Phytopathol. Mediterr. (2005) 44, 12–16

Grouping of potato isolates of PVY based on the 5′-UTR nucleotide sequence

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Summary. Potato isolates of *Potato virus Y* (PVY) were divided into two major groups based on nucleotide sequence homology in the 5'-end region of their genomes. Several characteristic nucleotide modifications differentiated the two virus groups. Sequence similarity within members of each group was 89–100%, whereas between members of the respective groups there was sequence divergence of up to 33%. The two groups were distinguished by restriction cleavage of the virus PCR products elicited by group-specific restriction endonucleases: *Xho* II and *Nsp* I (group I) and *Mbo* II, *Fok* I and *Nco* I (group II). The sites of the first three enzymes (group I-specific) were sequentially positioned so that a sequence of 16 nucleotides preceding the ATG codon could be determined in this way. Group I included members of the tobacco necrotic (N) and non-necrotic (O) strains of PVY, whereas the potato tuber necrotic isolate NTN-H belonged to the second group, which included also non-tuber and tobacco leaf necrotic members. It seems therefore that the biological characteristics of the tuber or tobacco leaf necrosis are not related to nucleotide modifications at the 5'-UTR but rather to group-specific differences.

Key words: strain differentiation, PTNRD, RFLP, PCR.

Introduction

Potato virus Y (PVY) belongs to the *Potyviridae* family, the largest and economically most important group of plant viruses (Barnett, 1992). PVY infects mainly potato, tomato and pepper (De Bokx and Huttinga, 1981). The family comprises three historic groups, PVY^{O}, PVY^{N} and PVY^{C}, of which the first two are the most common in potato. The N-strain causes vein necrosis on tobacco leaves, whereas members of the O-group induce mottling and mosaic symptoms. Within PVY^{N} an economically important variant is recognized: PVY^{NTN}, which causes potato tuber necrotic ringspot disease (PTNRD) and severely reduces tuber quality (Beczner *et al*., 1984). Although PVY^{NTN} belongs to the N-group it is serologically indistinguishable, and molecular methods have to be applied for its differential diagnosis. The full-length nucleotide sequences of the PVY-N-, O-, and NTN-strains were determined by Robaglia *et al*., (1989), Singh and Singh (1996) and Thole *et al*., (1993), respectively. Tordo *et al*., (1995) demonstrated that the 5'-end region of the PVY genome was most variable and could be used for classification of the virus isolates. Molecular methods based on nucleotide modifications at the 5'-end of the virus genome have been previously applied to this aim (Rosner and Maslenin, 1999a, b).

In the present paper we suggest that potato isolates of PVY are divided into two major groups,

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depending on the 5'-end nucleotide sequence of the PVY genome.

Materials and methods

Virus isolates

A potato tuber necrosis strain of PVY– Y^{NTN}-H (Nicola, Hessen) was kindly given to us by H-L. Weidemann (Braunschweig, Germany). Three virus isolates were kindly provided by Camille Kerlan (INRA, France): PVY^{N}-W- N242 (W-Fr.) (accession No. AF248499, Glais *et al*., 2002), isolated in Northern France; PVY^{N}-B203, isolated in Brittany, France (Glais *et al*., 2002); and PVY^{N} -N605 (accession No. X97895) a Swiss isolate. PVY^{N}–RB, an N-strain field isolate from Scotland, was supplied by Ian Barker (CSL, York, UK). PVY-52 (N-strain) and DL (O-strain) are two local field isolates from Israel.

Primers and RT-PCR

PCR primers:

(I) 5'-AATTAAAACAACTCAATACA-3'
(forward, position 1–20).

(II) 5'-AGTACCGTTCTTGAGCACAC-3'
(reverse, position 363–382).

(III) 5'-TG(CT)GA(CTA)CCACGCACTATGAA-3'
(reverse, position 955–974)

RT-PCR conditions were as described by Rosner and Maslenin (1999b).

Sequence analysis

PCR products of the virus isolates were cloned into pGEM-TEasy plasmid vector (Promega, Madison, WI, USA) according to the manufacturer's instructions. Sequencing was carried out at the Sequencing Service Centre of the Weizmann Institute of Science, Rehovot, Israel. The nucleotide sequence analysis was performed with the UWGCG program (Genetic Computer Group, University of Wisconsin, WI, USA) (Devereaux *et al*., 1984).

Results and discussion

The 5'-end region (950 nt long) of six PVY isolates was amplified by RT-PCR with primers I and III, cloned and sequenced, and the first 500 nucleotides were aligned (Fig. 1). A few mismatches were found within the first 50-nt region and their frequency gradually increased towards the ATG-translation initiation signal. Most variability was found between nucleotides 100 to 188. Sequences of isolates 52 (N-strain) and W-Fr. were identical to the PVY^{NTN}-H sequence (see Table 1), including the positions of the a- and b-boxes at positions 18 and 70 nt (Turpen, 1989) and the occurrence of the pentanucleotide TTTCA (Thole *et al*., 1993). Isolate W-Fr. did have nucleotide modifications within the P1 gene (not shown), but it exhibited 97% similarity with the NTN-sequence (see Table 1). The N605, N-strain contained more nucleotide modifications but nevertheless it was closer to the reference NTN-sequence (89% similarity) than to the PVY^{O} reference with which it shared only 70% sequence similarity. None of the above four isolates showed similarity greater than 70% with the PVY^{O} sequence. In contrast, isolates RB (an N-group) and DL (O-group) showed 95% identity with PVY^{O} (Table 1), and less than 70% similarity with the PVY^{NTN}-H- reference sequence. These PVY isolates could thus be divided into two major groups; one-related to the nucleotide sequence of PVY^{O} (group I), and the other to that of PVY^{NTN}-H (group II). In general, the PVY isolates varied by as much as 33% and they thus form two separate groups.

It should be noted that group I contained members of both the N- and O-strains. In addition, it was shown that the unique 5'-end sequence of PVY^{NTN}-H is shared by several tuber non-necrotic but tobacco leaf necrotic N-strains of PVY (Fig. 1 and Table 1). Other isolates, RB (an N-strain) and DL (O-strain) differed markedly in their nucleotide sequence from PVY^{NTN}-H, with which they showed low degree of similarity (see Table 1), but they were

Table 1. Percent of nucleotide sequence similarity. The nucleotide sequences (500 nt long) of PCR products of isolates 52, B203, W-Fr., N605, RB and DL (determined as described in the legend to Fig. 1) were aligned with the reference sequences of PVY^{NTN}-H and PVY^{O} and the percent similarity was determined.

Reference strains	Nucleotide sequence similarity (%)					
	52	B203	W-Fr.	N605	RB	DL
PVY^{NTN}-H	100	100	97	89	70	68
PVY^{O}	68	68	67	70	95	95

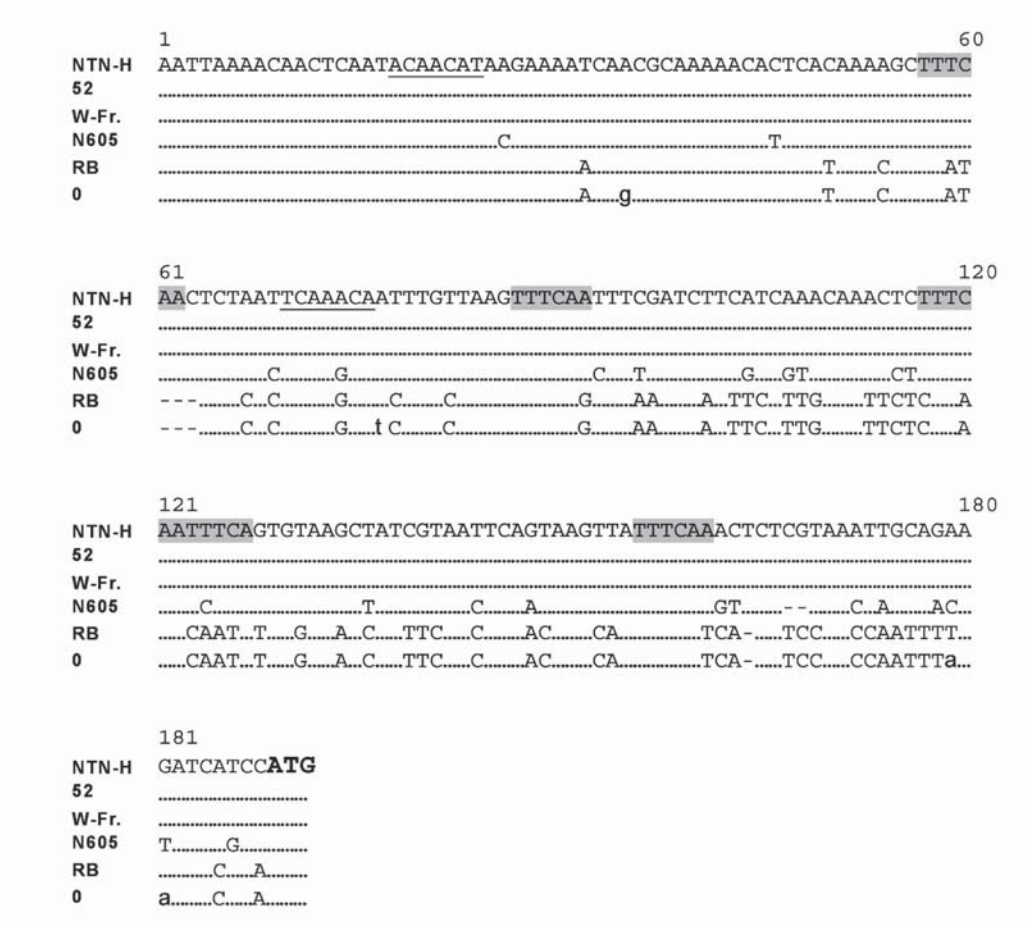

Fig. 1. The 5'-UTR nucleotide sequences of PVY isolates. The 5'-end region of several PVY isolates (as indicated in the figure) was amplified by PCR (with primers I and III) yielding a 950 nt long product, cloned, sequenced and the 5'-UTR nucleotide sequences were aligned. Identical nucleotides are marked with dots, modified nucleotides with letters, and a deletion with a dash. The upper and lower sequences of PVY^{NTN}-H (Thole *et al.,* 1993) and PVY^{O} (Singh and Singh, 1996) were used as references (accession No. M95491 and U09509 respectively). Nucleotide modifications unique to PVY^{O} are indicated with small letters. The a- and b-boxes at positions 70 and 162 respectively (Turpen, 1989) are underlined.

closely related to one another and to PVY^{O}. Since group I included both tobacco leaf-necrotic (N-) and leaf-non-necrotic (O-) strains, whereas group II contained both tobacco leaf necrotic (N) and tuber-necrotic (NTN) isolates, it was concluded that the nucleotide modifications were more related to differences between the groups than to the tobacco leaf-necrotic or the potato tuber-necrotic characteristics.

The nucleotide sequences flanking the ATG-translation initiation codon of the two virus groups was searched for restriction endondonuclease cleavage sites (Fig. 2). Three successive recognition sites were identified within the sequence of group II isolates: *Mbo* II, *Fok* I and *Nco* I. Overall, these sites covered a continuous sequence of 16 nt, except for a single T at position -6, which was situated between the recognition sequences of the first two enzymes (Fig. 2).

The actual cleavage patterns of PCR products by these enzymes are shown in Fig. 3. As expected, PCR products of NTN-H (belonging to group II), were cleaved by these three enzymes (Fig. 3C), whereas products of group I member-DL, remained uncut. These products were cleaved only by *Xho* II and *Nsp* I (Fig. 3B). Control un-cleaved PCR prod-

ucts of groups I and II are shown in Fig. 3A. From the known recognition sequences of the enzymes specific to group II an overall 16 nt-long sequence could be determined and the type group was identified in this way.

While the NTN-strain was included in the second group, the 5'-end sequence of this strain was not found to be unique to tuber necrotic isolates, but was also shared by tobacco leaf necrosis isolates belonging to the same group. The genomic region encoding the tuber necrosis characteristic has not yet been clearly defined (Glais *et al*., 1998). Until more information is available on the origin of this characteristic the classical biological test remains the most reliable criteria for their definition.

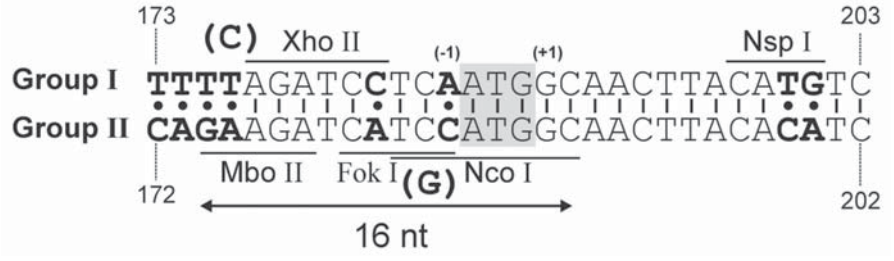

Fig. 2. The nucleotide sequences flanking the ATG in PVY groups I and II. Homologous nucleotides are indicated with a dash and mismatched ones with bold letters and dots - numbered downstream (-) and upstream (+) of the shaded ATG-translation initiation codon. The restriction endonuclease recognition sequences are under- and overlined. Nucleotides in brackets varied among virus isolates. The nucleotides are numbered according to the reference sequences of PVY^{O} (group I) and PVY^{NTN} (group II).

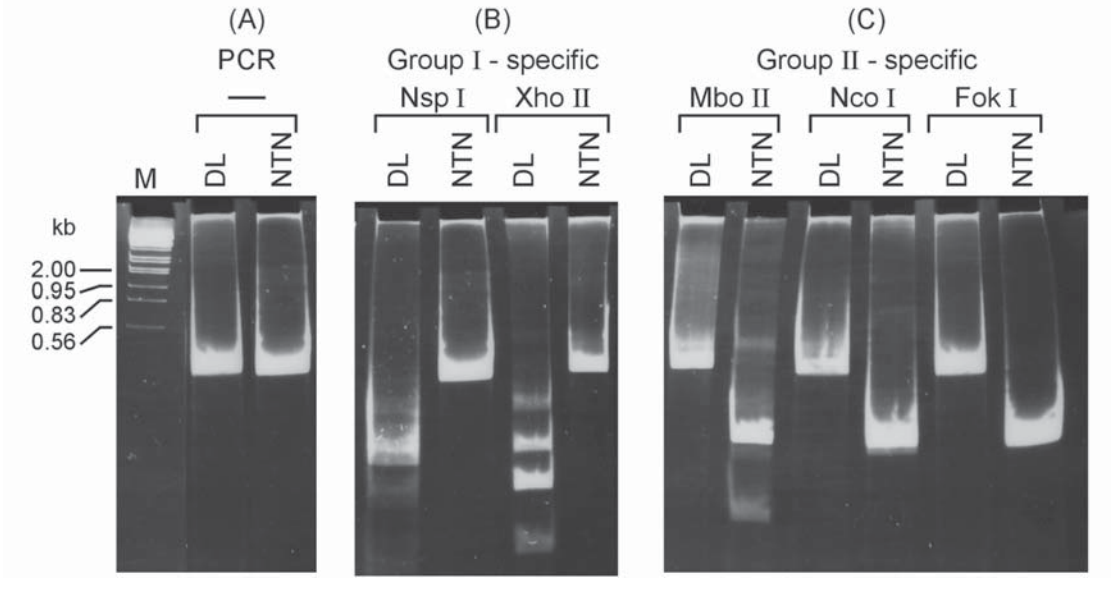

Fig. 3. Restriction endonuclease cleavage patterns of groups I and II. PCR products, amplified with primers I and II (380 nt long) of isolates DL (group I) and NTN-H (group II), were cleaved with the following restriction endonucleases: (A) *Xho* II and *Nsp* I (group I-specific), and (B) *Mbo* II, *Fok* I, and *Nco* I (group II-specific). The digests were fractionated in 6% polyacrylamide gel by electrophoresis and stained with ethidium bromide. M-size marker of *Eco*R I / *Hin*d III cleaved Lambda DNA.

Acknowledgement

This study was in part financially supported by the European Union Project number 92/2.

Literature cited

Barnett O.W., 1992. A summary of potyvirus taxonomy and definitions. *Archives of Virology* 5 (Supplement), 435–444.

Beczner L., J. Horvath, J. Romhanyi and H. Forster, 1984. Studies on the etiology of tuber necrotic ringspot disease in potato. *Potato Research* 27, 339–352.

De Bokx J.A. and H. Huttinga, 1981. Potato virus Y. *Description of Plant Viruses No. 242,* CMI/AAB, Kew, Surrey, UK, 6 pp.

Devereaux J., P. Heaberli and O. Smithies, 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Research* 12, 387–395.

Glais L., M. Tribodet, J.P. Gauthier, S. Astier-Manifacier, C. Robaglia and C. Kerlan, 1998. RFLP mapping of the whole genome of ten viral isolates representative of different biological groups of potato virus Y. *Archives of Virology* 143, 2077–2091.

Glais L., M. Tribodet and C. Kerlan, 2002. Genomic variability in potato virus Y (PVY): evidence that $PVY^{N}W$ and PVY^{NTN} variants are single to multiple recombinants between PVY^{O} and PVY^{N} isolates. *Archives of virology* 147, 363–378.

Robaglia C., M. Durand-Tardif, M. Tronchet, S. Astier-Manifacier and F. Casse-Delbart, 1989. Nucleotide sequence of potato virus Y (N strain) genomic RNA. *Journal of General Virology* 70, 935–947.

Rosner A. and L. Maslenin, 1999a. Differentiating PVY^{NTN} by unique single-restriction cleavage of PCR products. *Potato Research* 42, 215–221.

Rosner A. and L. Maslenin, 1999b. Transcript conformation polymorphism: a novel approach for differentiating PVY^{NTN}. *Journal of Phytopathology* 147, 661–664.

Singh N. and R.P. Singh, 1996. Nucleotide sequence and genomic organization of a Canadian isolate of the common strain of potato virus Y (PVY^{O}). *Canadian Journal of Plant Pathology* 18, 209–224.

Thole V., T. Dalmay and E. Balazs, 1993. Cloning and sequencing of potato virus (Hungarian isolate) genomic RNA. *Gene* 123, 149–156.

Tordo M-J.V., A.M. Chachulska, H. Fakhfakh, M. Le Romancer, C. Robaglia and S. Astier-Manifacier, 1995. Sequence polymorphism in the 5'-NTR and in the P1 coding region of potato virus Y genomic RNA. *Journal of General Virology* 70, 1951–1960.

Turpen T., 1989. Molecular cloning of a potato virus Y genome: nucleotide sequence homology in non-coding regions of Potyviruses. *Journal of General Virology* 70, 1951–1960.

Accepted for publication: November 22, 2004