cac ttt gga a
      T   N   L   K   G   H   F   G                              249
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Fig (5): The nucleotide sequence (835 bp) and derived amino acid sequence (249 aa) of 5'-NRT and P1 of present isolate of PVY (PVY-3) as obtained by DNA Star Laser gene (DNASTAR software).

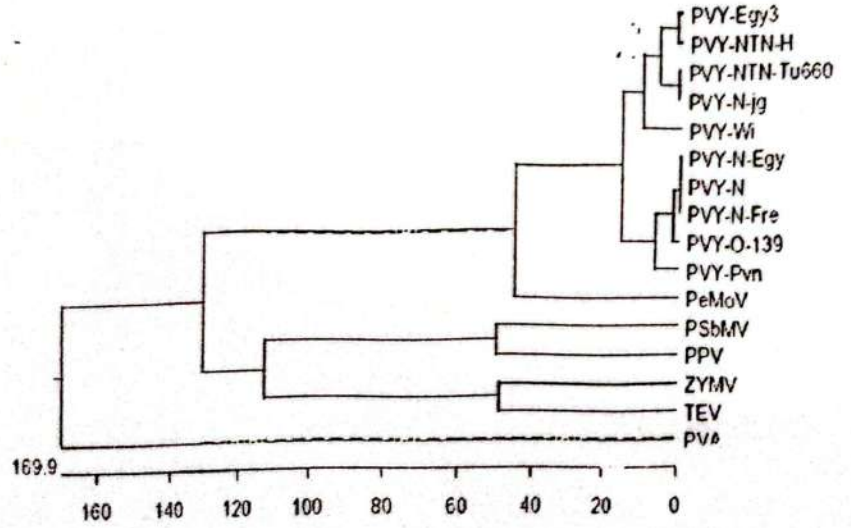

Fig (6): Phylogenetic tree of nucleotides of 5'-NRT and P1 of present the isolate (PVY-3), different PVY isolates and some of potyviruses obtained from GeneBank.

Table (2): Symptoms expression of PVY isolates (1 to 10) on the differential hosts.

Isolates / differential hosts	1	2	3	4	5	6	7	8	9	10
Chenopodium amaranticolor	CH.LL *	CH.LL $	CH.LL $	CH.LL $	CH.LL *, $	CH.LL #	CH.LL $	CH.LL *	CH.LL #	CH.LL #
Ch. quinoa	CH.LL *	CH.LL $	CH.LL $	CH.LL $	CH.LL *, $	CH.LL #	CH.LL $	CH.LL *	CH.LL #	CH.LL #
Datura metel	mM, VC	M	Mo, C	sM, LD	sM	LCS, M	Mo, VC	Mo, VC	M	M
N .tabacum cv.Samsun	M	M	VN , LF	M, LD	M, LD	M	VN, LF	VN, LF	M	M
N. tabacum cv.White burley	M	M	VN	M, LD	C, LD	M	VN	VN	M	M

CH.LL = Chlorotic local lesion LF = Leaf falling M = Mosaic VN = Veinal Necrosis LD = Leaf Deformation LCS = Leaf Cup Shape VC = Vein Clearing Mo = Mottle mM = mild Mosaic sM = severe Mosaic C= Crinkle * = large and irregular

Table (3): Nucleotide sequence and amino acid identities of the partial sequencing from 5'-NTR region and P1 gene of the studied isolate (PVY-Egy3) with other PVY isolates and potyviruses using DNASTAR software.

Accession number in GeneBank	Isolate used	Homology identity nt %	Homology identity aa %	References
NC 001616	PVYN	70.4 %	69.2 %	Robaglia *et al.*, 1989
AY166867	PVYN-jg	89.9 %	88.0 %	Nie and Singh, 2003
AY166866	PVYNTN-Tu660	90.0 %	88.4 %	Nie and Singh, 2003
AF237963	PVY-pvn	69.3 %	69.6 %	Unpublished
AF248500	PVY-Wi	84.2 %	82.8 %	Unpublished
AF522296	PVYN Egyptian isolate	70.4 %	69.2 %	Unpublished
D00441	PVYN French isolate	70.4 %	69.2 %	Robaglia *et al.*, 1989
M95491	PVYNTN Hungarian isolate	98.8 %	95.6 %	Thole *et al.*, 1993
U09509	PVYO-139 Canadian isolate	70.1 %	69.6 %	Singh and Singh, 1996
M11458	TEV	29.3 %	18.0 %	Allison *et al.*, 1985
NC 001517	PeMoV	44.5 %	33.1 %	Vance *et al.*, 1992
NC 001671	PSbMV	25.4 %	7.9 %	Johansen *et al.*, 1991
L31350	ZYMV	27.8 %	15.0 %	Wisler *et al.*, 1995
X81083	PPV	27.4 %	15.2 %	Unpublished
Z21670	PVA	35.2 %	21.1 %	Puurand *et al.*, 1992

nt= nucleotide aa= amino acid

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