ANALYSIS OF THE NUCLEOTIDE SEQUENCE OF THE COAT PROTEIN AND 3'-UNTRANSLATED REGION OF TWO BRAZILIAN *Potato virus* Y ISOLATES

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ABSTRACT

Two Brazilian *Potato virus Y* (PVY) isolates were biologically characterized as necrotic (PVY-NBR) and common (PVY-OBR) based upon symptoms on test plants. Additional characterization was performed by sequencing a cDNA corresponding to the 3' terminal region of the viral genome. The sequence consisted of 195 nucleotides (nt) coding part of the nuclear inclusion body b (NIb) gene, 804 nt of the coat protein (CP) gene, and 328 nt (PVY-OBR) or 326 nt (PVY-NBR) of the 3'-untranslated region (UTR). Translation of the sequence resulted in one single open reading frame with part of the NIb and a CP of 267 amino acids. The two isolates shared 95.1% similarity in the CP amino acid sequence. The CP and the 3'-UTR sequence of the Brazilian isolates were compared to those of other PVY isolates previously reported and unrooted phylogenetic trees were constructed. The trees revealed a separation of two distinct clusters, one comprising most of the common strains and the other comprising the necrotic strains. PVY-OBR was clustered in the common group and PVY-NBR in the necrotic one.

Key words: PVY, *Potyvirus*, phylogenetic analysis, taxonomy, coat protein.

RESUMO

Análise da seqüência de nucleotídeos da capa proteica e da região 3' não codificadora de dois isolados brasileiros de *Potato virus Y*

Dois isolados do vírus Y da batata (*Potato virus Y,* PVY) foram caracterizados biologicamente como pertencentes às estirpes necrótica (PVY-NBR) e comum (PVY-OBR) com base nos sintomas induzidos em plantas-teste. Caracterização adicional foi realizada com a determinação da seqüência de nucleotídeos do cDNA correspondente à extremidade 3' do genoma viral. A seqüência obtida continha 195 nucleotídeos (nt) que codificam uma parte do gene da inclusão nuclear b (NIb), 804 nt da capa proteíca (CP) e 328 nt em PVY-OBR e 326 nt em PVY-NBR da região 3' não traduzida (UTR). A análise da seqüência mostrou ser composta por uma única fase aberta de leitura contendo parte do NIb e a CP consistindo de 267 aminoácidos. Os dois isolados apresentaram uma similaridade de 95,1% na seqüência de aminoácidos da CP. A CP e a seqüência 3'-UTR dos isolados brasileiros foram comparados com as seqüências de outros isolados de PVY previamente relatados e árvores filogenéticas foram construídas. As árvores mostraram a delimitação de dois grupos distintos, um contendo a maioria das estirpes comuns e o outro com as estirpes necróticas. PVY-OBR foi agrupado no grupo comum e PVY-NBR no necrótico.

INTRODUCTION

Potato virus Y (PVY) is the type species of the genus *Potyvirus* from the family *Potyviridae.* This virus causes serious losses in many important crops worldwide, especially in solanaceous plants such as potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), and tobacco (*Nicotiana tabacum* L.). Three PVY strains are recognized by their distinct host responses: the common strain (PVY^O), which causes mosaic and mottling symptoms in *N. tabacum* plants; the tobacco veinal necrosis strain (PVY^N), causing necrotic symptoms in *N. tabacum*; and the stipple streak strain (PVY^C), characterized by the hypersensitive reaction caused in many potato cultivars (De Bokx & Huttinga, 1981). Recently, a new PVY isolate causing the potato tuber necrotic ring disease (PTNRD) was reported as caused by a new variant of PVY, PVY^{NTN} (Beczner *et al.*, 1984; Le Romancer *et al.*, 1994).

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Under Brazilian conditions, the occurrence of PVY^O and PVY^N strains (based upon reaction on potato and tobacco plants) are widely reported (Silberschmidt *et al*., 1954; Montenegro *et al*., 1968; Alexandre & Barradas, 1982).

The nucleotide or amino acid sequence of the viral genome has been especially important to classify the species and strains of the *Potyvirus* genus (Van der Vlugt *et al.*, 1993). The availability of this information is also a prerequisite for the success of new control measures, e.g. methods based on transgenic resistant plants expressing viral genes (Tennant *et al.*, 1994). Many of the transgenic approaches are based upon specific interactions between the transgene and the predominant virus in a given environment, which underlines the need for sequencing local virus strains.

Here, two Brazilian PVY isolates from potato were studied and the nucleotide sequence of part of the inclusion body b (NIb) gene, the coat protein (CP) gene and the 3'-untranslated region (UTR) were determined. The taxonomical position of these Brazilian PVY isolates are discussed in this report.

MATERIALS AND METHODS

PVY isolates

Thy PVY isolates, PVY-OBR and PVY-NBR, used in this study were originally collected from potato in the Federal District region more than 15 years ago. Since their collection, they were maintained in the greenhouse of Embrapa Vegetables in *N. tabacum* TNN plants by mechanical inoculation using 0.01M potassium phosphate buffer, pH 7.0, with 0.01M sodium sulfite as inoculation buffer.

Host response

The PVY isolates were mechanically inoculated to 14 test plants (Table 1). Inoculated plants were incubated under greenhouse conditions for symptom development up to 30 days after inoculation.

cDNA Cloning and sequencing of the viral 3' terminal region

Total RNA was extracted from infected *N. tabacum* TNN plants using Tri Reagent RNA extraction solution (Sigma). The total RNA was then used for cDNA synthesis primed with oligo d(T) (T-primed first-strand kit, Pharmacia). PCR was performed using *Taq* polymerase (GIBCO-BRL) and the primers PY1 and PY2 (Table 2). The amplified DNA fragments amplified of ca. 1.330 bases were cloned in pGEM-T vector (Promega) following recommendation of the manufacturer. The nucleotide sequence was determined by automated sequencing using vector (T7 and SP6) and internal (PY3, PY4, and PY5) primers (Table 2).

Nucleotide sequence analysis

The nucleotide sequences were compiled and analyzed using programs of the University of Wisconsin Genetics Computer group (GCG, Madison, USA) (Devereux *et al.*, 1984). PVY sequences were fetched from the GenBank and referred to as the locus name (Table 3), except for PVYNNL (Van der Vlugt *et al.*, 1989), PVYOH (Ohshima *et al.*, 1991) and PVYTH (Hataya *et al.*, 1990). The sequences were aligned and Clustal W (http://www2.ebi.ac.uk/clustalw) was used for phylogenetic tree construction, which was viewed by the program Treeview (http://taxonomy.zoology.gla.ac.uk/rod/treeview).

TABLE 1 - Reactions of test plants inoculated with the *Potato virus Y* isolates OBR and NBR

Test plant	PVY-OBR	PVY-NBR
Capsicum annuum L. 'Ikeda'	-	-
C. chinense Jacq. (PI 159236)	-	-
Chenopodium amaranticolor Coste & Reyn.	CLL, NLL	-
C. quinoa Willd.	CLL, NLL	-
Datura stramonium L.	-	-
Gomphrena globosa L.	-	-
Lycopersicon esculentum Mill. 'Rutgers'	VC, mMT	mMT
Nicandra physaloides (L.) Gaertn.	MT, VC	MT, VC
Nicotiana benthamiana Domin.	VC, M, LD	VC, M, LD
N. rustica L.	MT	MT, M
N. tabacum L. 'TNN'	VC, MT, YS	VC, VN, NS
Physalis floridana Rydn.	mM, M, YS, DL	MT
Solanum tuberosum L. 'Achat'	NS, VN	mNS, VC
S. tuberosum 'Bintje'	M	mMT

CLL: chlorotic local lesion; NLL: necrotic local lesion; VC: vein clearing; MT: mottling; M: mosaic; LD: leaf distortion; YS: yellow spot; DL: leaf dropping; NS: necrotic spot; VN: veinal necrosis; m: mild; -: no symptom.

TABLE 2 - Primers used for nucleotide sequence determination

Primer [a]	Sequence 5'®3' [b]	Position [c]
PY1	GGGGGATCCAAATCAGGAGATTC	8362-8377
PY2	CCCGGATCCGTCTCCTGATTGAAG	9704-9691
PY3	CTTAGGCAAATCATGGC	9038-9054
PY4	AAACCATATCGTGGCAT	9129-9113
PY5	CACAGTTTGATACGTGG	8835-8851

[a] The primers were designed according to the sequence of X12456
[b] Restriction enzyme site (*Bam*H I) is underlined
[c] Position of the primer according to X12456

RESULTS

Host response to PVY inoculation

PVY-OBR and PVY-NBR were originally isolated from potato fields in Brazil and maintained in *N. tabacum* plants. When inoculated into test plants, they showed typical symptoms of the common and necrotic strains, respectively (Table 1). *Datura stramonium* and pepper plants were not infected with either isolates and only PVY-OBR caused local lesions in *C. amaranticolor* and *C. quinoa*. *Nicotiana*

TABLE 3 - PVY sequences used for nucleotide sequence comparison

Access number	Locus name	Isolate
AF255659	-	PVY-OBR
AF225660	-	PVY-NBR
AJ223592	pvy223592	N 854, necrotic
AJ223593	pvy223593	0768, common
AJ223594	pvy223594	0803, common, Switzerland
AJ223595	pvy223595	0854, common, Switzerland
D12539	pvyocrna	Common, Japan
D12570	pvycp	PVY-T, necrotic, Japan
E03317	pvye03317	PVY-T
M22470	pvycpa	New Zealand N-PVY
M81435	pvypolypr	PVY
M95491	pvypolyp	NTN, Hungary
S74810	pvys74810	PVY-36, common, Japan
S74813	pvys74813	PVY-T13, necrotic, Japan
X12456	pvynxx	Necrotic, France
X14136	pvycoat	Strain Y, common, Argentina
X54058	pvypocp3	China
X54636	pvycapsi	Necrotic, Russian YN
X68221	pvycapa	NC 178, strain Chilean, from tobacco
X68222	pvycapb	NC 179, strain Potato US, from tobacco
X68223	pvycapc	NC 189, strain Europe-H, from tobacco
X68224	pvycapd	NC 78, strain NsNr, from tobacco, USA
X68225	pvycape	NC 138, strain MsNr, from tobacco, USA
X68226	pvycapf	PVY-O, from tobacco
X79305	pvygcp	NTN
X92078	pvycpgene	Substrain LB
X97895	pvygen	605, necrotic, Switzerland
U09508	pvyu09508	N27-92, necrotic, North America
U09509	pvyu09509	P07, common
U10378	pvyu10378	Nnp, from *Capsicum annuum*
U25672	pvyu25672	China
U91747	pvyu91747	N27, necrotic, North America
Z70237	pvycpnysa	PVY-N-Nysa, necrotic
Z70238	pvycpwilg	PVYN-Wilga, necrotic
*	pvynnl	Necrotic, the Netherlands
*	pvyoh	Common, Japan
*	pvyth	Necrotic, Japan

* not deposited in the GenBank

tabacum TNN showed distinct symptoms. PVY-NBR caused necrotic spots and veinal necrosis, while PVY-OBR caused vein clearing, mottling, and pearl spots. *Physalis floridana* infected with PVY-OBR showed more severe symptoms than when inoculated with PVY-NBR. PVY-OBR appeared to be more severe in *S. tuberosum*, with systemic necrosis in cv. Achat and mosaic in cv. Bintje. The infection of the test plants was confirmed by DAS-ELISA using a polyclonal antibody (Laboratory of Virology, Embrapa Vegetables) against PVY .

Nucleotide sequence analysis of the 3'end of the PVY genome

PCR amplification was directed to the conserved region in the nuclear inclusion body b (NIb) gene approximately 200 bases upstream of the CP gene, and to the last 15 bases of the 3'-UTR, which were completely conserved among all the PVY sequences analyzed. Amplified PCR products were cloned and the sequence of approximately 1.330 nucleotides was determined for both strains using two clones from different PCR amplifications. The comparison of the nucleotide sequence of both isolates, revealed 87.6% of identity to each other including the NIb, CP and the 3'-UTR (Figure 1). The amplified region of the NIb gene and the CP gene of both isolates were of the same size (nucleotides 1 to 195 for NIb and 196 to 999 for CP). However, the PVY-OBR 3'-UTR (nucleotides 1000 to 1327) was two nucleotides longer than of PVY-NBR (nucleotides 1000 to 1325). The PVY-OBR and PVY-NBR nucleotide sequence were deposited in the GenBank under the accession numbers AF255659 and AF255660, respectively.

Translation of the CP gene resulted in 267 amino acids for both isolates sharing 95.1% similarity. The amino acid sequences of 35 different PVY isolates were aligned and compared to the Brazilian isolates (data not shown). Most of the heterogeneity was found in the amino terminal region. The similarity between PVY-OBR and the other isolates varied from 94.4 to 99.2%. The highest similarity was found with PVY223594 (isolate 0803) and PVY223595 (isolate 0854), both common strains from Switzerland. PVY-NBR showed amino acid similarity from 93.3 to 99.7% when compared to other isolates. The highest degree of similarity with PVY-NBR was found with PVYCPNYSA (a necrotic isolate from Poland) and PVYU91747 (a necrotic isolate from North America).

A phylogenetic tree was constructed based on the alignment and displayed as an unrooted tree (Figure 2). PVY-OBR was clearly clustered with other common isolates, with the exception of two necrotic isolates (PVYNXX and PVYCPWILG). PVY-NBR was clustered with typical necrotic isolates and the two PVY^{NTN} isolates (PVYGCP and PVYPOLYP). A potato isolate from Russia (PVYCAPSI) was isolated in a separate branch close to the PVY^{N} cluster, as well as the common isolate from Argentina (PVYCOAT) and a tobacco isolate from Chile (PVYCAPA), both closer to the PVY^{O} cluster. Pepper and tobacco isolates formed a distinct branch including two tobacco isolates from the USA (PVYCAPD and PVYCAPE) and the causal agent of pepper veinal necrosis (PVYU10378).

The 3'-UTR nucleotide sequence of the Brazilian isolates was aligned with 18 available PVY sequences and used for construction of an unrooted phylogenetic tree (Figure 3). The consensus sequence found throughout the alignment (data not shown) formed two well defined groups, which were visualized in the phylogenetic tree (Figure 3). The first group comprised all PVY^{O}-like isolates, except for PVYNXX, the necrotic strain from France (Robaglia *et al.*, 1989), and included PVY^{NTN} (PVYPOLYP), PVY from pepper (PVYU10378), a PVY^{N} from Poland (PVYCPWILG), an unclassified PVY (PVYPOLYPR) and the PVY^{N} from Russia (PVYCAPSI). The second group comprised the PVY^{N} isolates and PVYNXX. The sequence of PVYNXX showed high identity with the PVY^{O} group in the first 90 nucleotides and with PVY^{N} for the remaining sequence (data not shown). PVY-OBR showed 98.2 to 99.4 % identity within the PVY^{O} group. The highest values were found with PVYPOLYP,

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PVY-OBR       1 TACTCATGGTTATTGCAACAGCAACCTTTTGCAACAATAGCGCAGGAAGG 50
                ||||||||||| |||||||||||||||||| |||| ||||| ||||||||
PVY-NBR       1 TACTCATGGTTGTTGCAACAGCAACCTTTTTCAACGATAGCACAGGAAGG 50

PVY-OBR      51 GAAGGCTCCTTATATAGCAAGCATGGCATTAAGGAAACTGTATATGGATA 100
                 || ||||| || ||||| |||||||||||| | ||| ||||| ||| |||
PVY-NBR      51 AAAAGCTCCATACATAGCGAGCATGGCATTGAAGAAGCTGTACATGAATA 100

PVY-OBR     101 GGACTGTGGATGAGGAAGAGCTAAGAGCCTTCACTGAAATGATGGTCGCA 150
                ||||  || |||||||| || ||  |  || |||||||||||||||||  ||
PVY-NBR     101 GGACAGTAGATGAGGAGGAACTGAAGGCTTTCACTGAAATGATGGTTGCC 150

PVY-OBR     151 TTAGACGATGAGTTTGAGTTTGACTCTTATGAAGTACACCATCAAGCAAA 200
                || || ||||| |||||||  ||  |||||||||| |||||||||| |||
PVY-NBR     151 TTGGATGATGAATTTGAGTGCGATACTTATGAAGTGCACCATCAAGGAAA 200

PVY-OBR     201 TGACACAATTGATGCAGGAGGAAGCAACAAGAGAGATGCAAAACCAGAGC 250
                |||||||||  |||  ||||||||||||   ||||  |||||||||| |||||
PVY-NBR     201 TGACACAATCGATACAGGAGGAAGCACTAAGAAGGATGCAAAACAAGAGC 250

PVY-OBR     251 AAGGCAGCATCCAGTCAAACCCGAACAAAGGAAAAGATAAGGATGTTAAT 300
                ||||  |||||  ||  ||||  |  |||||  | |||  || |||||  || |||
PVY-NBR     251 AAGGTAGCATTCAACCAAAACTCAACAAGGAAAAGGAAAAGGACGTGAAT 300

PVY-OBR     301 GCTGGCACATCTGGGACACATACTGTGCCGAGAATCAAGGCTATCACGTC 350
                | |||  ||||||||  ||  ||||||||||   ||||  ||  ||||||||||
PVY-NBR     301 GTTGGAACATCTGGAACTCATACTGTGCCACGAATTAAAGCTATCACGTC 350

PVY-OBR     351 CAAAATGAGAATGCCCAAAAGCAAGGGAGCAACCGTGCTAAACTTAGAAC 400
                ||||||||||||||||||  ||  ||  |||||  ||  |||||  ||  ||||
PVY-NBR     351 CAAAATGAGAATGCCCAAGAGTAAAGGTGCAACTGTACTAAATTTGGAAC 400

PVY-OBR     401 ATTTGCTTGAGTATGCTCCACAACAAATTGATATTTCAAATACTCGGGCA 450
                | ||  ||  ||||||||||||||  |||||||||  ||  ||||||||||  |||
PVY-NBR     401 ACTTACTCGAGTATGCTCCACAGCAAATTGACATCTCAAATACTCGAGCA 450

PVY-OBR     451 ACTCAATCACAGTTTGATACGTGGTATGAGGCAGTGCGGATGGCATACGA 500
                |||||||||||||||||||||||||||||  |||||  |    | |||||||||
PVY-NBR     451 ACTCAATCACAGTTTGATACGTGGTATGAAGCAGTACAACTTGCATACGA 500

PVY-OBR     501 CATAGGAGAAACTGAGATGCCAACTGTGATGAATGGGCTTATGGTTTGGT 550
                ||||||||||||||  ||||||||||||||||||||||||||||||||||||
PVY-NBR     501 CATAGGAGAAACTGAAATGCCAACTGTGATGAATGGGCTTATGGTTTGGT 550

PVY-OBR     551 GCATTGAAAATGGAACCTCGCCAAATGTCAACGGAGTTTGGGTTATGATG 600
                ||||||||||||||||||||||||||  ||||||||||||||||||||||||
PVY-NBR     551 GCATTGAAAATGGAACCTCGCCAAACATCAACGGAGTTTGGGTTATGATG 600

PVY-OBR     601 GATGGGGATGAACAAGTTGAGTACCCGTTGAAACCAATCGTTGAGAATGC 650
                |||||  ||||||||||||  ||  |||||  |||||||||||||||||||||||
PVY-NBR     601 GATGGAGATGAACAAGTCGAATACCCACTGAAACCAATCGTTGAGAATGC 650

PVY-OBR     651 AAAACCAACCCTTAGGCAAATCATGGCACATTTCTCAGATGTTGCACAAG 700
                |||||||||  |||||||||||||||||||||||||||||||||||||||  |||
PVY-NBR     651 AAAACCAACACTTAGGCAAATCATGGCACATTTCTCAGATGTTGCAGAAG 700

PVY-OBR     701 CGTATATAGAAATGCGCAACAAAAAGGAACCATATATGCCACGATATGGT 750
                ||||||||||||||||||||||||||||||||||||||||||||||||||||
PVY-NBR     701 CGTATATAGAAATGCGCAACAAAAAGGAACCATATATGCCACGATATGGT 750

PVY-OBR     751 TTAATTCGGAATCTGCGGGATGTGGGTTTAGCGCGTTATGCCTTTGACTT 800
                |||  ||||  ||||||||  ||||    ||||  ||  ||  |||||  ||||||||
PVY-NBR     751 TTAGTTCGTAATCTGCGCGATGGAAGTTTGGCTCGCTATGCTTTTGACTT 800

PVY-OBR     801 TTATGAGGTCACATCACGAACACCAGTGAGGGCTAGGGAAGCGCACATTC 850
                ||||||  ||  ||||||||  ||||||||||||||||||  ||  ||  |||||||
PVY-NBR     801 TTATGAAGTTACATCACGGACACCAGTGAGGGCTAGAGAGGCACACATTC 850
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PVY-OBR      851 AAATGAAGGCCGCAGCATTGAAATCAGCCCAACCTCGACTTTTCGGGTTG 900
                 ||||||||||||||| || |||||||| ||| |||||||||||||| |||
PVY-NBR      851 AAATGAAGGCCGCAGCTTTAAAATCAGCTCAATCTCGACTTTTCGGATTG 900

PVY-OBR      901 GACGGTGGCATCAGTACACAAGAGGAGAACACAGAGAGGCACACCACCGA 950
                 || |||||||| |||||||||||||| |||||||||||||||||||||||
PVY-NBR      901 GATGGTGGCATTAGTACACAAGAGGAAAACACAGAGAGGCACACCACCGA 950

                                                              -*-
PVY-OBR      951 GGATGTCTCTCCAAGTATGCATACTCTACTTGGAGTCAAGAACATGTGA. 999
                 |||||| |||||||||||||||||||||||||||| ||||||||||||
PVY-NBR      951 GGATGTTTCTCCAAGTATGCATACTCTACTTGGAGTGAAGAACATGTGAT 1000

PVY-OBR     1000 TGTAGTGTCTCTCCGGACGATATATAAGTATTTACATATGCAGTAAGTAT 1049
                 |||||||||| |||||||||| |||  ||||||  | ||||||||||||
PVY-NBR     1001 TGTAGTGTCTTTCCGGACGATACATAGATATTTATGTTTGCAGTAAGTAT 1050

PVY-OBR     1050 TTTGGCTTTTCCTGTACTACTTTTATCATAATTAATAATCAGTTTGAATA 1099
                 |||||||||||||||||||||||||  |||||||||| |||||||||
PVY-NBR     1051 TTTGGCTTTTCCTGTACTACTTTTATCGAAATTAATAATC.GTTTGAATA 1099

PVY-OBR     1100 TTACTAATAGATGGAGGTGGCAGGGTGATTTCGTCATTGTGGTGACTCTA 1149
                 |||||   |||| | ||||| |  | |||| |||| |||| |||||  ||
PVY-NBR     1100 TTACTGGCAGATAGGGGTGGTATAGCGATTCCGTCGTTGTAGTGACCTTA 1149

PVY-OBR     1150 TCTGTTAATTCCGTATTATTAAGTCTTAGATAAAAGTGCCGGGTTGTCGT 1199
                  ||||   ||| ||||||||| || ||   |||||||||||||||| ||
PVY-NBR     1150 GCTGTCGTTTCTGTATTATTATGT.TTGTGTAAAAGTGCCGGGTTGTTGT 1198

PVY-OBR     1200 TGTTGTGGATGATTCATCGATTAGGTGATGTTGCGATTCTGTCGTAGCAG 1249
                 |||||||| ||||  |||||||||||||| ||||||| ||||||||||
PVY-NBR     1199 TGTTGTGGCTGATCTATCGATTAGGTGATGCTGCGATT.TGTCGTAGCAG 1247

PVY-OBR     1250 TGACTATGTCTGGATCTATCTGCTTGGGTGGTGTTGTGATTTCGTCATAA 1299
                 |||||||||||||| ||  | ||||||| || |||||||  ||||||
PVY-NBR     1248 TGACTATGTCTGGATTTAGTTACTTGGGTGATGCTGTGATTCTGTCATAG 1297

PVY-OBR     1300 CAGTGACTGTAAACTTCAATCAGGAGAC 1327
                 ||||||||||||||||||||||||||||
PVY-NBR     1298 CAGTGACTGTAAACTTCAATCAGGAGAC 1325
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FIG. 1 - Nucleotide sequence comparison of the 3' genome terminal region of PVY-OBR and PVY-NBR cDNAs. Identical nucleotides are linked by vertical bars. Dots were added to the nucleotide sequence to optimize the alignment. The partial sequence of the nuclear inclusion b gene is shown in italics. The coat protein gene is underlined. The stop codon (*) of the polyprotein is shown at position 997-999.

PVYU10378, and PVYCPWILG. The identity ranged from 85.6 to 86.5% with the PVY^N group and was 89.0% with PVYNXX. The identity of PVY-NBR within the PVY^N group ranged from 97.3 to 98.8% and was 94.4% with PVYNXX. The highest values were found with the necrotic isolates from Switzerland (PVYGEN) and New Zealand (PVYCPA). The identity ranged from 85.2 to 86.5% with the PVY^O group. The two groups showed a high degree of homogeneity within the group and the variability between the two groups was found in stretches of one to three nucleotides scattered throughout the sequence (data not shown).

DISCUSSION

The host range and nucleotide sequence of the Brazilian PVY-OBR and PVY-NBR isolates confirmed their inclusion in the PVY^O and PVY^N group, respectively. A comparison of the amino acid sequence of the CP with other PVY isolates showed that PVY-OBR was closely related to European isolates than the PVY^O from Angentina (Bravo-Almonacid & Mentaberry, 1989). Regarding the 3'-UTR, PVY-OBR was closer to necrotic or pepper isolates from Central Europe. PVY-NBR, on the other hand, showed a higher correlation with the necrotic isolates from North America by CP comparison and with the necrotic isolates from Switzerland by 3'-UTR comparison. The lower similarity of the CP sequence from the Brazilian isolates with those from South American, PVYCOAT from Argentina (95.9% with PVY-OBR and 93.3% with PVY-NBR) and PVYCAPA from Chile (96.3% with PVY-OBR and 94.0% with PVY-NBR), is well justified by the high rate of seed potato importation from Canada and Europe (SECEX/DECEX, 1999). This result confirmed that seed potato importation constitutes an easy entrance of new viruses or viral isolates

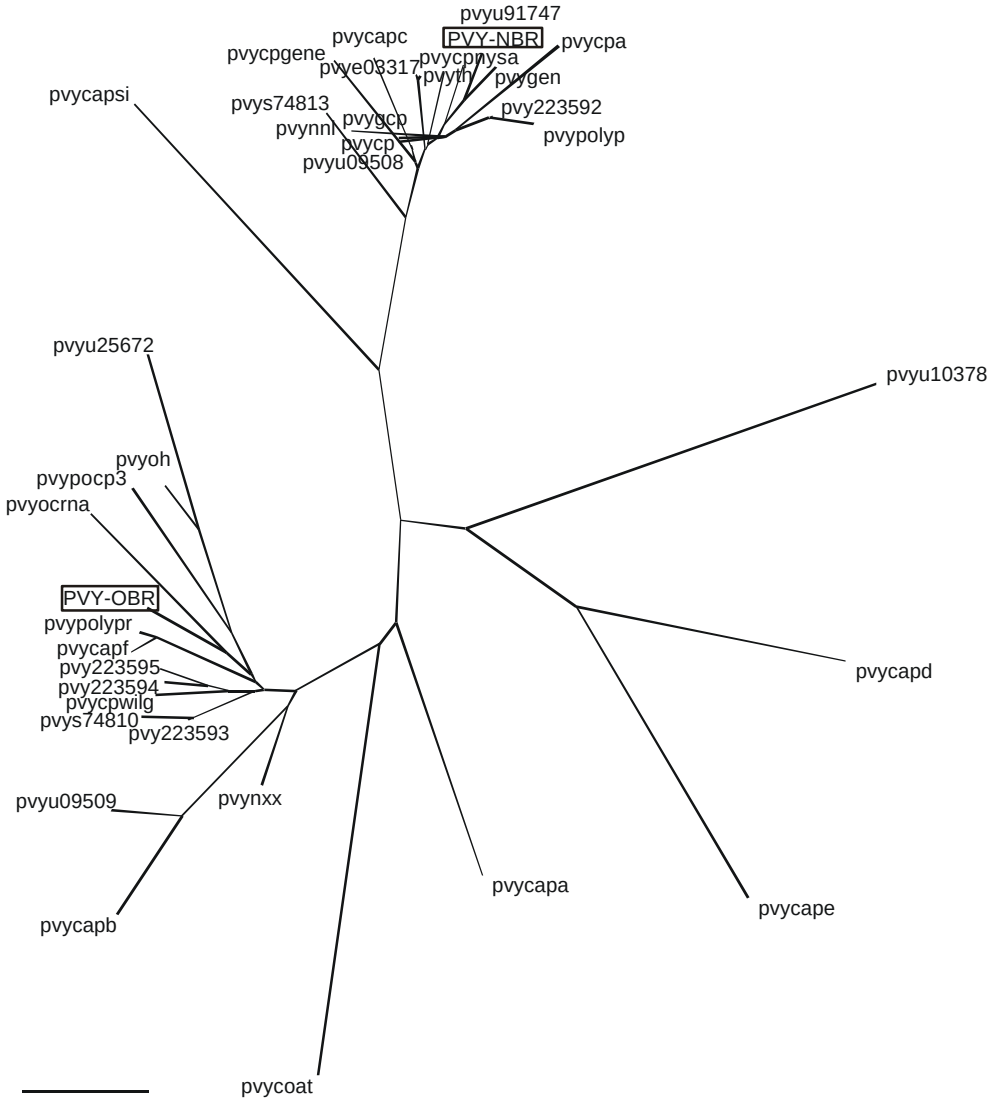

FIG. 2 - Unrooted phylogenetic tree of 37 coat protein amino acid sequences from different PVY accessions (Table 3). The Brazilian isolates, PVY-OBR and PVY-NBR, are boxed. Bar represents 0.01 amino acid substitutions per site.

into the country (Figueira *et al*., 1998), which may quickly spread throughout potato production areas.

The analysis of the phylogenetic trees from the CP and 3'-UTR sequence revealed two distinct clusters, one comprising most of the PVY^O sequences and the other with PVY^N ones. In the CP analysis, the two clusters showed a good correlation with the biological properties of the isolates, with the exception of PVYNXX and PVYCPWILG, two necrotic isolates grouped in the PVY^O cluster. This tree separated the potato strain from Russia (PVYCAPSI), the pepper strain (PVYU10378), the tobacco isolates (PVYCAPD, PVYCAPE and PVYCAPA) and the PVY^O from Argentina (PVYCOAT) from the two clusters. The separation of the isolates from Russia, Argentina and Chile (PVYCAPA) may indicate isolated geographical evolution of these viruses. Pepper and tobacco isolates are still poorly studied, but they formed a separate group suggesting a possible correlation among them. This result confirms the grouping of PVY isolates in PVY^O, PVY^N and non-potato isolates according to RFLP analysis of the CP (Blanco-Urgoiti *et al.*, 1996).

Thirty seven PVY sequences were analyzed for the CP alignment, but only 20 were available for the 3'-UTR analysis. The 3'-UTR tree showed a well defined grouping of PVY^O and PVY^N and a separate and isolated sequence of PVYNXX, a PVY^N isolate from France. Although this isolate was necrotic to *N. tabacum* (Robaglia *et al.*, 1989), it was consistently clustered with the PVY^O isolates when the CP gene was analyzed (Figure 2). This result confirms the previous alignment of 21 CP sequences, which showed the grouping of PVYNXX in the PVY^O group (Van der Vlugt *et al.*, 1993). The analysis of the PVYNXX 3'-UTR region showed a mixture of a PVY^O–like 5'-region followed by a highly divergent stretch of nucleotides and a PVY^N-like sequence at its 3' termini (data not shown). The PVYCPWILG

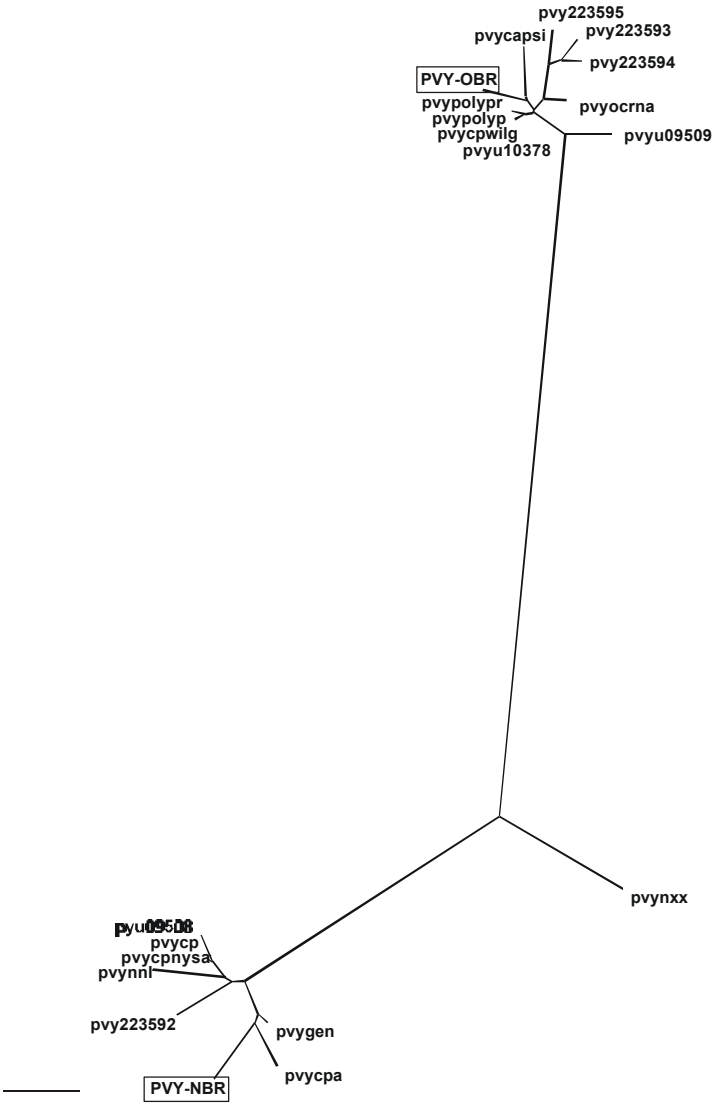

FIG. 3 - Unrooted phylogenetic tree of 20 3'-UTR nucleotide sequences from different *Potato virus Y* accessions (Table 3). The Brazilian isolates, PVY-OBR and PVY-NBR, are boxed. Bar represents 0.01 nucleotide substitutions per site.

was also a necrotic isolate but which was clustered in both trees with PVY^{O}-like isolates. The PVY^{NTN} isolates (PVYPOLYP and PVYGCP) were grouped in PVY^{N} cluster by the CP analysis, but the 3'-UTR of PVYPOLYP was closer to PVY^{O}. Therefore, the nucleotide sequence of CP and 3'-UTR was not useful to distinguish PVY^{NTN} from other PVY isolates. PVY^{NTN} is also indistinguishable from PVY^{N} by serology and host response (Weilguny & Singh, 1998). These results confirm the report that the necrotic reaction determinants in PVY are not localized in the 3' half portion of the genome (Chachulska *et al.*, 1997).

The nucleotide sequence determination of the CP is postulated as the basis for identification and classification in the *Potyvirus* genus (Shukla & Ward, 1988). The comparison of two PVY strains with several PVY isolates confirmed the overall clustering into PVY^{O} and PVY^{N} groups. However, the existence of many variants with distinct biological responses and nucleotide sequences showed that characterization studies of new isolates must include a description of their biological properties.

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