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Article *in* Plant Pathology · January 2002

DOI: 10.1046/j.1365-3059.1999.00384.x

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Plant Pathology (1999) **48**, 648–654

Characterization of a potyvirus from eggplant (*Solanum melongena*) as a strain of potato virus Y by N-terminal serology and sequence relationships

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A potyvirus (eggplant mottle virus, EMoV) causing mosaic mottling in eggplant (*Solanum melongena*) was characterized on the basis of biological, serological and partial nucleotide sequence properties. EMoV infected *Chenopodium amaranticolor* and members of the Solanaceae. Polyclonal antiserum against EMoV showed antigenic relationship with henbane mosaic potyvirus (HMV) and potato Y potyvirus (PVY). Virus-specific antibodies directed to the N-terminal region of EMoV cross-reacted only with PVY. Determination and comparison of nucleotide sequence of the coat protein (CP) and the 3′-untranslated region (UTR) of EMoV with other potyviruses showed that the level of homology was highest with PVY isolates. Comparative sequence analyses of the CP amino acid and 3′-UTR sequences with distinct PVY isolates placed EMoV within the PVY^O subgroup.

Keywords: DNA sequence, eggplant, N-terminal serology, potyvirus

Introduction

Eggplant (*Solanum melongena*) is an important vegetable in various parts of the world. Five different potyviruses, namely eggplant green mosaic virus (Ladipo *et al*., 1988a), brinjal mild mosaic virus (Naqvi & Mahmood, 1976), eggplant severe mottle virus (Ladipo *et al*., 1988b), pepper veinal mottle virus (Igwegbe & Waterworth, 1982) and potato virus Y (PVY)(Sastry *et al*., 1974), are reported to infect eggplant naturally. Of these, brinjal mild mosaic virus and PVY are reported from India. These were identified as distinct potyviruses from their host range, transmission characteristics and serological relationships. However, identification of potyviruses based on these parameters has resulted in considerable confusion in the past. For reliable identification and differentiation of distinct members and strains, additional criteria such as virus specific antibodies directed towards the N-terminus of the coat protein (CP), peptide profiles of the CP, and genomic relationships based on nucleic acid hybridization, or sequence homology of the 3′-terminal region are increasingly being used (Shukla *et al*., 1994).

In 1988, a virus was isolated from naturally infected eggplant from experimental fields of the Indian Agricultural Research Institute, New Delhi. Preliminary studies showed this to be a potyvirus, based on its flexuous particle morphology, mean length of 696 nm and presence of typical pinwheel inclusions in ultrathin sections of infected leaves. The virus was designated as eggplant mottle virus (EMoV) (Rajamannar & Varma, 1988). Serological and molecular characteristics of EMoV are reported here.

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Accepted 8 April 1999.

Materials and methods

Virus isolates

Potyviruses used in this study were: cowpea aphid-borne mosaic virus (CABMV) in *Vigna unguiculata* cv. V-218, EMoV in *Nicotiana tabacum* cv. White Burley, henbane mosaic virus (HMV) in *N. glutinosa*, papaya ringspot virus (PRSV) in *Carica papaya* cv. Honey Dew, peanut stripe virus (PStV) in peanut, PVY^O in *N. tabacum* cv. White Burley, sugarcane mosaic virus (SCMV) in *Sorghum vulgare* cv. CHS-9 and sweet potato feathery mottle virus (SPFMV) in *Ipomoea setosa*. The plants were maintained in an insect-proof glasshouse.

Host range

Five plants each of *Chenopodium amaranticolor*, *Capsicum annuum*, *Lycopersicum esculentum*, *Nicotiana*

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Table 1 Symptoms induced by eggplant mottle virus on selcted plant species

Family/host species	Symptoms
Chenopodiaceae	
Chenopodium amaranticolor	local lesions
Solanaceae	
Capsicum annuum	Systemic mosaic/mottling
Lycopersicum esculentum	Systemic mosaic/mottling
Nicotiana benthamiana	Systemic mosaic/mottling
N. glutinosa	Necrosis of midrib and veins
N. tabacum cv. White Burley	Systemic mosaic/mottling
N. tabacum cv. Xanthi	Systemic mosaic/mottling

benthamiana, *N. glutinosa*, *N. tabacum* cv. White Burley and *N. tabacum* cv. Xanthi (Table 1), and *Chenopodium album*, French bean, lima bean, cowpea, peanut and soybean, were mechanically inoculated with extracts from EMoV-infected White Burley tobacco and maintained for at least four weeks for symptom development.

Virus purification

Virus particles were purified using a modified protocol of Moghal & Francki (1976), as described in Bhat *et al.* (1997).

Antiserum production

Antiserum was prepared by administering purified EMoV intramuscularly into a rabbit four times at 10 day intervals. On each occasion, 0·5 mg of virus emulsified with an equal volume of Freund's complete adjuvant was injected. Blood was collected 15 days after the last injection (Ball *et al.*, 1990).

Purification of N-terminal specific antibodies

N-terminal specific antibodies from polyclonal antiserum against EMoV were separated by affinity chromatography as described by Shukla *et al.* (1989). The surface located N-terminal peptide region from 3 mg of purified virus was removed by a 30-min incubation with lysyl endoproteinase (Boehringer Mannheim, Germany). The resulting enzyme-resistant particles (presumed to be devoid of the N-terminus) were concentrated by centrifugation and then dissociated using formic acid followed by coupling to 0·75 g of cyanogen bromide-activated Sepharose 4B (Pharmacia, Sweden) as per manufacturer's instructions. EMoV antiserum (200 μL) was passed through the column and antibodies that did not bind to the column were collected and used in the immunoassay.

Serological assays

Direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) and electroblot immunoassay (EBIA) were performed to determine the reactivity of EMoV with selected potyviruses (Table 2).

DAC-ELISA was carried out as described by Clark & Bar-Joseph (1984) using polystyrene plates (Corning, New York, USA). Sap extract was prepared by grinding leaf tissue in 10 volumes (w/v) of coating buffer containing 2% polyvinyl pyrrolidone, filtered through cheese cloth and stored at 4°C overnight or 25 ± 2°C for 1 h, followed by centrifugation at 3000 ***g*** for 5 min. The supernatant was used in ELISA. Antigen, antibody and antirabbit immunoglobulin-alkaline phosphatase (Sigma Chemical Co., St.Louis, USA) were used at 1 : 10, 1 : 1000 and 1 : 2000 dilutions, respectively. The reactions of ELISA were read at 405 nm, 1 h after adding the substrate (p-nitrophenyl phosphate).

EBIA was performed after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by O'Donell *et al.* (1982) using nitrocellulose membrane and alkaline phosphatase conjugated anti-rabbit antibodies (Sigma Chemical Co., St. Louis, USA). After electroblotting at 200 V for 2–4 h, the membranes were processed as described earlier (Bhat *et al.*, 1997). Antiserum and alkaline phosphatase-labeled antirabbit IgG (Sigma Chemical Co., St. Louis, USA) were used at 1 : 1000 and 1 : 2000 dilutions, respectively. Prestained

Table 2 Reactivities of unfractionated and affinity-purified N-terminal-specific antibodies of eggplant mottle virus (EMoV) against selected potyviruses[b] in direct antigen-coated ELISA (DAC-ELISA)[a] and electroblot immunoassay (EBIA)[c]

Detecting antibody	Antigens CABMV	EmoV	HMV	PRSV	PStV	PVY°	SCMV	SPFMV
unfractionated								
DAC–ELISA	–	++++	+	–	–	+	–	–
EBIA	–	+++	+	–	–	++	–	–
N-terminus								
DAC–ELISA	–	+++	–	–	–	+	–	–
EBIA	–	++	–	–	–	–	–	–

[a]Reaction strength (A_{405} readings after deducting the values obtained for healthy samples) was classified as: ++++, > 0·91; +++, 0·61–0·90; ++, 0·31–0·60; +, 0·10–0·30; –, < 0·10.

[b]Acronyms for the potyviruses tested are given in Materials and methods.

[c]+++, strong; ++, medium; +, mild; –, no reaction.

marker proteins (Bio-Rad, Richmond, CA, USA) were used as size standards.

RNA isolation, cDNA synthesis and cloning

EMoV RNA was isolated from 1 mg of purified virus preparation as described by Hammond & Lawson (1988). The resulting viral RNA preparation was used as a template in cDNA synthesis by using a ZAP-cDNA Synthesis Kit (Stratagene, LaJolla, CA, USA) according to the manufacturer's instructions, and cloned into *Eco*RI and *Xho*I sites in Lambda ZAP II (using Uni-ZAP XR Vector Kit, Stratagene, LaJolla, CA, USA) and packaged using Gigapack II Gold Packaging Extract (Stratagene, LaJolla, CA, USA). Recombinant clones were identified by restriction endonuclease digestion followed by Southern hybridization using a heterologous cDNA probe (PVY CP supplied by Dr R.P. Singh, Agriculture and Agri-Food Canada, Frederickton). Hybridization at 65°C for 16 h was followed by washing the membrane in 2×SSC and 0·5% SDS three times for 10 min each. Phagemid pBlueScript excised along with the insert from the recombinant lambda genome was used for further studies. Viral specificity of the selected clones was confirmed using northern hybridization and total RNA isolated from infected and healthy plants following standard molecular biological techniques (Sambrook *et al*., 1989).

Nucleotide sequencing

A cDNA clone with an approximately 1·4 kb insert was sequenced at the DNA Sequencing Core Facility of the Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL. Sequence data were initially compiled using Seqaid II (Rhoads & Roufa, 1985). Multiple alignments were made using CLUSTALW (Thompson *et al*., 1994). Sequence phylograms were constructed using PHYLIP and unrooted trees were generated using TreeView (Page, 1996). Other PVY CP and 3′-UTR sequences used for comparison (Table 3) were obtained from GenBank (Benson *et al*., 1996). The BLAST program (Altschul *et al*., 1990) was used to identify related sequences available from the GenBank database.

Table 3 Source of coat protein amino acid and 3′-untranslated region sequences used for comparisons

Isolate	GenBank accession number	Strain
PVY-C27	AF012026	PVY-C2[a]
PVY-C28	AF012027	PVY-C1[a]
PVY-C30	AF012028	PVY-C2[a]
PVY-45	AF012029	PVY-C1[a]
PVY-21	AJ005639	PVY-NP[a]
PVY-N854	AJ223592	PVY-N
PVY-O768	AJ223593	PVY-O
PVY-O803	AJ223594	PVY-O
PVY-O854	AJ223595	PVY-O
PVY-Fr	D00441	PVY-N
PVY-O4	D12539	PVY-O
PVY-T	D12570	PVY-N
PVY-Nz	M22470	PVY-N
PVY-US	M81435	PVY-O
PVY-Hu	M95491	PVY-NTN
PVY-36	S74810	PVY-O
PVY-VN	U06789	–
PVY-Can1	U09509	PVY-O
PVY-Can2	U09508	PVY-N
PVY-PepN	U10378	PVY-NP[a]
PVY-Chin2	U25672	–
PVY-N27	U91747	PVY-N
PVY-O1	X14136	PVY-O[b]
PVY-Chin1	X54058	PVY-O[b]
PVY-Ru	X54636	PVY-N
PVY-Chil	X68221	PVY-N/ PVY-NP[c]
PVY-PotUS	X68222	PVY-O
PVY-EurH	X68223	PVY-N
PVY-NsNr	X68224	PVY-NP[c]
PVY-MsNr	X68225	PVY-NP[c]
PVY-MsMr	X68226	PVY-O
PVY-NTN	X79305	PVY-NTN
PVY-LB	X92078	–
PVY-605	X97895	PVY-N
PVY-Nysa	Z70237	PVY-N
PVY-Wil	Z70238	PVY-N
PVY-LW	Z70239	PVY-O
PVY-In	Kumar *et al.* (1997)	PVY-O
EMoV	AF118153	This study
PRSV[d]	AF063220	PRSV-P

[a]According to Blanco-Urgoiti *et al.* (1998).
[b]According to Van der Vlugt *et al.* (1993).
[c]According to Blanco-Urgoiti *et al.* (1996).
[d]Papaya ringspot virus.

Results

Host range

Of the 15 plant species tested for susceptibility to EMoV by sap inoculation, one species belonging to the family Chenopodiaceae and all species of Solanaceae were susceptible (Table 1). The virus caused systemic mild mosaic or mottling on *N. tabacum* cvs. White Burley and Xanthi, *N. benthamiana*, *Capsicum annuum* and *Lycopersicon esculentum*. Inoculated leaves of *N. glutinosa* developed necrosis of the midrib and veins, resulting in drooping of the leaves. *C. amaranticolor* developed chlorotic local lesions. No symptoms were observed on French bean, lima bean, cowpea, *C. album*, peanut or soybean, and no virus was recovered when extracts of these test plants were back inoculated onto White Burley tobacco.

Virus purification

Highest yields of purified virus were obtained when fresh leaf tissue was harvested three weeks after inoculation.

Climatic conditions during the growth of the propagation host also resulted in variation of virus yield (0·82–3·17 mg kg^{-1} of tissue), the highest being obtained during spring and autumn, and the lowest during summer, when temperature often exceeded 40°C.

Serological relationships

Of the different potyviruses tested by DAC-ELISA and EBIA for cross-reactivity with the unfractionated EMoV antiserum, only HMV and PVY showed positive reactions (Table 2). The affinity-purified, EmoV-specific N-terminal antibodies cross-reacted only with PVY in DAC-ELISA, and no reaction was seen with PVY in EBIA (Table 2).

Cloning and sequencing

The 3′ 1426 bp terminal region of EMoV was cloned and sequenced (Fig. 1). The sequenced region contained a single open reading frame (ORF) of 1099 nucleotides that could potentially encode a polyprotein of 365 amino acids. The ORF was followed by an untranslated region (UTR) of 328 nucleotides, excluding the poly (A) tail. The polyprotein included a part of the nuclear inclusion b (NIb) protein consisting of 98 amino acids. Assuming Q/A as the proteolytic cleavage site between NIb and CP (Shukla *et al*., 1994), the CP consisted of 267 amino acid residues (Fig. 1). The amino acid sequences conserved among the majority of potyviruses (MIEA/SWG, Pappu *et al*., 1994; and WCIEN and QMKAAA, Pappu *et al*., 1993) were also present in EMoV (Fig. 1).

Sequence comparisons

GenBank searches identified several PVY isolates as

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GCACGAGCAGACTTGCCCGAACACAGGCTTGAGGCGATTTGCGCAGCTATGATAGAGTCCTGGGGTTATT
 A  R  A  D  L  P  E  H  R  L  E  A  I  C  A  A  M  I  E  S  W  G  Y  S

CTGAACTAACACACCAAATCAGGAGATTCTACTCATGGTTATTGCAACAGCAACCTTTTGCAACAATAGC
  E  L  T  H  Q  I  R  R  F  Y  S  W  L  L  Q  Q  Q  P  F  A  T  I  A

GCAGGAAGGGAAGGCTCCTTATATAGCAAGCATGGCATTAAGGAAACTGTATATGGATAGGGCTGTGGAT
 Q  E  G  K  A  P  Y  I  A  S  M  A  L  R  K  L  Y  M  D  R  A  V  D

GAGGAAGAGCTAAGAGCCTTCACTGATATGATGGTCGCATTAGACGATGAGTTTGAGTTTGACTCCTATG
E  E  E  L  R  A  F  T  D  M  M  V  A  L  D  D  E  F  E  F  D  S  Y  E

AAGTGCACCATCAAGCAAATGACACAATTGATGCAGGAGGAAGCAACAAGAAAGATGCAAAACCAGAGCA
  V  H  H  Q  A  N  D  T  I  D  A  G  G  S  N  K  K  D  A  K  P  E  Q

AGGCAGCATCCAGTCAAACCCGAACAAAGGAAAAGATAAGGATGTGAATGCTGGTACATTCGGGACACAT
 G  S  I  Q  S  N  P  N  K  G  K  D  K  D  V  N  A  G  T  F  G  T  H

ACTGTGCCGAGAATCAAAGCTATCACGCCCAAAATGAGAATGCCCAAAAGCAAGGGAGCAACCGTGCTAA
T  V  P  R  I  K  A  I  T  P  K  M  R  M  P  K  S  K  G  A  T  V  L  N

ATCTAGAACACTTGCTTGAGTACGCTCCACAACAAATTGATATTTCAAATACTCGGGCAACTCAATCACA
  L  E  H  L  L  E  Y  A  P  Q  Q  I  D  I  S  N  T  R  A  T  Q  S  Q

GTTTGATACGTGGTATGAGGCAGTGCGGATGGCATACGACATAGGAGAAACTGAGATGCCAACTGTGATG
 F  D  T  W  Y  E  A  V  R  M  A  Y  D  I  G  E  T  E  M  P  T  V  M

AATGGGCTTATGGTTTGGTGCATTGAAAATGGAACCTCGCCAAATGTCAACGGAGTTTGGGTTATGATGG
N  G  L  M  V  W  C  I  E  N  G  T  S  P  N  V  N  G  V  W  V  M  M  D

ATGGGAATGAACAAGTTGAGTACCCGTTGAAACCAATCGTTGAGAATGCAAAACCAACCCTTAGGCAAAT
  G  N  E  Q  V  E  Y  P  L  K  P  I  V  E  N  A  K  P  T  L  R  Q  I

CATGGCACATTTCTCAGATGTTGCAGAAGCGTATATAGAGATGCGCAACAAAAAGGAACCGTATATGCCA
 M  A  H  F  S  D  V  A  E  A  Y  I  E  M  R  N  K  K  E  P  Y  M  P

CGATATGGTTTAATTCGAAATCTGCGGGATGTGGGTTTAGCGCGTTATGCCTTTGACTTTTATGAGGTCA
R  Y  G  L  I  R  N  L  R  D  V  G  L  A  R  Y  A  F  D  F  Y  E  V  T

CATCACGAACACCAGTGAGGGCTAGGGAAGCGCACATTCAAATGAAGGCCGCAGCATTGAAATCAGCCCA
  S  R  T  P  V  R  A  R  E  A  H  I  Q  M  K  A  A  A  L  K  S  A  Q

ACCTCGACTTTTCGGGTTGGACGGTGGCATCAGTACACAAGAGGAGAACACAGAGAGGCACACCACCGAG
 P  R  L  F  G  L  D  G  G  I  S  T  Q  E  E  N  T  E  R  H  T  T  E

GATGTTTCTCCAAGTATGCATACTCTACTTGGAGTCAAGAACATGTGATGTAGTGTCTCTCCAGATGATA
D  V  S  P  S  M  H  T  L  L  G  V  K  N  M  < 365

TATAAGTATTTACATATGCAGTAAGTATTTTGGCTTTTCCTGTACTACTTTTATCATAATTAATAATCAG
TTTGAATATTACTAATAGATAGAGGTGGCAGGGTGATTTCGTCATTGTGGTGACTCTATCTGTTAATTCC
GCATTATTAAGTCTTAGATAAAAGTGCCGGGTTGTCGTTGTTGTGGATGATTCATCGATTAGGTGATGTT
GCGATTCTGTCGTAGCAGTGACTATGTCTGGATCTATCTGCTTGGGTGATGTTGTGATTTCGTCATAACA
GTGACTGTAAACTTCAATCAGGAGAC 1426
```

Figure 1 Nucleotide (shown as DNA) and deduced amino acid sequences of the 3′-terminal region of eggplant mottle virus (EMoV). The conserved regions in the nuclear inclusion b (NIb) and the coat protein (CP) are underlined. The potential cleavage site between NIb and CP genes (Q/A) and the DAG triplet potentially associated with aphid transmissibility are doubly underlined.

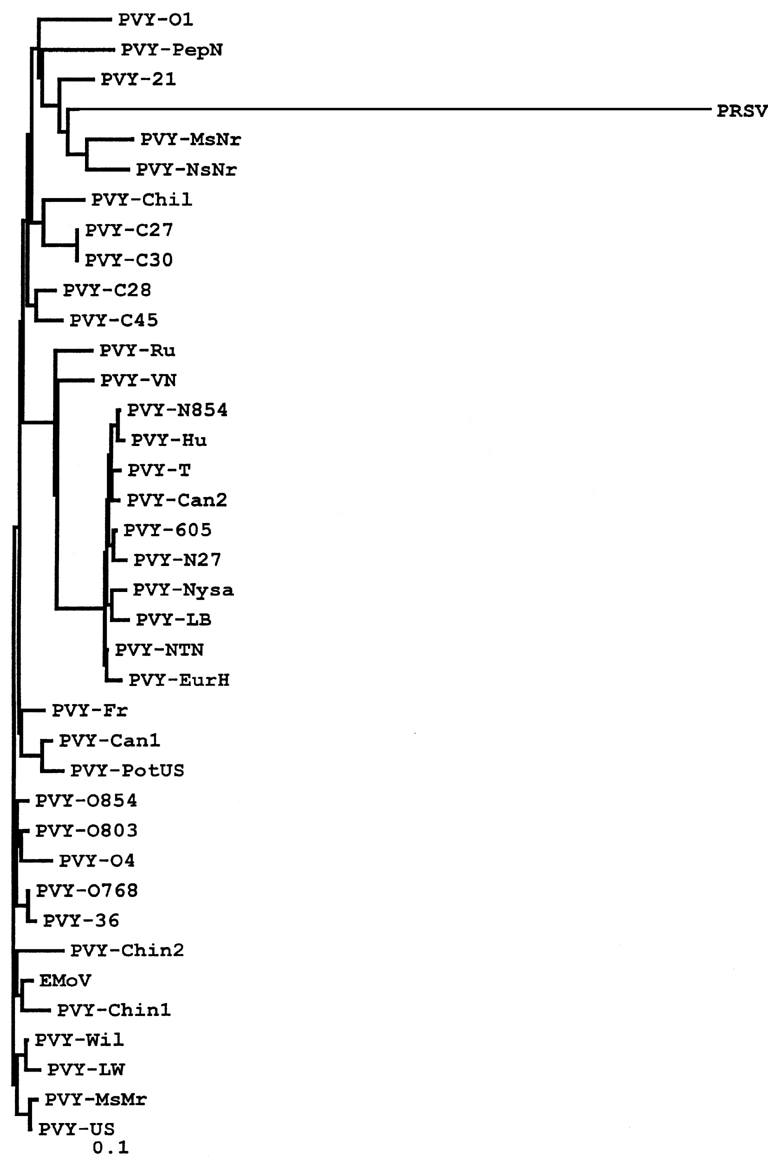

Figure 2 Cluster dendrogram illustrating phylogenetic relationships based on the multiple alignments of the coat protein (CP) amino acid sequences of 36 distinct potato virus Y isolates and eggplant mottle virus (EMoV). Sequences for comparisons were obtained from GenBank (Benson *et al.*, 1996). The CP amino acid sequence of papaya ringspot virus (PRSV) is included as an outgroup. The designation given to each of the isolates and their GenBank accession numbers are given in Table 3.

closely related to EMoV. A detailed comparison of the CP sequence and 3′-UTR with 38 geographically distinct PVY isolates available from GenBank (Table 3) was carried out. Multiple alignment of the EMoV CP amino acid sequence with other PVY CP sequences revealed a high level of sequence identity (92–98%), the N-terminus being the most divergent (not shown). Sequence comparisons grouped EMoV within the PVY^{O} subgroup. Furthermore, the first amino acid of the CP of EMoV was alanine, a feature of members of the PVY^{O} subgroup (Fig. 1) (Blanco-Urgoiti *et al.*, 1996). This grouping of EMoV within the PVY^{O} subgroup was confirmed in the cluster dendrograms based on the CP amino acid sequences (Fig. 2). The presence of a unique *Hin*fI restriction site at position 137 and the lack of *Dde*I and *Eco*RV restriction sites in the CP gene of EMoV also supports inclusion of the virus in the PVY^{O} subgroup (Blanco-Urgoiti *et al.*, 1996).

Similarly, multiple alignment of EMoV 3′-UTR with the 23 geographically distinct PVY isolates available from GenBank (Table 3) revealed high sequence identity (84–99%). A cluster dendrogram based on 3′-UTR sequences also clearly placed EMoV within the PVY^{O} subgroup (Fig. 3).

CP amino acid and 3′-UTR sequence analysis of a large number of geographically distinct isolates of PVY also brought out some interesting findings. As observed by Blanco-Urgoiti *et al.* (1996) for some PVY isolates, it was found that PVY-Wil, originally described as a PVY^{N} strain, clearly falls into the PVY^{O} subgroup, based on both CP amino acid and 3′-UTR sequence comparisons (Fig. 2; Fig. 3). PVY-Hu, grouped as PVY^{N} on CP amino acid sequence (Fig. 2) and restrictotyping (Blanco-Urgoiti *et al.*, 1996), falls into PVY^{O} subgroup according to the 3′-UTR sequence comparisons (Fig. 3). This type of contrast clustering

Figure 3 Cluster dendrogram illustrating phylogenetic relationships based on the multiple alignment of the 3′-untranslated region (UTR) of 23 distinct potato virus Y isolates and eggplant mottle virus (EMoV). Sequences for comparisons were obtained from GenBank (Benson *et al*., 1996). The UTR sequence of papaya ringspot virus (PRSV) is included as an outgroup. The designation given to each of the isolates and their GenBank accession numbers are given in Table 3.

(Blanco-Urgoiti *et al*., 1996) was also seen for the PVY-PepN isolate (Fig. 2; Fig. 3)

Discussion

A virus naturally infecting eggplant was characterized at biological, serological and molecular levels. The host range was restricted to Solanaceae and *C. amaranticolor.* The virus showed a serological relationship with PVY and HMV. According to the criteria of Shukla *et al.* (1989), the cross-reactivity of EMoV N-terminal specific antibodies with PVY in DAC-ELISA suggests that EMoV and PVY are strains of the same virus. Similarly, N-terminal specific antibodies of PVY also reacted with EMoV, confirming the N-terminal epitope similarity between PVY and EMoV (Bhat *et al*., 1997). However, there was a lack of cross-reaction of N-terminal specific antibodies of EMoV with PVY in EBIA. The SDS-mediated dissociation of virions may have disrupted the epitope(s) usually recognized by the EMoV antibodies. Similar results were obtained for pepper mottle virus and PVY, which are considered to be strains of the same virus (Shukla *et al*., 1992). However, similarities are also reported for N-terminal regions of CPs of biologically distinct potyviruses (Shukla *et al*., 1989; Khan *et al*., 1990; Fortass *et al*., 1991). Therefore, for unequivocal identification of EMoV, its CP gene and 3′-UTR were cloned, sequenced and compared with other potyviruses to assess its status either as a distinct potyvirus or a strain of PVY.

The high degree of EMoV CP amino acid sequence identity with those of PVY isolates (92–98%) confirmed EMoV as a strain of PVY. Further more, the 3′-UTR of EMoV showed 84–99% identity with UTR sequences of distinct PVY isolates, thus complying with the cut-off range of 83–99% for identifying strains of the same virus as described by Frenkel *et al.* (1989). Cluster dendrograms based on CP amino acid as well as 3′-UTR sequences placed EMoV within the PVY^{O} subgroup (Fig. 3).

Based on the genetic distances estimated from RFLP patterns of the CP gene, Blanco-Urgoiti *et al.* (1996) have proposed the PVY^{NP} subgroup for nonpotato isolates. Although EMoV was originally isolated from eggplant, cluster dendrogram based on the CP amino acid sequences showed that it is closer to the PVY^{O} subgroup than to PVY^{NP}. This was further confirmed by the presence of a unique *Hin*fI recognition site that is characteristic of the PVY^{O} subgroup (Blanco-Urgoiti *et al*., 1996). EMoV also shared 98% identity with a partial CP sequence and 99% identity with the 3′-UTR (Fig. 3) of a PVY^{O} isolate of potato from New Delhi, India (Kumar *et al*., 1997). These two are the only PVY isolates sequenced, to date, from India. On the basis of the CP gene and 3′-UTR sequence analysis data, EMoV is considered to be a member of the PVY^{O} subgroup.

Acknowledgements

A.I. Bhat is supported by a BOYSCAST fellowship from the Department of Science and Technology, Government of India. Access to sequence analyses software and GenBank was provided by the Research Computing Resource Center, University of Georgia. The nucleotide sequence data reported in this paper have been submitted to GenBank under accession no. AF118153. We thank Dr Sita Pappu for critical reading of the manuscript and Dr R.P. Singh for a cDNA probe of the PVY CP gene.

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