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Research Article

Phylogenetic Analysis of *Potato Virus Y* (PVY) Isolate from Upper Egypt Proves the Widespread of PVY^{-NTN} Strain Causing PTNRD Disease in Egypt

Osama A. Abdalla*, Amal I. Eraky, Safynaz A. Mohamed, and F.G. Fahmy

Department of Plant Pathology, Assiut University, Egypt

***Corresponding author**

Osama A. Abdalla, Department of Plant Pathology, Assiut University, Egypt, Email: osama-mohammad@utulsa.edu

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Abstract

Severe viral disease was observed on potato crops growing in Assiut governorate, Upper Egypt. Serological and Molecular identification proved the causal pathogen of this disease is *Potato virus Y*. comparison of nucleotide and amino sequences of coat protein genes of PVY isolate from Assiut (Upper Egypt) and PVY worldwide isolates revealed that PVY-Assiut shared the highest identity with PVY^{-NTN} isolates and this identity was up to 99% at nucleotide level. Phylogenetic analysis confirmed this close relationship among PVY-Assiut and PVY^{-NTN} strain. These data provided a proof that PVY-Assiut is a PVY^{-NTN} strain. PVY-^{NTN} occurrence has been reported before in North of Egypt but this is the first time to be reported in South of Egypt (Upper Egypt). These data proved the wide spread of this virus all over Egypt and indicated to this strain is replacing the old PVY stain especially PVY^{-O}, PVY^{-N} and presents a serious threat to potato production in Egypt. This study proposes that using nucleotide and amino acid sequences of coat protein gene is a potential tool for identification of PVY strains.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops [1]. It is the fourth worldwide largest food cultivated crop [2]. Potato is infected by at least 40 viruses and viroids [3], some of these viruses are restricted to certain geographical region, while the others infect potato worldwide [4]. Among these viruses PVY is the major viral threat to potato cultivation, it is probably the most widespread virus infecting potato and affecting both yield and tuber quality and results in severe yield losses in production up to 80% [5-10], and also causing a major diseases in other solanaceous crops including, tobacco, pepper and tomato as well as many non-solanaceous weeds [11].

In potato, PVY can induce a range of different symptoms, the most common ones are leaf mosaic, vein clearing and stunting [12]. PVY is transmitted by vegetative propagation of infected material and by aphid transmission [10], more than 40 aphid species are known to transmit PVY [13].

Potato virus Y (PVY) is a type member of the genus potyvirus in the family potyviridae [14]. It has a monopartite genome composed of single stranded positive sense RNA molecule of about 9.7 kb.[12]. During the infection process, this RNA is translated into a large precursor polyprotein that is cleaved into to mature 10 proteins [15].

PVY was first classified in 1931 [16], and since that time, three main strains of PVY (PVY^{-O}& PVY^{-N} & PVY^{-C})were recognized according to their reaction to certain potato cultivars bearing resistance genes [17,8]. PVY^{-C} is identified by hypersensitive reaction in potato cultivars bearing Nc resistant gene, while PVY^{-O} infecting the same cultivars without inducing HRs, but inducing HRs in other crops bearing Ny gene, and PVY^{-N} causing mild symptoms in most potato cultivar and overcome Ng without eliciting HRs [18-20]. Another strain called PVY-Z was assigned a new PVY strain along with the previous three historical strains PVY^{-C}& PVY^{-O}& PVY^{-N} [21].

Recently, A new PVY recombinant causing a potato tuber necrosis ringspot disease (designated PTNRD) was first reported in Hungary in 1980s [22]. This new recombinant has a different pathogenicity than PVY^{-N} [23], and is now distributed in most potato producing countries including Europe [24-26], North America [27-28], Japan [29,30], Brazil [31], Egypt [32], and North Africa [33].

PVY^{NTN} characterized by the appearance of external rings on the surface of affected potato tuber, at first these rings protrude and then sunken and causing a potato tuber necrotic ring spot disease PTNRD [34]. This disease can cause losses s up to 90% in the sensitive potato varieties and present serious threat to potato production worldwide [34].

In Egypt, PVY became PVY a major problem of potato production and PVY^{NTN} has reported before in different area [32], several studies have been conducted before on PVY infecting potato in Egypt [35-37], but all of these studies investigated PVY isolates from north of Egypt, and to the best of our knowledge, this study is the first one about PVY isolate from Upper Egypt, and aims to characterize PVY isolate causing necrotic symptoms on potato tubers growing in Assiut governorate (Upper Egypt), identify its strain and determine its relation with other worldwide strains as well as the degree of genetic variation among this isolate and other worldwide PVY isolates, these information are useful to establish a permanent control strategy against this pathogen, as knowledge about degree of variability in plant virus is prerequisite for the success of many control strategies [38].

MATERIALS AND METHODS

Virus Source

During 2012-2013 growing season, potato samples showing mosaic and vein clearing were collected from experimental farm of faculty of Agriculture - Assiut University, serological and molecular identification revealed that the causal pathogen of this disease is *Potato virus Y* [39].

Reverse Transcription-Polymerase Chain reaction

Total RNA was extracted from leaves according to the Tri-Reagent procedure (Molecular Research Center Inc.) and was used in reverse-transcription polymerase chain reaction (RT-PCR) using the Superscript III Reverse Transcription Kit (Invitrogen) according to the manufacturer's instructions.PCR amplification was carried out using virus-specific forward and reverse primers to amplify the coat protein (CP) gene of PVY, included forward PVY: 5′-GATGGTTGCCTTGGATGATG ′3 and Reverse PVY: 5′-TAAAAGTAGTAC- AGGAAA AGCCA as described by [40].

Sequencing

Sequencing was carried out in both directions using Big-Dye terminator cycle according to [41], at the core facility of Molecular Biology Unit, Assiut University, Assiut, Egypt using a sequencing instrument DNA Sequencing Applied Biosystem.

Calculation of Amino Acid and Nucleotide Identity

Sequence identity among CP genes of PVY from Upper Egypt and Worldwide PVY isolates were calculated using the following Formula ID= 100x (Identical residues / sequences length). The calculation conducted using available analysis tools at SIAS Immmunomedicie group (http://imed.med.ucm.es/Tools/sias.html). And evolutionary divergence between sequences also was estimated according to the number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model [42].

Phylogenetic Analysis

Nucleotide and amino acid sequences of 69 PVY isolate Table (1) were extracted from genbank database. These isolates were aligned along with PVY-Assiut (Upper Egypt) using the multiple sequences alignment ClustalW [43]. Phylogenetic analysis were conducted according to neighbor-Joining algorithm [44], using 1000 bootstrap as recommended by [45], and evolutionary analysis were implemented using Mega 7 program [46].

RESULTS

Comparison of CP Gene of PVY Isolate from Upper Egypt and Worldwide Isolates

Analysis revealed that coat protein gene of PVY isolate from Upper Egypt (PVY-Assiut) shared identity ranged from 99.6 – 88.7 % at nucleotide level (nt) with 69 worldwide PVY isolates used in this study. PVY-Assiut shared the highest identity at nt level with PVY^{NTN} strain from South Africa, while shared the least identity with PVY^{O} strain from Poland in case (Table 2). PVY-Assiut shared identity ranged from 99.8 -87 % at amino acid sequences (aa) with these worldwide PVY isolates. PVY-Assiut shared the highest identity at aa level with was PVY^{NTN} isolate from South Africa and shared the least identity with PVY^{NNP} from Spain (Table 2).

In context of comparing PVY-Assiut with each strain separately, PVY-Assiut isolate shared the highest identity with PVY^{NTN} strain regardless their geographical or host origin, this identity with PVY^{NTN} strain ranged from 99.6-98.5% and 99.8-95.58 % at nt and aa sequences respectively, followed by isolates of PVY^{N} strain with identity ranged from 98.03- 89.7%and 99.4-88.2% in case of nt and aa sequences, respectively (Table 2). Also, PVY-Assiut shared high identity with PVY recombinant N/P(PVY$^{N/P}$) and recombinant N-waliga (PVY^{NWi}), and this identity ranged from 99.01-87.7% and 98.52-87.64%at nt and aa sequences, respectively (Table 2).

While, PVY-Assiut isolate shared the least identity with PVY^{O} strain and ranged from 89.21-86.76 and 92.58- 87.55 % at nt and aa sequences, respectively, followed by PVY^{C} strain with identity about 89.21-88.72 % and 97.05 -89.11%at nt and aa sequences, respectively (Table 2).

Phylogenetic Analysis of PVY Isolate from Upper Egypt and Worldwide Isolates

Phylogenetic analysis of nt sequences of the CP genes of PVY-Assiut isolate and 69 PVY worldwide isolates was conducted using tools implemented in Mega 7 program. A Neighbor joining trees were constructed from these CP genes and showed that worldwide isolates clustered into two different groups (Figure 1). Group one included mainly PVY isolates belong to PVY^{NTN}, PVY^{N}, PVY$^{N/P}$ and PVY^{NW} strains. Whereas, group 2 included PVY isolates belong to PVY^{C} and strain PVY^{O}. PVY-Assiut isolate clustered in group one with PVY^{NTN}, PVY^{N}, with close proximity PVY^{NTN} form South Africa (Figure 1).

Similar results were obtained when neighbor joining tree was constructed from aa sequences of CP gene of PVY-Assiut and 69 worldwide isolates (Figure 2). As two groups was formed, with

Table 1: List of PVY isolates obtained from Gene bank and used in PVY phylogenetic analysis.

No	Accession	Isolate	Strain	Host	Country	References
1	KX348009	PVY-Asuit		Potato	Egypt	This study
2	AJ890332	SON 41	NTN	Tomato	Spain	[74]
3	AJ890330	-	NTN	Tomato	Spain	[74]
4	AJ890328	P15D	NTN	Tomato	Spain	[74]
5	AJ890324	D3D	NNP	Tomato	Spain	[74]
6	AJ890321	B3	N	Tomato	Spain	[74]
7	AY792597	-	O	Potato	China	[75]
8	AY061994	_	O	Potato	India	Ghosh et al., Unpublished
9	GQ496607			Latvia	Lativa	[76]
10	GU980964	Egypt	NTN		Egypt	Unpublished
11	AY841269	XCH46	-	Tobacco	China	[48]
12	AF228635	-	NTN	Potato	Czech	[77]
13	AB295477	SYR-D4	NTN	Potato	Syria	[78]
14	EU161658	v0619802	NTN	Potato	United Kingdom	Gow et. al.
15	EF027886	v983585	N	Potato	United Kingdom	Gow et. al.
16	KJ174515	-	N-Wi	-	China	[79]
17	JN034580	CN2"		Potato	Austrlia	[73]
18	JF698682	Medhat		Potato	Egypt	[36]
19	EU252529	-	C	Potato	France	[40]
20	AF012028	-	C	potato	Spain	[47]
21	AF012027	-	C	potato	Spain	[47]
22	AF012026	-	C	potato	Spain	[47]
23	GQ853667	CC24_5	N-Wi	POtato	S Africa	[80]
24	GQ853666	TT138E_1	N	POtato	S Africa	[80]
25	GQ853664	NN300_	N	POtato	S Africa	[80]
26	GQ853662	="DD122A	N-Wi	POtato	S Africa	[80]
27	GQ853659	DD051_7	O	POtato	S Africa	[80]
28	GQ853652	CC55_8_	N-Wi	POtato	S Africa	[80]
29	GQ853634	N484_1	O	POtato	S Africa	[80]
30	GQ853629	NN333B	NTN	POtato	S Africa	[80]
31	GQ853628	NN300_7	NTN	POtato	S Africa	[80]
32	GQ853623	CC62_2	NTN	POtato	S Africa	[80]
33	GQ853621	CC9_12_1	NTN	POtato	S Africa	[80]
34	GQ853607	PVYNTN3	NTN	POtato	S Africa	[80]
35	GQ853603	DD103A_	N	POtato	S Africa	[80]
36	GQ853601	SS082A_	N	POtato	S Africa	[80]
37	GQ853599	NN459_	N	POtato	S Africa	[80]
38	GQ853596	NN300_	N	POtato	S Africa	[80]
39	AB042811		NTN	Potato	Japan	[29]
40	AB025417	TNK	NTN	Potato	Japan	[29]
41	DQ000988		NTN	Potato	Ceske	[81]
42	KR816245		NTN	Potato	Russia	Unpublished
43	KR816240		N	Potato	Russia	Unpublished
44	KR816233		O	Potato	Russia	Unpublished
45	KJ746455	HP5	N Wi-P	Tomato	Poland	[82]
46	KJ746453	W4	N Wi-P	Tomato	Poland	[82]
47	KJ746449	PC1	N	Tomato	Poland	[82]
48	KJ746446	PS4	O	Tomato	Poland	[82]
49	KJ746442	S5	NTN	Tomato	Poland	[82]
50	KJ746440	PC6	NTN	Tomato	Poland	[82]
51	AJ585342	NIB-NTN	NTN	Potato	Slovenia	Unpublished
52	AJ390308	S-RB96	NTN	Potato	United Kingdom	[34]
53	AJ390304	v951175	N	Potato	United Kingdom	[34]
54	AJ390303	v97005	-	Potato	United Kingdom	[34]

55	AJ390302	PVY-C-CM	C	Potato	United Kingdom	[34]
56	AJ390301	O-Gov	O	Potato	United Kingdom	[34]
57	AJ390300	Hung95	NTN	Potato	United Kingdom	[34]
58	AJ390299	53-49	N	Potato	Denmark	[34]
59	AJ390298	53-29	NTN	Potato	Denmark	[34]
60	AJ390297	NN-UK-O	O	Potato	United Kingdom	[34]
61	AJ890343	Gr99	NTN	Potato	Poland	[83]
62	AF255659	PVY-OBR	O	potato	Brazil	[5]
63	AB461482	SYR-II-L3	NTN	Potato	Syria	[84]
64	AB331550	NTNHO95	NTN	Potato	Japan	[30]
65	AB714134	OH	O	Potato	Japan	[85]
66	HQ631374	HN1	NTN	Potato	China	[86]
67	AJ890347	Satina	NTN	Potato	Germany	[83]
68	JQ954381	PVYNTN1	NTN	Potato	South Africa	[9]
69	U09508		N	Potato	Canada	[87]
70	DQ925437	-N/P	N	Potato	Viet Nam	[88]

Table 2: Percentage of coat protein gene identity between PVY isolate from Upper Egypt (PVY-Assiut) and worldwide isolates (at both nucleotide and Amino acid sequences).

PVY strain	Nucleotide sequences of CP	Amino acid sequences
AJ890328_NTN_Spain	99.3	98.52
AJ890324_NNP_Spain	87.74	87.64
AJ890321_N_Spain	89.7	92.05
AY792597_O_China	89.21	90.58
AY061994_O_India	86.76	87.55
AF228635_NTN_Czech	98.27	94.87
AB295477_NTNT_Syria	98.7	98.2
EU161658_NTN_United_Kingdom	99.5	98.52
EF027886_N_United_Kingdom	98.03	94.11
EU252529_C_France	89.21	97.05
AF012028_C_Spain	88.72	90.58
AF012027_C_Spain	88.23	89.11
AF012026_C_Spain	89.21	92.05
GQ853667_NW_South_Africa	99.01	98.52
GQ853666_N_South_Africa	94.11	92.35
GQ853665_N_South_Africa	98.52	97.05
GQ853664_N_South_Africa	94.6	93.82
GQ853662_NW_South_Africa	89.21	90.58
GQ853660_O_South_Africa	89,21	90.58
GQ853659_O_South_Africa	88.72	89.11
GQ853652_NWi_South_Africa	89.21	90.68
GQ853643_NWi_South_Africa	89.21	90.58
GQ853640_South_Africa	89.2	90.58
GQ853634_O_South	88.72	89.11
GQ853630_N/P_South Africa	99.4	98.52
GQ853629_NTN_South	99.5	98.53
GQ853628_NTN_South	99.01	97.05
GQ853624_N/P_South_Africa	99.5	99.4
GQ853623_NTN_South_Africa	99.6	99.6
GQ853621_NTN_South_Africa	99.5	99.8
GQ853619_N/P_South_Africa	99.5	98.52
GQ853603_N_South_Africa	99.01	98.52
GQ853601_N_South_Africa	98.03	95.58
GQ853599_N_South_Africa	99.5	98.58
GQ853596_N_South_Africa	99.01	98.53
AB042811_NTN_South_Africa	98.03	94.11
AB025417_NTN_Japan	98.52	95.58
KR816257_NWi_Belarus	88.72	89.11
KR816256_O_kyrgyzstan	88.72	89.11
KR816240_N_Russia	99	89.2
KR816233_O_Russia	89.21	90.58
KJ746455_NWi_Poland	89.21	90.58
KJ746453_NWi_Poland	88.72	89.11
KJ746451_NWi_Poland	88.23	89.11
KJ746446_O_Poland	88.52	89.11
KJ746442_NTN_Poland	99.5	98.55
JN635310_O_China	88.72	89.11
AJ390304_N_United_Kingdom	98.52	95.58
AJ390303_NTN_United_Kingdom	99.01	97.05
AJ390302_C_United_Kingdom	89.21	92.05
AJ390301_O_United_Kingdom	88.72	92.58
AJ390299_N_Denmark	98.03	94.11
AJ390298_NTN_Denmark	98.03	94.11
AJ390297_O_United_Kingdom	87.74	89.11
AB331550_NTN_Japan	98.52	95.58
AB331540_NTN_Syria	98.52	95.58
AB331543_NTN_Japan	98.52	95.58
JN936425_N_South_Africa	99.01	97.05

clear demarcation between old strains PVY^{-C} and PVY^{-O} (group 2) and other necrotic and recombinant strains PVY^{-NTN}, PVY^{-N}, PVY$^{-N/P}$ and PVY^{-NW} strains (group 1). PVY-Assiut clustered with other recombinant PVY isolates in group with close proximity to PVY^{-NTN} from South Africa, Russia and Poland (Figure 2).

Effect of Host Origin on Relationship among PVY Worldwide Isolates

This study revealed that Neighbor joining trees were constructed from both nt and aa sequences of PVY-CP isolates infecting different hosts, and showed formation of two groups in case of nt and aa (Figure 3 a,b), with sub-clustering of PVY isolates in group A into two sub-groups in case of aa tree (Figure 3b). Although, in case of nt tree there was no clear clustering depending on the host origin, but in case of aa trees, isolates clustered partially according to their host origin to form different

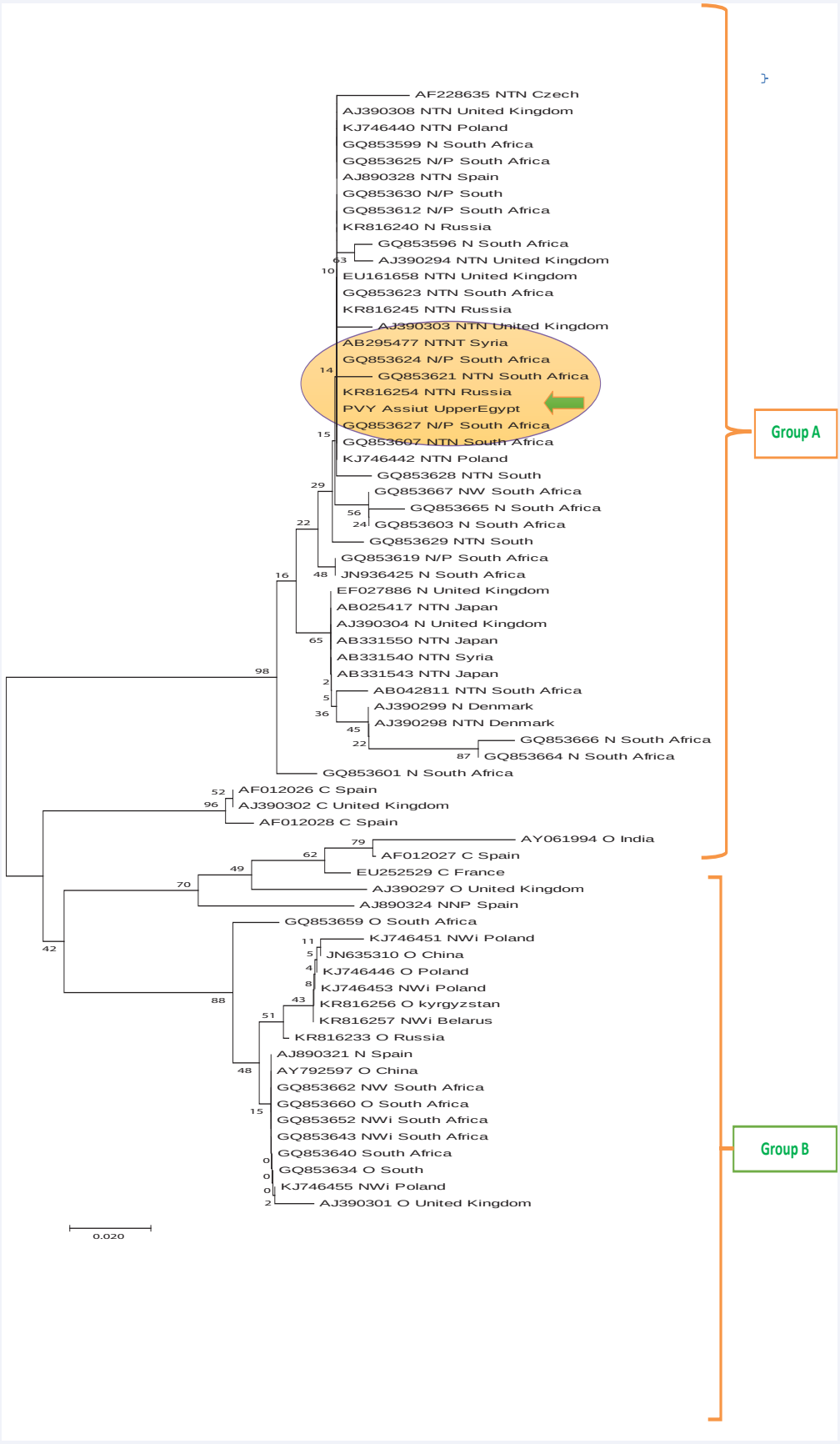

Figure 1 Neighbor-Joining tree constructed from nucleotide sequences of coat protein genes of PVY isolate from Upper Egypt ((PVY-Assiut) and 69 worldwide isolates.

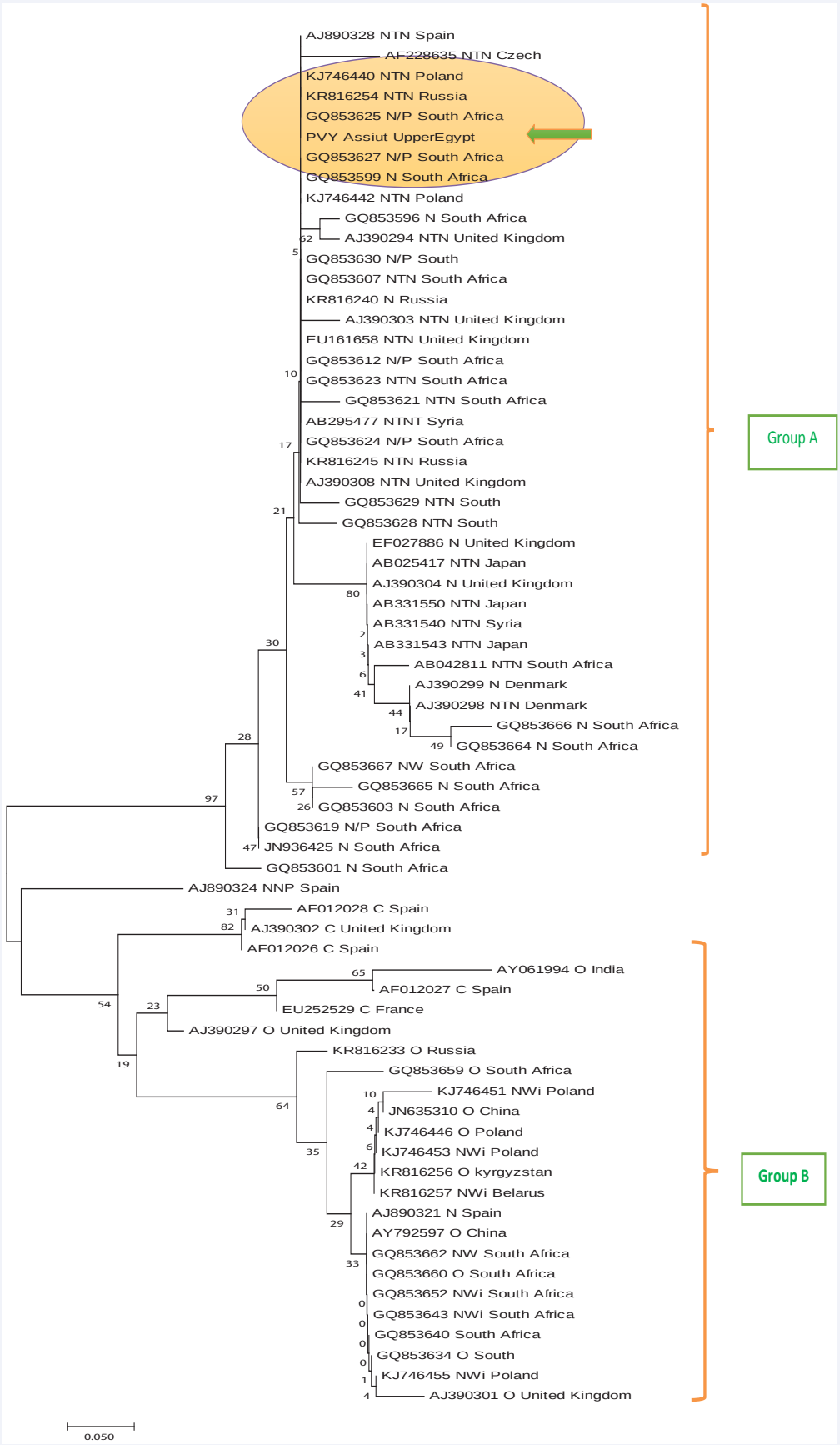

Figure 2 Neighbor joining tree constructed from amino acid sequences ofcoat protein genes of PVY isolate from Upper Egypt (PVY-Assiut) and 69 worldwide isolates.

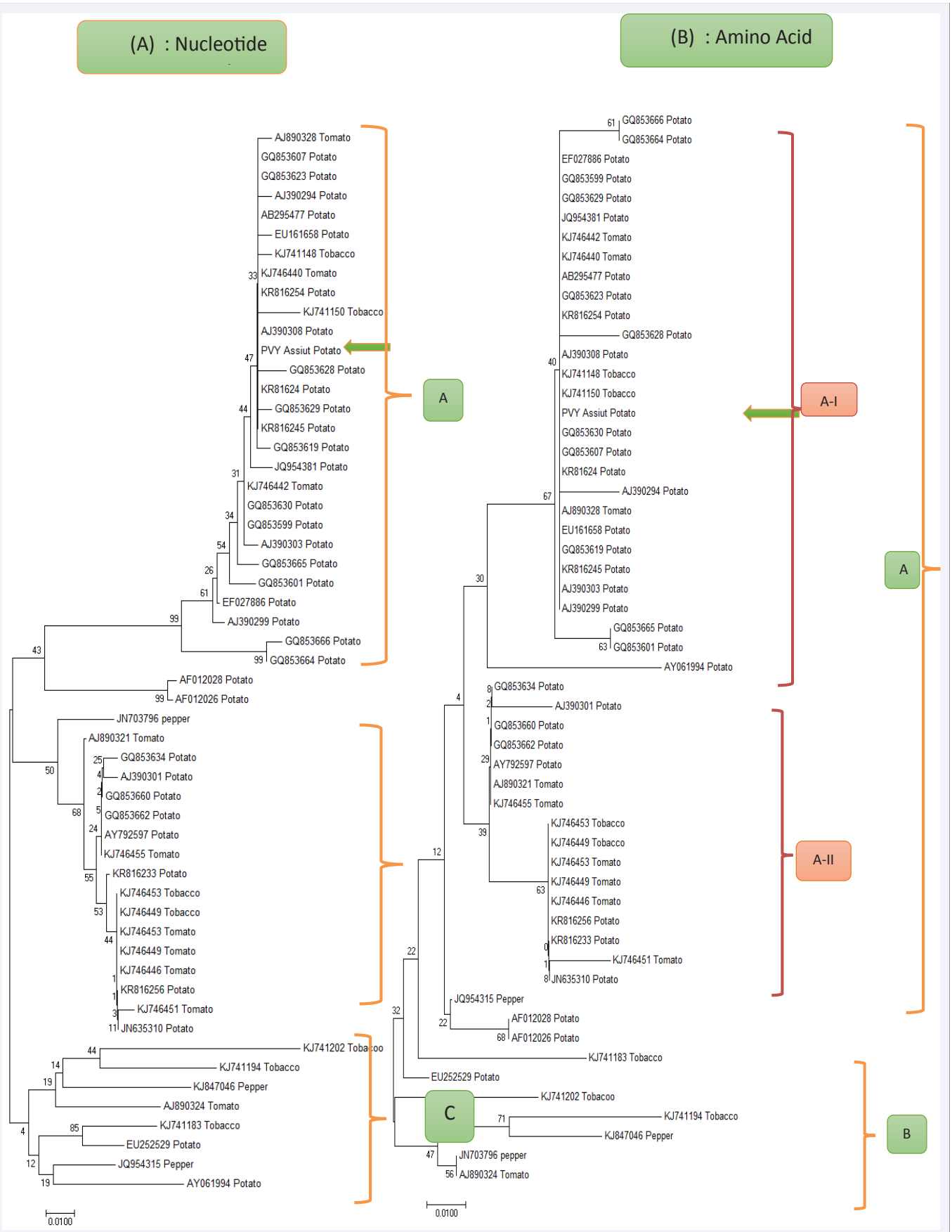

Figure 3 Effect of Host determent on PVY isolates clustering (A: Nucleotide sequences & B: Amino acid sequences.

sub-groups. Interestingly, when a neighbor joining tree was constructed from nt sequences of recombinant PVY isolates (PVY-^{NTN}), these isolates clustered into three main groups A & B & C according to their host origin, as group A contained PVY isolates infecting potato, group B contained PVY isolates infecting pepper, tomato, and group C contained PVY isolates infecting tobacco (Figure 4).

To study the effect of CP gene region on the relationship among PVY population, CP was divided into two main regions; C-terminal region and N-terminal region. Neighbor joining trees were constructed from nt sequences of both regions separately (Figure 5a,b). The phylogenetic analysis showed that PVY isolates formed two main groups (group A& group B) in both cases of C-terminal and N-terminal region. Surprisingly, in case of N-terminal region Assiut isolates was very close to PVY isolates belong to PVY^{-C} strain and PVY^{-NTN} strain (Figure 5b), while in case of C-terminal region, PVY-Assiut isolate clustered with PVY isolate categorized as PVY^{-NTN} and PVY$^{-N/P}$ (Figure 5a).

Phylogenetic Analysis of PVY Isolates from Egypt

Analysis of PVY-Assiut isolates and other Egyptian isolates available in gene bank (Figure 6) showed that these isolates clustered in two different groups in both cases of nt and aa nucleotide sequences (Figure 5a&b). PVY-Assiut clustered with PVY isolates infecting potato in Giza governorate. Analysis showed that PVY-Assiut was the ancestor these two isolate, these isolate from Giza were previously described as PVY^{-NTN} strain, while the group two contained isolate from North of Egypt.

DISCUSSION

During 2012-2013 growing season, severe symptoms were observed in potato crop growing in Assiut Governorate-Upper Egypt. Serological and molecular identification proved the causal virus of this disease is *Potato virus Y* (PVY), and this isolate was called PVY-Assiut. Coat protein gene (CP) of this isolate was amplified, sequenced and compared with other 69 PVY worldwide isolates representing the three main old PVY strains (PVY^{-O}, PVY^{-C} and PVY^{-N}) as well as the new PVY recombinants

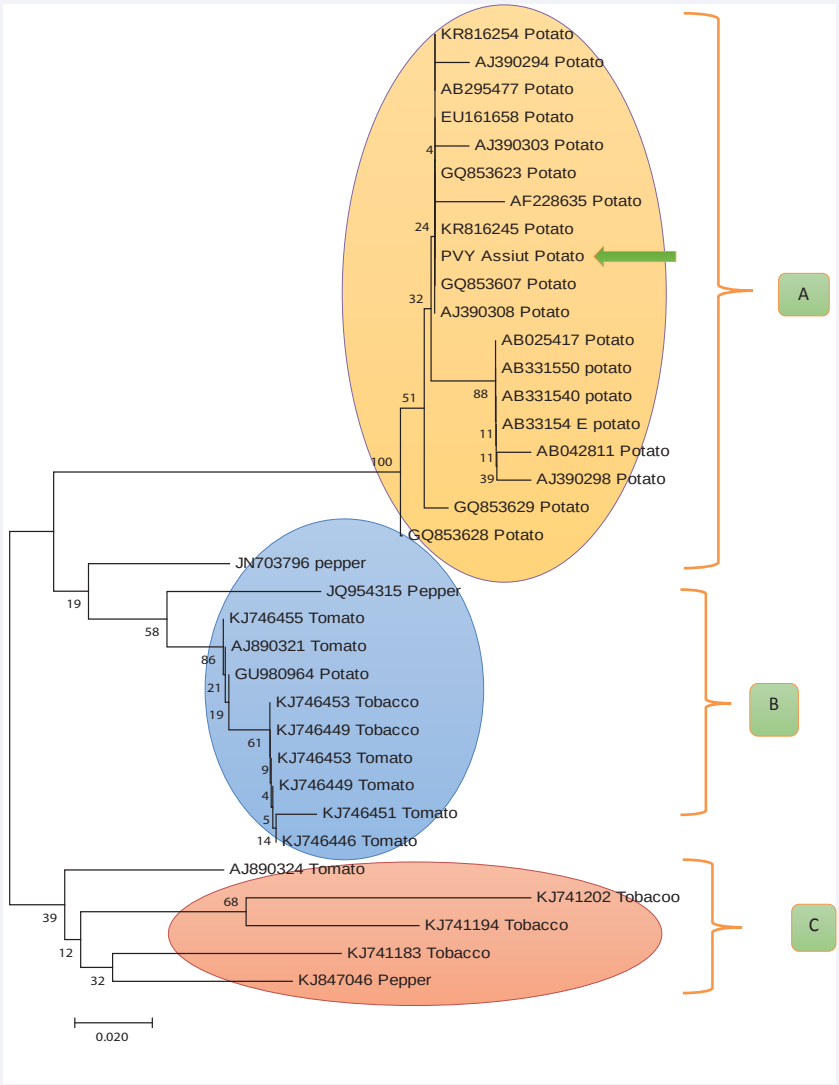

Figure 4 Effect of Host determent of clustering of PVY isolates N & NTN Strains.

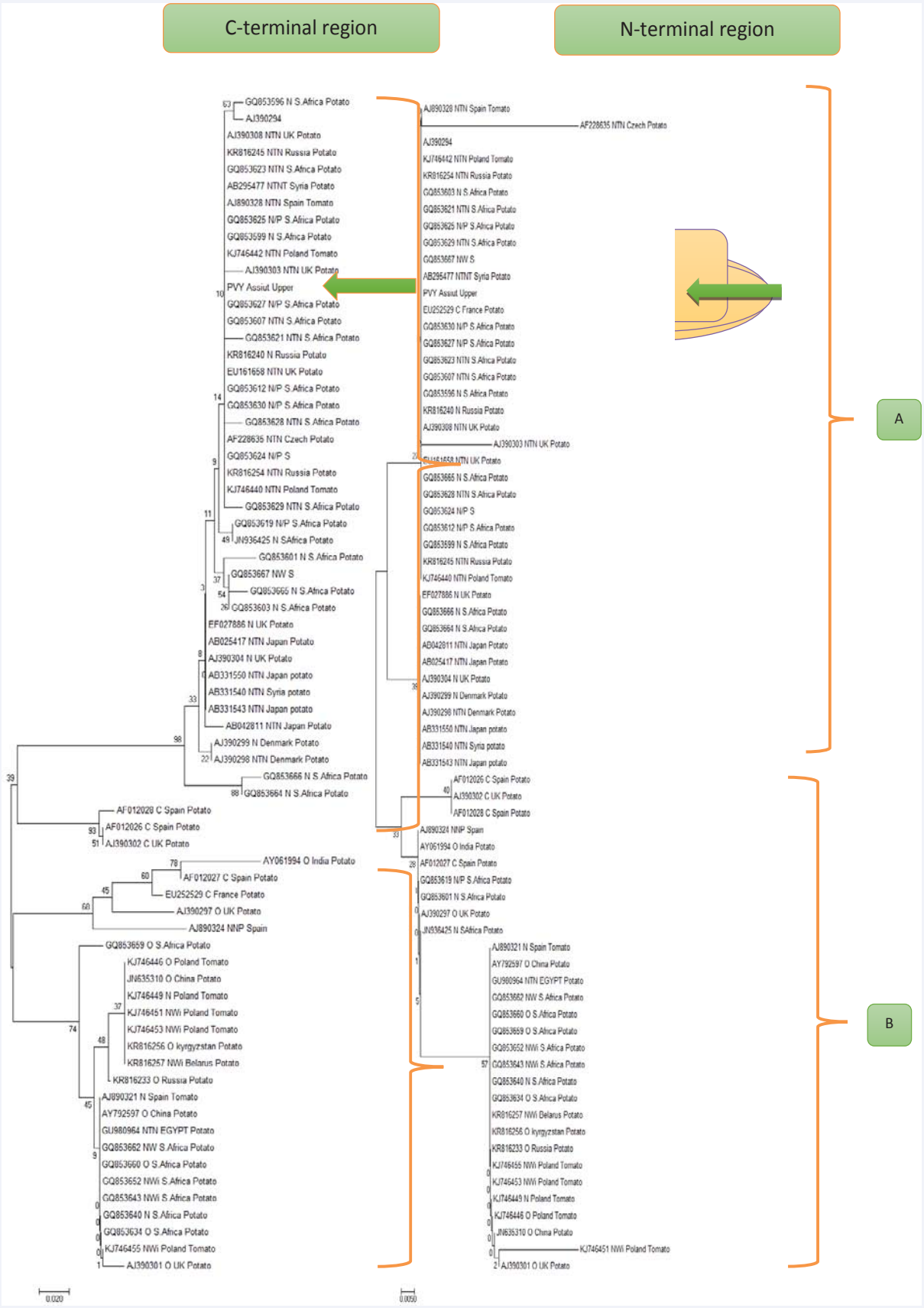

Figure 5 Neighbor joining tree constructed from nucleotide sequences of C-terminal & N-terminal region of CP genes of PVY isolate from Upper Egypt (Assiut) and 69 worldwide isolates.

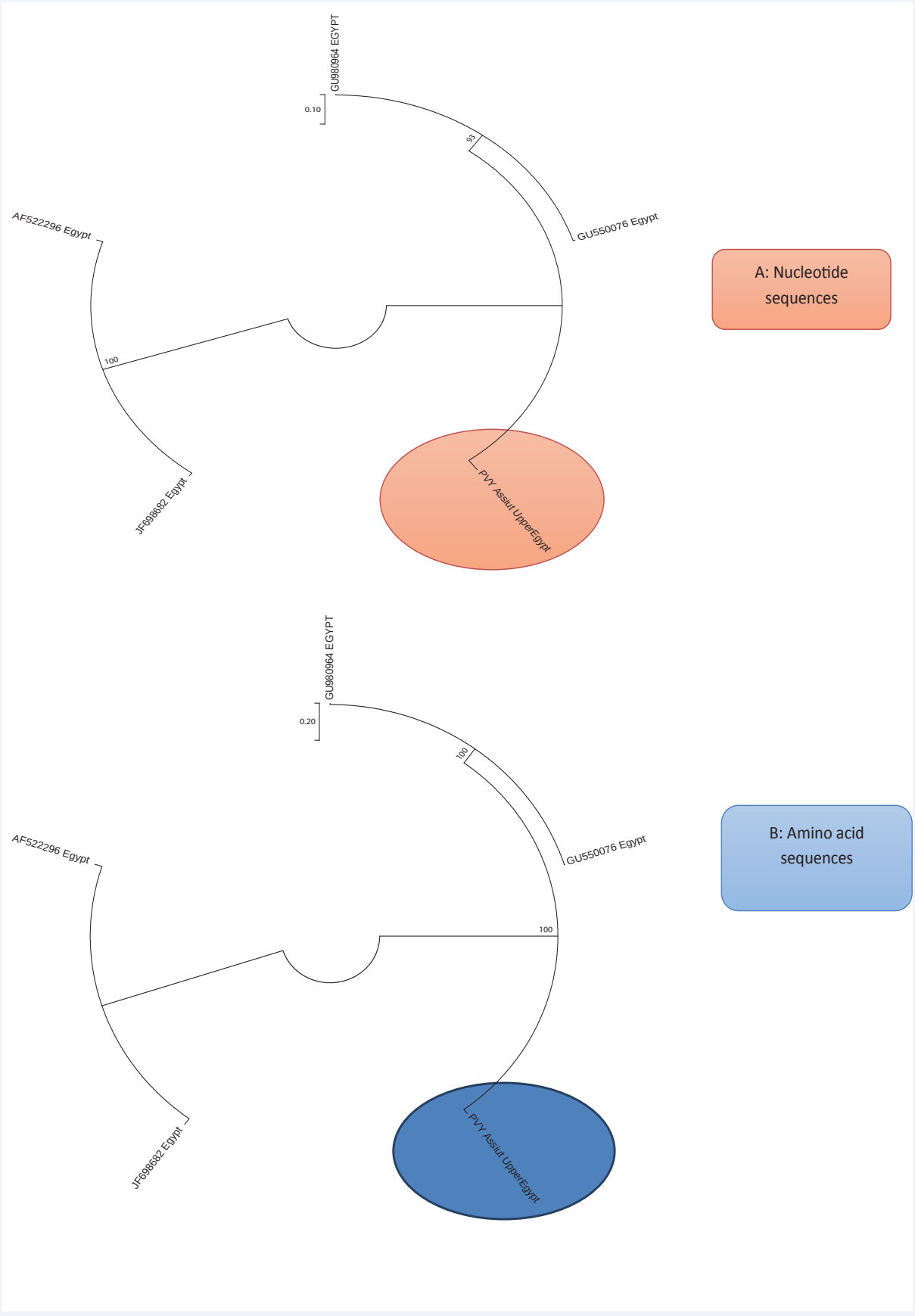

Figure 6 Rooted tree constructed from Coat protein gene sequences of PVY isolates from Egypt; A: Nucleotide sequences & B: Amino acid sequences.

(PVY^{-NTN}, PVY^{-NWi}, PVY$^{-N/P}$). Comparison of CP genes of these isolates showed that PVY-Assiut shared identity ranged from 88.7- 99.6% and 87-99.8%with other PVY worldwide isolates at nucleotide (nt) and amino acid (aa) level respectively. This high variation rate in PVY was reported before in several studies which found sharing identity ranged from 88.8- to 99.5%in nt sequences of CP gens among PVY^{-N} isolates [47], 92.5% among PV^{Y-NTN} strain [30], and 90.6 % identity in nt sequence among CP genes of isolates representing different strains [64].While, identity between PVY^{-O} and PVY^{-N} was about 92% [8].The result of this study also in partial accordance with findings reported by [48], who found PVY isolates from tobacco sharing low identity with average of 83.2% at nt level and 87.6 % at aa level.

High degree of variation was reported before in other Potyviruses, like *Yam mosaic virus* [49], and *Rice yellow mottle virus* [50], which showed variation in CP up to 28 and 22.4%, at nt and aa sequences, respectively, and other RNA viruses like *Alfalfa mosaic virus* which showed variation up to 10 % in nt of CP gene [51].

The diversity Potyviruses is probably due to the lack of proof reading during RNA replication [52],which result to high mutation rate and emergence of new variants [27], in addition to high mutation rate, also recombination and re-assortment can play a main roles in variation of RNA viruses [53,11,54,20,55,56], all of these three mechanisms produce a vast pool of virus genomes [11,57], and thus create this high variation rate in virus population.

Whereas, other studies mentioned a smaller degree of variation among PVY population, including studies about PVY population from Brazil, Czech Republic, Pakistan, Spain and USA, where these studied showed that PVY population in these countries showed identity ranged from94.4-99.2 %, 96-100%, 99-99.7%, 98.1-99.2%and 99-99.5% at nt sequences of CP gene of PVY isolates from these countries, respectively [5,59-61,7]. While [62], estimated the identity in CP gene level between PVY^{-N} strain and PVY^{-NTN} strain to be 98 % and 97.4 % at nt and aa, respectively. This high identity reported in PVY- CP from these studies may be due to this analysis was conducted on fewer number of PVY isolates or these isolates belonging to one strain or infecting only one host, while to calculate the actual variation in PVY, it is necessary to incorporate large number of PVY isolates representing all the strains and infecting different hosts.

PVY-Assiut shared the highest identity with PVY isolates identified as PVY^{-NTN} strain and this identity was high up to 99 % at both nt and aa level. This close proximity among PVY-Assiut and PVY^{-NTN} strains from different geographic regions provides a strong indication that PVY-Assiut isolate belongs to PVY^{-NTN} strain.

Phylogenetic analysis was conducted among PVY-Assiut isolate and other 69 worldwide isolates, it provided another indication about the close relationship between PVY-Assiut and the other isolates belong to PVY^{-NTN} strain. As, neighbor joining (NJ) tress constructed from these isolates showed PVY-Assiut closely clustered with PVY^{-NTN} either in case of nt or aa sequences. This study found that PVY worldwide isolates clustered into two different groups, (either in case of nt or aa sequences), one group included isolates belong to PVY^{-NTN},PVY^{-N}, PVY^{-NWi} and PVY$^{-N/P}$ strains. While, the other group included isolates belong to PVY^{-O} and PVY^{-C} strains. PVY-Assiut isolate was incorporate among isolate in group one (giving a proof that this isolate could be identified as PVY^{-NTN} strain). These results are in agreement with other studies which mentioned that PVY population could be divided into two different groups, one contains necrotic and recombinant isolates (PVY^{-NTN}, PVY^{-N}, PVY^{-NWi} and PVY$^{-N/}$), while the second group included the old strain (PVY^{-O} and PVY^{-C}) [63-66,5,34,67].

This study revealed the host origin of PVY (infecting host) plays a role in PVY clustering, this role may be less significant than the role of PVY strain, but it was revealed that PVY isolates belonging to the same strain could be divided according to their infecting host, and thus PVY^{-NTN} isolates clustered into three different groups according to their infecting hosts (either potato or tomato or tobacco). These results proved that PVY isolates could evolve separately to adapt into different hosts, and became different than other isolates infecting the other hosts. Similar conclusion was reported by [10], who mentioned genetic difference among PVY could be reinforced by host barrier, and form separate clads like those observed among PVY infecting potato and PVY infecting pepper [10], and confirm previous findings that environmental factors like plant host of PVY can exert forces on PVY evolution [12].

Geographic origin of the PVY isolates does not play an significant role in relationship among PVY as was shown in this study, PVY isolates always clustered according to their strain and infecting hosts not according to their geographic origin, and it was exhibited in this study that PVY isolates from different countries and continents clustering together in the same clad as long as they belonged to the same strain and infecting the same host, this explain the high proximity which PVY-Assiut isolates shared with PVY^{-NTN} isolates from distant geographic regions like South Africa and Russia, as these isolates belonged to the same PVY^{-NTN} strain ,and infecting the same host. This phenomenon of insignificance of geographic origin was observed before in several RNA viruses including *Watermelon mosaic virus* [67], and *Alfalfa mosaic virus* [68].

PVY-Assiut isolate clustered with PVY isolate categorized as PVY^{-NTN} and PVY$^{-N/P}$ when NJ trees was constructed from C-terminal region of CP gene, while it was clustered with isolates belong to PVY^{-C} strain in case of N-terminal region. This result proves a recombination event occurred in CP gene of PVY-Assiut, and this explains the discrepancy in clustering of PVY-Assiut isolate in case of C-terminal and N-terminal region of CP gene, and indicates that PVY-Assiut may emerged a s result of a possible recombination between PVY^{-C} and PVY^{-N} or PVY^{-NTN} strains. Similar situation was observed by [48], who found that C-terminal region was more conservative than N-terminal region of CP gene, it was suggested that recombination may occur in N-terminal region to make PVY adapt into new hosts and new environment as this region is essential for replication and cell to cell movement of some potyviruses [70].

PVY-Assiut and other Egyptian PVY isolates (available genbank database) clustered in two different groups, one group included PVY-Assiut and PVY isolates infecting potato from Giza

governorate, while the second group contained isolates from Lower Egypt (North of Egypt). It was shown that PVY-Assiut is the ancestor of other PVY isolates (in group one) which were previously described as PVY^{-NTN} strain, this may indicates to PVY^{-NTN} spread in Egypt from south to the north, and close relationship which PVY-Assiut shared with PVY^{-NTN} from South Africa may refer to this strain was introduced into Egypt from the south and moved into north of Egypt. While, the source other PVY isolates in north of Egypt may be from Europe as previous study mentioned that PVY isolates from Borg El-Arab (Alexandria) shared the highest similarity with isolate form France and Germany [37].

The results of this study prove the spread of PVY^{-NTN} strain in Upper Egypt, This strain (PVY^{-NTN}) was reported before in different potato production area in north of Egypt [32,35,36], and confirm the statement that PVY^{-NTN} strain become dominant and replacing PVY old strains especially PVY^{-C}& PVY^{-O} as have been previously reported in many worldwide potato production area [28,30,31,33,70]. It appears that the incidence of PVY^{-NTN} is increasing globally and creates a big concern in most potato growing countries and regions [61].

Forces stand behind emergence and spread of these new PVY recombinant strains is not completely clear, but there are increasing evidence of positive selection events occurring in the coat protein gene of PVY [12], to increase the efficiency of virus survival as CP gene plays vital role in both vector transmission and systemic plant colonization [71,72]. This positive selection mainly promoting the emergence and spread of the current recombinant strains [9]. Also, other environmental factors like using susceptible cultivars increase the possibility of emergence new PVY variants and lead to PVY exists as a complex of strains [70,18], this complex of PVY provides a huge pool of virus genome for the selection of the best variant in each environmental [73], and increase the possibility recombination event among these variants.

With this situation, it is urgent to develop an efficient diagnostic strategy to efficiently identify the PVY strains, it is impossible to use a single detection method for correct diagnosis of PVY strain [73]**.** It was proposed that nucleotide and amino acid sequence of CP to bea basis for identification and classification in Potyviruses [62,64]. And thus the classification and PVY should be according to the biological and molecular information [18,74].The data from this study support the proposal of using CP sequences as basis of identification of PVY strain along with biological characters of PVY strain in plant host.

CONCLUSION

This study revealed that PVY-Assiut isolates shared the highest identity with PVY PVY^{-NTN} isolates. Phylogenetic analysis proved this close relationship among PVY-Assiut and PVY^{-NTN} strain. These data provide a strong indication that PVY-Assiut isolate belong to PVY^{-NTN} strain. This study support the proposal of using nucleotide and amino acid sequences of coat protein gene along with biological characters as a tools for identification of PVY strain.

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