Egyptian J. Virol. 9(1), (2012) 359-371

Phylogenetic relationship of some *Potyviridae* members that found in Egypt.

Mervat M. Fath- Allah, Amal A. Ahmed, Hala A. Amin
Virus & Phytoplasma Research Department. Plant Pathology Research Institute Agricultural Research Center. 12619 Giza – Egypt.

ABSTRACT

Five different plant samples showing different *Potyviridae* symptoms were previous isolated and identified. All infected plant samples were examined using ELISA using against, number of potyviruses polyclonal antibodies. The ELISA results gave a positive reaction with *Bean yellow mosaic virus* (BYMV), *Carnation vein mottle virus*(CVMV), *Potato virus Y, Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic* virus (ZYMV) of potyviridae family. A potyvirus universal degenerate primer was used to amplify the polyprotein region of members of *Potyviridae*. The RT-PCR revealed 335 bp amplified products with only infected plant samples corresponding to viruses BYMV, CVMV, PVY, WMV and ZYMV, and according to virus symptoms and ELISA results. The nucleotide sequence analysis of the polyprotein gene of PVY-ME2 (the present Egyptian isolate) showed 98% homology with the PVY-isolate strain N of Poland. In turn the PVY-ME2 showed low homology with the other characterized *Potyvirus* members. Furthermore the PVY-ME2 had much more distantly related (91%) to the previously published Egyptian and France isolates. The WMV-ME2 isolate (Egyptian isolate) has a high similarity (98%) with France, Turkey and Chile isolates. On the other hand, ZYMV –ME2completely separated from MM- isolate of China and it showed only 87% of homology and 98% of identity with JOR-B5 isolate of Jordan. However, the phylogenetic homology tree based on the multiple sequence alignments revealed that the BYMV-ME2 polyprotein was closely related to Japanese isolates (S.22C and MB4) since it showed 98 – 97% of similarity . In turn the BYMV-ME2 appeared far from the BYMV- MC and Alaska isolates of Japan and USA respectively. Since it showed only 92-89% of identity. The CVMV grouped in PVY group but in a separate subcluster since it has a slight similarity with PVY virus (66%).

Introduction

The *Potyviridae* is the largest of the plant virus families currently recognized. It contains almost 200 definite and possible members (about 20% of all classified plant viruses). Most members of the *Potyviridae* have restricted, or very restricted, host ranges, but a few occur naturally in a wide range of monocotyledonous and/or dicotyledonous species. However, members of the *Potyviridae* infect many important crop species, many of which cause significant losses in agricultural, pasture, horticultural and ornamental crops. Some induce no conspicuous symptoms in infected plants, but most cause mosaic or mottle symptoms in leaves; many also induce colour-breaking in flowers, mottled and/or distorted fruits and seeds, and some cause considerable losses of crop yield and quality. The family consists of 6 genera based on their transmission by fungi

(*Bymovirus*), whiteflies (*Ipomovirus*), aphids (*Macluravirus, Potyvirus*) or mites (*Rymovirus, Tritimovirus*) [Skula *et al*., 1998 and Chen and Adams, 2001).

All members have filamentous particles 650–900 nm or 500–600 and 200–300 nm in length and11–13 nm in width, made up of about 2000 units of a single structural coat protein surrounding a linear, single-stranded positive sense monopartite or bipartite RNA genome of 8,500–12,000 nucleotides with a poly (A) tail at the 3-terminus and probably a genome-linked protein (VPg) at its 50-terminus. The genome or genome segments are translated into polyproteins which are subsequently processed by virus encoded proteases into functional proteins. The viruses induce characteristic pinwheel or scroll-shaped inclusion bodies in the cytoplasm of the infected cells [Edwardson and Christie, 1996 and Gibbs *et al*, 2003]. These cylindrical inclusion (CI) bodies are formed by a virusencoded protein and can be considered as the unique phenotypic criterion for assigning viruses to the family [Ward and Shukla, 1991].

Since the early 1990 at least seven attempts have been made to design universal primers to detect all potyviruses (Langeveld *et. a*l., 1991; Nicolas and Laliberte, 1991; Colinet and Kummert, 1993; Pappu *et. al.*, 1993; Rossolini *et al*., 1994; Gibbs and Mackenzie, 1997; Chen and Adams, 2001,and Ha *et al*., 2008). Universal primers for virus genera are often highly degenerate and designed by finding conserved sequences in an alignment by eye. They permit the detection of a range of species with different sequences as well as previously unknown viruses. Universal primer design depends on the sequence data of the viruses available at the time, so primers developed using a small sample of sequences may not cover variations of unknown Species in the virus group. In this study, we review the phylogeography of some potyviruses isolated from Egypt plants for polyprotein gene sequences which are publicly available. In these analyses, the phylogenies of various sets of potyviruses have been determined using gene sequences, and we have searched the trees for clusters of potyviruses with restricted geographic distributions to obtain clues about relationship between these viruses.

Material and method

Source of Viruses isolated:

Carnation vein mottle virus(CVMV), *Bean yellow mosaic virus* (BYMV),*potato virus Y* (PVY), *Watermellon mosaic virus* (WMV) and *Zucchini yellow mosaic* virus (ZYMV) used in this study were isolated from naturally infected plants. Samples proved to be singly infected with viruses and gave higher absorbance values at 405 nm were used as the primary source for these isolates. The five isolates were isolated and identified by **(**Amer, et.al.;2004 ; Amal, A. Ahmed,2006.; Younes et.al.;2008 and Fegla et.al.;2009). The viruses were maintained on **Cucumber cv.**

Egyptian J. Virol. 9(1), (2012) 359-371

Betaalfa, Carnation, faba bean cv. Nobaria 1, *Nicotiana glutinosa* and Squash cv Eskandrany plants that served as a virus source for this study under greenhouse conditions.

Indirect ELISA Testing:

Source of antisera

Polyclonal antibodies (PAb) of five viruses: *Carnation vein mottle virus*(CVMV), and *Watermelon mosaic virus* (WMV), provided by Bio-Rad Laboratories. *Bean yellow mosaic virus* (BYMV), *potao virus Y* (PVY) and *Zucchini yellow mosaic* virus (ZYMV) were locally prepared, using indirect enzyme linked immunosorbent assay (indirect-ELISA).

Indirect-ELISA reported by Koeing (1981) The ELISA values were measured by Multi Skan Ex ELISA reader and expressed as absorbance at 405 nm. Absorbance values of at least double that of healthy control, were considered positive.

Reverse transcription polymerase chain reaction (RT- PCR)

Isolation of total nucleic acid

RNA was extracted from the same plants identified by indirect ELISA test. Total RNAs extraction from virus infected samples were carried out according to the instruction manual of "Total RNA isolation Nucleospin® RNA II (Macherey-Nagel, Germany).

RT-PCR:

The universal primers to PVY designed according to Langeveld *et al* .(1991**)** were used as fellows: the upstream U335[5' GAAT TCAT GGTA TGGT GCAT AGAC AACGG 3'] and downstream primer D335 [5' GAGC TCGC AGCC TTCA TCTG CGAT ATACG 3', Biometra), The cDNA synthesis was based on the method given in the Thermo Scientific Verso 1-Step RT-PCR kit. 40 reaction cycles were performed with 3 min at 94°C and 30 sec 94°C for denaturation, 5 min at 60°C for primer annealing, 1 min at 72°C for synthesis with 7 min at 72°C for final extension (Langeveld *et al.*1991). Ten microliter aliquots of RT-PCR were analyzed on 2% agarose gels in TBE buffer (89mM tris-HCL, 89mM boric acid, 2.5mM EDTA, pH 8.5) at 9o volt for 90min. A100bp ladder was used to determine the size of RT-PCR products. Gels were stained with ethidium bromide 10µg-ml (agarose gels) and visualized by UV illumination (Sambrook *et al*., 1989).

Sequencing of polyprotein gene of five viruses:

Partial sequence of polyprotein gene of five viruses was carried out in the cDNA sequencing facility of the Macrogen Inc, Korea, by using the forward primer U335 with the purified PCR product of five virusese.

Sequence comparison and phylogenetic analysis:

Computer-based sequence comparison of the five viruses cDNA sequence with partial sequence of polyprotein in other known BYMV, CVMV,PVY, WMV and ZYMV isolates were carried out using NCBI and CLUSTALW (1.82) http://www.ebi.ac.uk/clustalw (Thompson *et al*., 1994). Bootstrap neighbour-joining tree was generated using MEGA software version 4.0 (Tamura *et al.*, 2007) from CLUSTALW alignment.

Results

Detection of isolates by indirect ELISA:

Five different samples were reacted. Positively with specific virus antisera, (Table 1).

Detection of the viruses by RT-PCR:

The RT-PCR, using universal degenerate primers, revealed an approximately 335bp amplified products with the 5 tested viruses as an expected size. (Fig 1)

Table (1). Reaction of infected samples to different antisera as determined by indirect ELISA.

Virus source		Absorbance value at 405nm				
		BYMV	**CVMV**	**PVY**	**WMV**	**ZYMV**
Carnation	**I**	0.264	**0.989**	0.235	0.373	0.339
	H	0.331	0.341	0.307	0.405	0.249
Cucumber	**I**	0.236	0.336	0.229	0.428	**0.886**
	H	0.382	0.336	0.380	0.332	0.291
Faba bean	**I**	**1.241**	0.298	0.245	0.398	0.225
	H	0.221	0.303	0.318	0.341	0.322
Nicotiana glutinosa	**I**	0.276	0.382	**1.003**	0.393	0.356
	H	0.272	0.385	0.389	0.409	0.287
Squash	**I**	0.338	0.401	0.367	**1.103**	0.773
	H	0.312	0.354	0.326	0.369	0.321

I= Infected sample H=Healthy sample

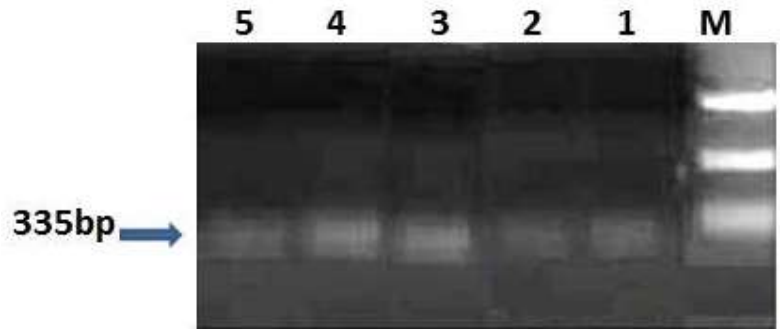

Fig. (1): Agarose gel electrophoresis showing the PCR amplification of the polyprotein gene for five different *Potyvirus* members. Lane M: 100bp ladder, (1) PVY, (2) ZYNV, (3) BYMV, (4) WMV and (5) CVMV.

Nucleotide sequence analysis:

One of our main objectives is to study the nucleotide sequencing of the present isolates and to compare them with the worldwide isolates of the potyvirus members. We used the universal primers to amplify the polyprotein of the *Potyviridae* family from infected plant samples representing five different viruses in the *Potyvirus*.

The nucleotide sequence of the polyprotein gene analysis of PVY-ME2 (the present isolate) showed 98% homology with the PVY-isolate strain N of Poland with the Accession No., in (Table 2).In addition the PVY-ME2 isolate also showed that the sequence was very similar (98%)

Identical nucleotides to PVY – isolate N- strain of Ditta and NH2 (Table 2) of Poland and Chinese isolates. On the other hand, the PVY-ME2 showed low homology with the other characterized potyvirus members, it showed only 66% and 54-52% homology with the other present isolated viruses, CVMV-ME2 ZYMV-ME2, WMV-ME2 and BYMV-ME2 respectively.

Despite the high variability of polyprotein region of different potyvirus isolates (5 Egyptian viruses), the clustering analysis of the 5 isolated viruses here (Fig 2) can be arranged in two main groups, the PVY group and ZYMV group.

The CVMV virus grouped in PVY group but in a separate

subcluster since it has a slightly similarity with PVY virus (66%). Furthermore, it yielding a low percentage of similarity ranking between 56-58% with the rest of the Egyptian viruses (ZYMV, BYMV and WMV respectively) Fig (2).On the other hand, the rest of viruses (WMV, ZYMV and BYMV) are closely related and grouped together in one group.

The WMV has 70% similarity with ZYMV and grouped together in subclster and in the same time the WMV and ZYMV have 66% similarity with BYMV which grouped in a separate subcluster.

The general clustering (Fig 3) showed that the Potyviruses found in Egypt and also in other parts of the world fall into Two main groups: PVY group and potymonus group which contains the rest of all Egyptian viruses (ZYMV, CVMV,BYMV and WMV groups). The PVY isolates are clearly separated from other viruses in a separate group. In the PVY group there is a cluster composed by two isolates: the PVY isolate of the previously published Egypt isolate (N-strain) (Table 2 and Fig. 3) with France isolate [with accession No. AF522298 and X12456 respectively (Table 2). According to multiple sequence alignments (data not shown), our PVY- ME2 present isolate had much more distantly related (91%) to the previously mentioned Egyptian and France isolates (N- strain). In turn, the PVY-ME2 (Egyptian isolate) showed a clustering with N-Nysa and 34/ 01(N- strain) isolates belonging to Poland (with accession No., FJ666337 and AJ890342 respectively) and it showed 98% of similarity (Table2 and Fig 3). In addition the PVY-ME2 isolate sparated from the cluster of PVY isolates from other parts of the world, USA, Germany and China (Table 2 and Fig.3).

In the potymonus group, the WMV isolates clustered together separately from the ZYMV, BYMV and WMV- ME2 isolates subcluster composed by Two isolates: the WMV-ME2 isolate (Egyptian isolate) with France isolate (with accession No., AY 437609) and having the highest similarity (98%) and also clustered with Turkey and Chile isolates (Turk 91 and CHl0243, respectively (table 2) and also showed 98% of identify. In turn the WMV-ME2 isolate separated from the Pakistan and Spain

isolates (Table 2 and Fig 3) with accession No.AB218280 and AJ579503, respectively and yielding 96-94% percentage of similarity.

The ZYMV –ME2showed 98% of identity with JOR-B5 isolate of Jordan (Table 2 and Fig.3) and subclustered together. On the other hand it completely separated from the MM isolate of China (Table 2 and Fig 3) and it showed only 87% of homology. According to the phylogenetic homology tree (Fig 3), the potymonus group, ZYMV and BYMV are grouped together but the CVMV subclustered together with the BYMV-ME2 and related to the BYMV of Australia (PVB-1), Indian (NBR1) and Japanese (S.22C) isolates with Accession No. EU0821 w23, EU249374 and AB029436 respectively). However, the phylogenetic homology tree (Fig 3) based on the multiple sequence alignments revealed that the BYMV-ME2 polyprotein was closely related to Japanese isolates (S.22C and MB4) with Accession No., AB029436 and D83749 since it showed 98 – 97% of similarity . In turn the BYMV-ME2 appeared far from the BYMV- MC and Alaska isolates of Japan and USA, respectively (with accession No., AB 004545 and GU126690, respectively. Since it showed only 92-89% of identity.

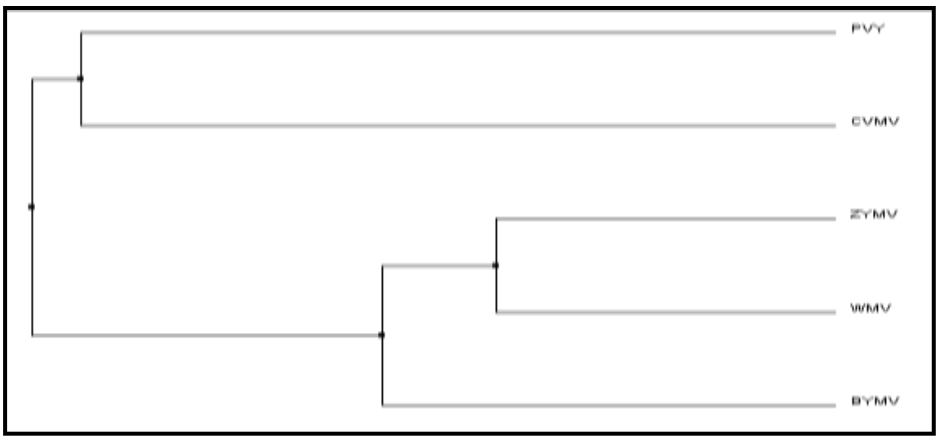

Fig. (2): The phylogenetic homology tree based on multiple sequence alignments of the BYMV[5]ME2, CVMV-ME2, PVY-ME2, WMV-ME2 and ZYMV-ME2 Egyptian isolates.

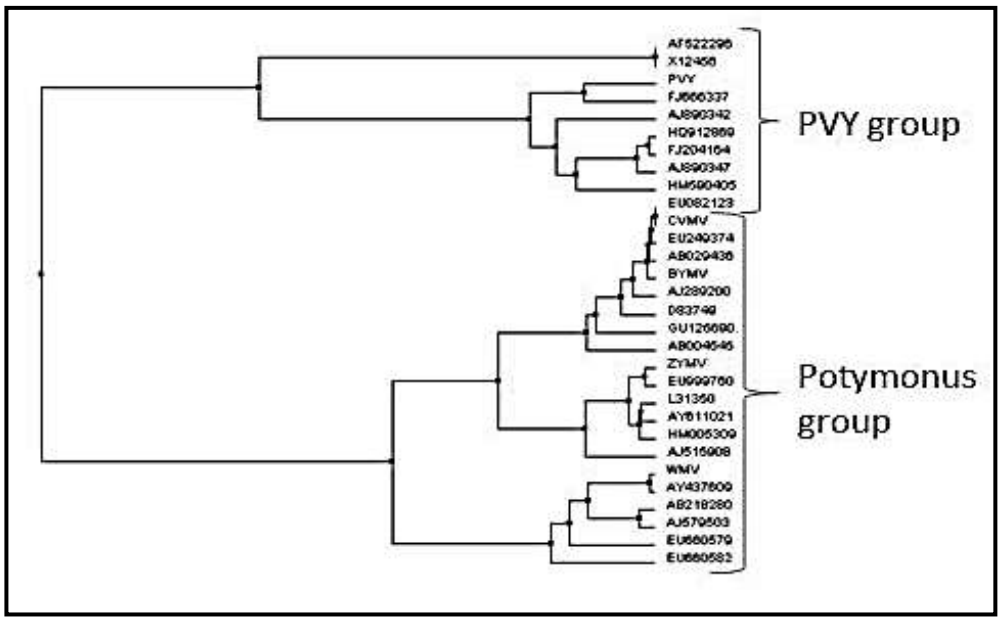

Fig. (3): The phylogenetic homology tree based on multiple sequence alignments of the BYMV, ME2, CVMV-ME2,PVY-ME2, WMV-ME2 and ZYMV-ME2 Egyptian isolates compared to previously sequenced isolates.

Egyptian J. Virol. 9(1), (2012) 359-371

Table (2): Twenty-six strains and isolates of BYMV, CVMV, PVY, WMV and ZYMV sequences from NCBI used in the comparison and analysis of polyprotein sequences.

Accession No./NCBI reference sequences	Isolate	Strain	Country
PYV:			
HQ912869	ID155	PVY-NTN	USA
FJ204164	N4	NTN	USA
HM590405	Guiding-3	N	Chine
FJ666337	N-Nysa	N	Poland
AJ890342	34/01	N	Poland
AJ890347	satina	NTN	Gernany
X12456	-	N	France
AF522296	-	N- EGYpt	Egypt
BYMV:			
AB029436	S-22C	-	Japan
EU249379	NBR1	-	India
EU082133	PVB-1	-	Australia
AJ289200	BBA99710	-	Germany
GU126690	Alaska	-	USA
AB004545	NC	-	Japan
D83749	-	MB4	Japan
WMV:	-		
AY437609	Turk91	WMV-ER	France
EU660579	CH102-481	-	Turkey
EU660582	-	-	Chile
AB218280	BAD95.2	WMV-PK	Pakistan
AJ579503		-	Spain
ZYMV:			
EU999767	SYR-B4	-	Syria
EU999760	JOR-B5	-	Jardan
Ay611021	CH99/116	-	Chine
HM005309	11SPNO2	-	Mali
L31350	-	california	USA
AJ515908	MM	-	Chine

Discussion

Five viruses of *Potyvirus* isolated from different hosts, BYMV, CVMV, PVY, WMV and ZYMV, were identified on the basis of symptomatology on different indicator host plants serological reaction by indirect ELISA and by using of universal oligonucleotide primers in reverse transcription-polymerase chain reaction (RT-PCR). Analysis of PCR products in agarose gel electrophoresis revealed amplification of band approximately 335 bp (base pair) which were in agreement with size of the fragment expected from the sequence data reported by (Langeveld *et. al*.,1991 and Zheng *et. al*., 2010)

Most recent reports of new potyvirus sequences include some form of phylogenetic analysis, but different sets of viruses, genes and parts of genes have been used in different analyses, and so comparisons between analyses cannot be made. Standardization is essential for making comparative phylogeographic analyses (Gibbs *et.al*., 2008).

The clustering analysis of the 5 isolated viruses here can be arranged in two main groups, the PVY group and ZYMV group

Comparison of partial sequences and phylogenetic analysis relationship among potyviruses isolated and twenty-six strains and isolates previously described in GenBank revealed that PVY appeared closely related to isolate HQ912869 and FJ204164 (Karasev, *et. al.,* 2011 and Hu *et.al*. 2009) while it had much more distantly related (91%) to the previously reported Egyptian isolates (N- strain), AF522296 (Abdel El-Mohsen *et.al*. 2003). BYMV-ME2 polyprotein was closely related to isolates AB029436 and D83749 (Wada *et.al*., 1999 and Nakamura *et.al*., 1994). The ZYMV – ME2showed 98% of similarity with isolation of EU999760, while it completely separated than the AJ515908 isolate (Al-Tamimi *et.al*., 2008 and Zhao *et.al*., 2003). The WMV-ME2 isolate having the highest similarity (98%) with isolates, AY437609, EU660579 and EU66582 (Desbiez and Lecoq, 2004and Desbiez and Lecoq, 2008).

Egyptian J. Virol. 9(1), (2012) 359-371

Refrence

Abdel El-Mohsen, N.M.A.;Gamal El-Dinn,A.S.; Sohair,I.E.-A.; Sadik,A.S. and Abdelmaksoud, H.M (2003). Characterization of potato virus Y strain 'N-Egypt' Ann. Agric. Sci. 48 (2), 485-504.

Al-Tamimi, N.A.; Kawas, H.; Akel, M. and Arabiat, A.H.(2008). Biological and Molecular Characterization of Some Zucchini Yellow Mosaic Virus (ZYMV) Isolates from Southern Syria and Jordan Valley. Unpublished.

Amal Abo El-Ela, A.; M. A. Amer and F. Abo El- Abbas.(2006). *Celery Yellow Mosaic Potyvirus* Affecting Umberlliferae Plants in Egypt. Egyptian J.Virol.3(1),1-18.

Amer, M.A., El-Hammady, M. H., Mazyad, H.M., Shalaby, A. A.and Abo-El-abbas, F.M. (2004). Cloning, Expression and Nucleotide Sequence of Coat Protein Gene of an Egyptian Isolate of *Potato Virus Y* Strain NTN Infecting Potato Plants. Egyptian J. Virol. 1, 39-50.

Chen, J.and Adams, M.J. (2001). A universal PCR primer to detect members of the Potyviridae and its use to examine the taxonomic status of several members of the family. Archives of Virology 146, 757–66.

Coline, D. and Kummert, and J. (1993). Identification of a sweet potato feathery mottle virus isolate from China (SPFMV-CH) by the polymerase chain reaction with degenerate primers. Journal of Virological Methods 45, 149–59.

Desbiez,C. and Lecoq, H .(2004). The nucleotide sequence of Watermelon mosaic virus (WMV, Potyvirus) reveals interspecific recombination between two related potyviruses in the 5' part of the genome.Arch. Virol. 149 (8), 1619-1632.

Desbiez,C. and Lecoq, H. (2008). Evidence for multiple intraspecific recombinants in natural populations of Watermelon mosaic virus (WMV, Potyvirus). Arch. Virol. 153 (9), 1749-1754.

Edwardson, J.R.and Christie R.G (1996). Cylindrical inclusions. Bull Agric Exp Sta Univ Fl no. 894.

Fegla, G. I., Younes, H. A., Fath-Allah, M. M., El-Samra, I. A. and El-Shair, S. M. (2009). Occurrence, Distribution and Relative Incidence of Seven Viruses Infecting Cucurbits in Northern Egypt. Egyptian J. of Virol. 6:303-322.

Gibbs, A. and Mackenzie, A. (1997). A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. Journal of Virological Methods 63, 9–16.

Gibbs, A.J.; Mackenzie, A.M. and Gibbs, M.J. (2003). The 'potyvirid primers' will probably provide phylogenetically informative DNA fragments from all species of Potyviridae. J .Virol Methods. 112:41–44.

Gibbs, A. J.; Mackenzie, A. M. Wei, K.-J. and Gibbs, M. J. (2008). The potyviruses of Australia. Arch Virol.155 (10), 1007- 1016.

Ha, C.; Coombs, S.; Revill, P.A.; Harding, R.M.; Vu.M.and Dale, J.L.(2008). Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses. Archives of Virology 153, 25–36.

Hu, X.; Meacham, T.; Ewing, L.; Gray, S.M. and Karasev, A.V.(2009). A novel recombinant strain of Potato virus Y suggests a new viral genetic determinant of vein necrosis in tobacco. Virus Res. 143 (1), 68-76.

Karasev, A.V.; Hu, X.; Brown, C.J.; Kerlan, C.; Nikolaeva, O.V.; Crosslin, J.M. and Gray, S.M. (2011). Genetic Diversity of the Ordinary Strain of Potato virus Y (PVY) and Origin of Recombinant PVY Strains. Phytopathology 101 (7), 778-785.

Koenig, R. (1981). Indirect ELISA methods for the broad specificity detection of plant viruses. J. Gen. Virol., 55:53- 62.

Langeveld, S. A.; Dore, J. M.; Memelink, J.; Derks, A. F. L. M.; Van der Vlugt, C. I. M.; Asjes, C. J. And Bol, J. F. (1991). Identification of *potyvirus* using the polymerase chain reaction with degenerate primers. J. gen. Virol. 72: 1531-1541.

Nakamura, S.; Honkura,R.; Ugaki,M.; Ohshima,M. and Ohashi, Y.(1994). Nucleotide sequence of the 3'-terminal region of bean yellow mosaic virus RNA and resistance to viral infection in transgenic Nicotianabenthamiana expressing its coat protein gene. Ann. Phytopathol. Soc. Jpn. 60, 295-304.

Nicolas, O.; Laliberte, J.F. (1991). The use of PCR for cloning of large cDNA fragments of turnip mosaic potyvirus. Journal of Virological Methods 32, 57–66.

Pappu, S.S.; Brand, R. and Pappu, H.R. (1993). A polymerase chain reaction method adapted for selective amplification and cloning of 3’ sequences of potyviral genomes: application to dasheen mosaic virus. Journal of Virological Methods 41, 9–20.

Rossolini, G.M.; Cresti, S.; Ingianni, A.; Cattani, P.; Riccio, M.L. and Satta, G. (1994). Use of deoxyinosine-containing primers vs degenerate primers for polymerase chain reaction based on ambiguous sequence information. Molecular and Cellular Probes 8, 91–8.

Sambrook, J.; Fritsh, E. F. and Maniatis, T. (1989). Molecular Cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Shula, D.D.; Ward, C.W.; Brunt, A.A. and Berger, P.H. (1998). Potyviridae family. AAB

Descriptions of Plant Viruses No. 366.

Tamura, K.; Dudley, J.; Nei, M. and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596-1599.

Thompson, J. D.; Higgins, D. G. and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Wada, Y.; Iwai, H.; Ogawa, Y. and Arai, K. (1999). Comparison of pathogenicity and nucleotide sequences of 3'-terminal regions of bean yellow mosaic virus isolates from different gladiolus ecotypes and broad(E-mail:pathst 1@farm. agri. Kagoshima-u.ac.jp).

Ward, C.W. and Shula, D.D. (1991). Taxonomy of potyviruses – current problems and some solutions. Intervirology 32: 269–296.

Younes, H. A. and G. I. Fegla. S. I. M. Atta Alla and E. E. M. Efaisha. (2008). Purification of Bean yellow mosaic virus and Broad bean mottle virus and production of their antisera. Egyptian J. of Virol. 5:149-156.

Zhao, M.F.; Chen, J.; Zheng, H.Y.; Adams, M.J. and Chen, J.P.(2003). Molecular analysis of Zucchini yellow mosaic virus isolates from Hangzhou, China. J. Phytopathol. 151, 307-311.

Zheng, L.; Rodoni, B. C.; Gibbs, M. J. and Gibbs, A. J.(2010). A novel pair of universal primers for the detection of Potyviruses Plant Pathology. 59, 211–220.