European Journal of Plant Pathology **102:** 655–662, 1996.

Molecular characterization of potato virus Y^N isolates by PCR-RFLP

Differentiation of PVY^N isolates by PCR-RFLP

L. Glais[1], C. Kerlan[1], M. Tribodet[1], V. Marie-Jeanne Tordo[2], C. Robaglia[2] and S. Astier-Manifacier[2]

[1] *INRA, Station de Pathologie Végétale, Domaine de La Motte, BP 29, 35650 Rennes-Le Rheu, France;* [2] *INRA, Station de Pathologie Végétale, Route de Saint-Cyr, 78026 Versailles, France (Fax: 88 285180)*

Accepted 22 April 1996

Key words: PVY, PVY^{NTN}, PTNRD, genome, RNA, polymorphism

Abstract

Based on the sequence polymorphism in the 5′ terminal part of the viral genome, a range of PVY^N isolates were characterized by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). Three pairs of primers selected in the 5′ non-translated and P1 protein region were tested. Two of them yielded PCR products of about 1Kb from all isolates tested. Restriction analysis of the PCR products gave two distinct electrophoretic patterns, whichever of the three enzymes was used. In this way, the 18 isolates were separated into two easily identifiable subgroups. All tuber necrosing isolates (PVY^{NTN}) were clustered in the same subgroup.

Introduction

Potato virus Y (PVY) is the type-species of the genus *Potyvirus*, one of the four genera making up the family *Potyviridae* (Barnett, 1992) which is the largest and economically the most important group of plant viruses. All members of this family comprise flexuous long rod-shaped particles containing a monopartite single-stranded positive sense RNA genome of approximately 10 Kb long with a small protein (VPg) covalently linked to the 5′ terminal nucleotide (Hollings and Brunt, 1981) and a poly (A) tail at the 3′ terminus (Hari, 1981). Two non-coding sequences, at the 5′ and the 3′ end, flank one unique large open reading frame which encodes a single polyprotein. The polyprotein is proteolytically processed to produce nine proteins, among which are the P1 protein, the helper component, the cytoplasmic and nuclear inclusions and the capsid protein (Dougherty and Carrington, 1988; Robaglia et al., 1989; Riechmann et al., 1992). The N-terminal part of the protein capsid contains the major virus-specific amino-acid sequences while the core protein is highly conserved between various *Potyviridae* members (Shukla et al., 1988; Shukla and Ward, 1989). The 3′ non-coding region displays a high level of sequence polymorphism (Lain et al., 1988; Frankel et al., 1989).

PVY (De Bokx and Huttinga, 1981) is a ubiquitous plant virus capable of infecting a large range of plants in several families, for example the *Solanaceae* such as potato, tobacco, tomato and pepper. In connection with this host diversity, PVY presents an important variability which firstly affects biological properties such as host range, symptomatology or aphid transmission. The best known strains, those naturally infecting potatoes, are commonly separated into three groups according to their reaction on potato and tobacco: PVY^O group (ordinary or common strains) which generally induces severe systemic symptoms of crinkle, rugosity or leafdrop streak in potato and systemic mottling on tobacco; PVY^N group (tobacco veinal necrosis strains) which produces very mild mottling in almost all potato cultivars and severe systemic veinal necrosis in tobacco; PVY^C (stipple streak strains) which evokes a hypersensitivity reaction on many potato cultivars, a systemic mosaic on the other cultivars and PVY^O-like

symptoms in tobacco (Beemster and De Bokx, 1987). These three groups are heterogeneous, several variants having been described in each of them (Watson, 1956, Thompson et al., 1987, Chrzanowska, 1994).

The PVY^N strain group has particular economic interest. It was responsible for severe potato crop epidemics in the 1950's and 1970's in many countries in Europe (Weidemann, 1988). Recent outbreaks of PVY^N have also been reported in North-America (Singh, 1992; Mc Donald and Kristjansson, 1993). Since 1984, the importance of PVY^N has been increasing in Europe (Chrzanowska, 1994). Two subgroups of new widely spread PVY^N isolates have been described. The first one, now called PVY^{NTN} (following a decision of the European Association for Potato Research Virology Section) (Le Romancer et al., 1994; Van Den Heuvel et al., 1994) is associated with a very harmful disease on potato, now referred to as ≪ potato tuber necrosis ringspot disease ≫ (PTNRD) (Beczner et al., 1984). The second group may be classified as having a particular virulence on potato, causing very mild mosaic symptoms on leaves, but no necrosis on tubers (Chrzanowska, 1991).

PVY^O and PVY^C are not serologically distinguishable, but it is possible to identify PVY^N by using monoclonal antibodies that recognize an epitope specifically present in most PVY^N isolates (Gugerli and Fries, 1983; Oshima et al., 1990; Sanz et al., 1990; Singh et al., 1993). At the genomic level, comparisons of capsid protein and 3′ NTR nucleotide sequences of a large number of PVY isolates confirmed the division between the two major groups, Y^O and Y^N, based on biological and serological properties (Van der Vlugt et al., 1993). Sequence studies of the coat protein cistron in PVY^N tuber necrosing isolates (Le Romancer, 1993; Thole et al., 1993; Van den Heuvel et al., 1994) also revealed a high homology with a standard PVY^N isolate (Robaglia et al., 1989) and confirm that PVY isolates inducing PTNRD belong to the PVY^N strain group. Conversely, the 5′ terminal segment comprises the most variable region of the PVY genome, as shown by sequence comparisons between two PVY^N isolates which display only about 70 % identity in this region (Marie-Jeanne Tordo et al., 1995). A further study in this part of the viral genome on a range of PVY isolates resulted in the dividing of these isolates into three groups, the five PVY^N isolates being put into two of these groups (Marie-Jeanne Tordo et al., 1995).

The objective of the present study was to confirm this genomic diversity in the 5′ terminal part of the viral RNA on a larger range of PVY^N isolates and by using faster molecular techniques than sequencing. The approach was based on the polymerase chain reaction with two steps: the first one was to obtain an amplicon with all PVY isolates, with an internal control; the second one involved restriction enzyme digestion of PCR products in order to get differentiating electrophoretic patterns.

Materials and methods

Virus isolates

Eighteen PVY isolates were compared: two reference tuber necrosing isolates, Y^{NTN}-Lb (our collection) and Y^{NTN}-H (German collection); eleven isolates obtained from tubers harvested in various countries and showing typical symptoms of PTNRD, i.e. superficial necrotic ringspots or arches; five standard PVY^N isolates never found associated with necrosis on potato tubers, Y^N 602, Y^N 605, Y^N 607 (these Dutch isolates derived from the IPO-DLO collection), Y^N C3VN (SCRI collection) and Y^N Nysa (≪ old N Polish ≫-type isolate, CHRZANOWSKA's collection). The characteristics of these isolates are summarized in Table 1. All isolates were maintained on *Nicotiana tabacum* var Xanthi in a regulated insect-proof greenhouse.

Serological reagents

Specific monoclonal antibodies anti-Y^N (from Bioreba Company) and anti-Y^O (from Rennes – Le Rheu laboratory) were used in DAS-ELISA (Clark and Adams, 1977).

Oligonucleotide primers

The sequence of the primers and their genomic location, shown in the Figure 1, were deduced from already known PVY 5′ region sequences (Marie-Jeanne Tordo et al., 1995). The degeneracy of the primer *d* allows it to recognize all PVY isolates tested so far. The region limited by the external primers (*c* and *d*) showed approximately 85% nucleotide sequence identity between NTN and non-NTN RNAs (Marie-Jeanne Tordo et al., 1995).

RNA extraction and first strand cDNA synthesis

Total RNA was extracted from plant leaf tissue as described by Robaglia et al. (1993). First strand cDNAs were synthesized with the *Avian Myeloblastosis Virus* reverse transcriptase (Boehringer) using the 3′oligonucleotide *d* (Figure 1). About 1 μg of total

Table 1. Details of the eighteen viral isolates used

Isolates		Cultivars	Symptoms on original samples	Countries	Source
No.	Ref.				
1	Y^{NTN}-Lb	Lola	TNR[1]	Lebanon	Rennes laboratory
2	Y^{NTN}-H	~	TNR	Hungary	BBA Branschweig
3	FrOrl	Rosalie	TNR	France	Rennes laboratory
4	FrVo1	~	TNR	France	ITPT[2]
5	AlGm1	Hybrid	TNR	Germany	Germicopa
6	PorGm1	Yesmina	TNR	Portugal	Germicopa
7	OsHer1	Hermes	TNR	Austria	Schiessendoppler
8	OsHer4	Hermes	TNR	Austria	Schiessendoppler
9	OsNic1	Nicola	TNR	Austria	Schiessendoppler
10	OsNic7	Nicola	TNR	Austria	Schiessendoppler
11	CzNic1	Nicola	TNR	Czech Republik	Dedic
12	CzNic3	Nicola	TNR	Czech Republik	Dedic
13	CzLuk1	Lukava	TNR	Czech Republik	Dedic
14	Y^{N} 602	Bintje	*	The Netherlands	Van Den Heuvel
15	Y^{N} 605	Gineke	*	The Netherlands	Van Den Heuvel
16	Y^{N} 607	Record	*	The Netherlands	Van Den Heuvel
17	Y^{N} C3VN	~	$	Scotland	Solomon-Blackburn
18	Y^{N} Nysa	Nysa	$	Poland	Chrzanowska

Meaning of abbreviations:
[1] TNR: typical superficial necrotic ringspots or arches on potato tubers. $: no symptoms on potato tubers.
[2] ITPT: Institut Technique de la Pomme de Terre (France).
*: freeze-dried leaf material.

RNA was used for the first strand cDNA synthesis. The samples were adjusted to 50 mM Tris HCl pH 8–9, 50 mM KCl and 7.5mM $MgCl_2$, to which 28 pmol of the 3′primer *d*, 80 μM of each dNTPs and 4 units of AMV reverse transcriptase were added in a 20 μl final reaction volume and incubated for 1 h at 42 °C.

Polymerase chain reaction

For the external amplification, five μl of the reverse transcriptase reaction were amplified in a total volume of 50 μl containing 1.5 mM $MgCl_2$, 40 μM of each dNTPs, 28 pmol each of primers *d* and *c*, 1 unit of *Taq* DNA polymerase (Promega) in the buffer recommended by the manufacturer (Promega). 30 cycles of amplification in an automatic thermal cycler were carried out using the following programme: 1 min denaturation at 94 °C; 1 min annealing at 57 °C and 1 min extension at 72 °C.

Then for the hemi-nested amplification, 0.3 μl of the external PCR reaction was added to an amplification mixture as before, containing 28 pmol of the 3′ primer *d* and 28 pmol of the 5′ primers *a* or *b* (Figure 1) as indicated. Cycling was the same as for the external amplification.

Restriction digests

PCR aliquots (10 μl) were analysed in 1.2% agarose gels in the presence of 0.5 μg ml^{-1} ethidium bromide, using 45 mM Tris-borate pH 8, 1mM EDTA (TBE), as an electrophoresis buffer.

10 μl aliquots of the PCR products amplified with primer *c* were restricted with 4 units of *Taq I* endonuclease at 65 °C for 2 h or with 7 units of *Hinc II* at 37 °C for 2 h in a final volume of 12 μl in the buffer supplied by the manufacturer (Biolabs).

Otherwise 5 μl aliquots of the PCR products amplified with primer *c*, mixed with 10 μl of sterile water, were restricted with 4 units of *Ava II* at 37 °C for 2 h in a final volume of 17 μl.

Results

Reaction induced on tobacco and serological grouping

All tested isolates belonged to the PVY^{N} group according to their behaviour on *Nicotiana tabacum* cv. Xanthi and their serological properties. They induced a typical vein necrosis and distortion of the leaves in tobacco

PRIMER	SEQUENCE 5'-3'	GENOMIC LOCATION *
a	AACACTCACAAAAGCTTTCA	43-62
b	T(CT)A(CT)AAAC(AG)CT(CT)ATT(CT)TCAC	48-70
c	AATTAAAACAACTCAATACA	1-21
d	TG(CT)GA(CTA)CCACGCACTATGAA	955-974

* : numbered in Y^{NTN}-H [Thole *et al.*, 1993]

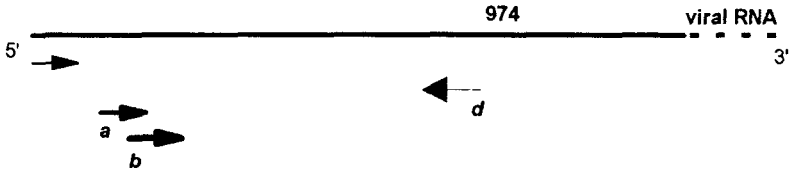

Figure 1. Sequence and genomic location of the oligonucleotide primers.

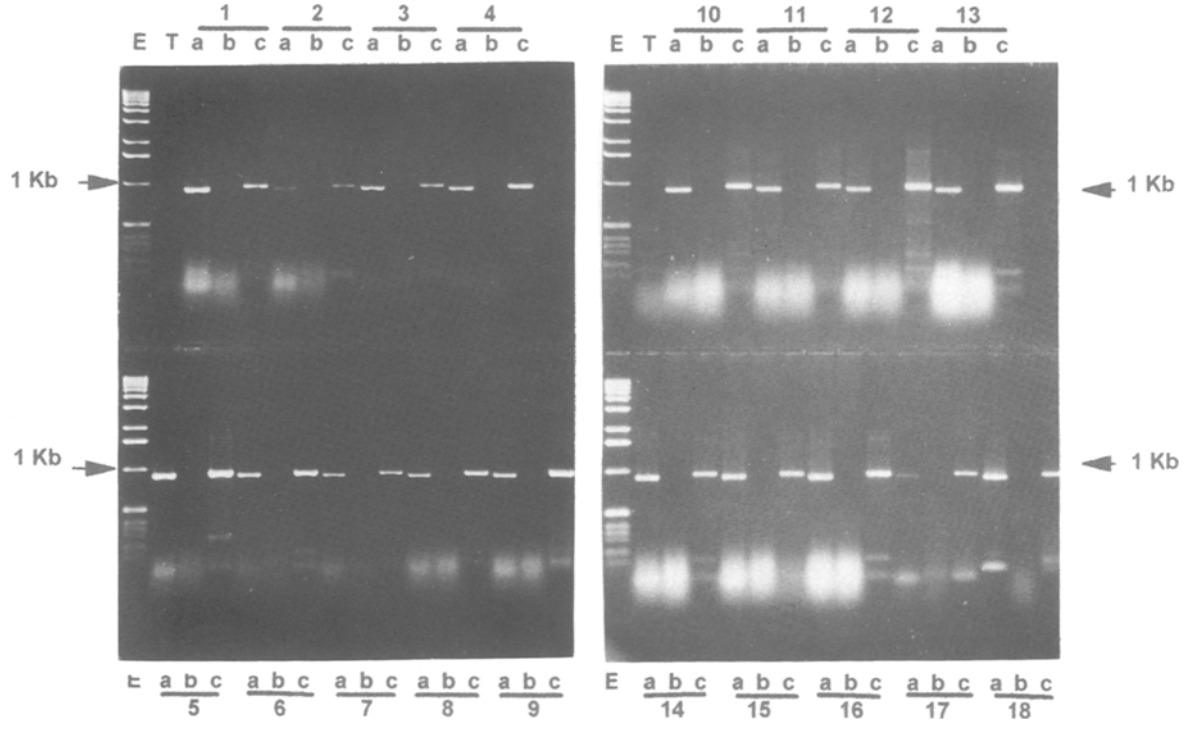

Figure 2. 1% agarose gel electrophoresis analysis of RT-PCR amplified cDNA using primers *a*, *b* or *c*. Lines E, 1 Kb DNA Ladder; lines T, total RNA from an healthy tobacco plant amplified with the primer *c*; lines 1 to 18, total RNA from tobacco plants infected with isolates 1 to 18 listed in the Table 1, respectively and amplified with primers *a*, *b* or *c*.

(Table 2). They clearly reacted with anti-PVY^N specific Mabs, but not with anti-PVY^O specific Mabs (Table 2), demonstrating that none of them was a mixture of serotypes.

Selection of oligonucleotide primers

The selection of the primers (Figure 1) was based on the multiple sequence alignment of different PVY isolates in the 5'NTR and P1 protein region of the genome (Marie-Jeanne Tordo et al., 1995). The pair of external primers *c* and *d* allowed us to recognize all PVY isolates tested so far. In this region, a three bases insert distinguished three PVY^N isolates, two of them inducing necrosis on tubers; the primer *a* was designed to contain this insert. All the other isolates listed in this alignment were amplified with the primer *b* (Astier-

Table 2. Biological and serological typing of the eighteen PVY isolates tested

Isolates		Symptoms on tobacco		Serology*	
No.	Ref.	VN	LD	Y^N	Y^O-Y^C
1.	Y^{NTN}-Lb	s	s	+	–
2.	Y^{NTN}-H	m	m	+	–
3.	FrOrl	m	s	+	–
4.	FrVo1	m	s	+	–
5.	AlGm4	m	s	+	–
6.	PorGm1	m	s	+	–
7.	OsHer1	m	m	+	–
8.	OsHer4	m	s	+	–
9.	OsNic1	m	s	+	–
10.	OsNic7	m	s	+	–
11.	CzNic1	m	s	+	–
12.	CzNic3	s	s	+	–
13.	CzLuk1	s	s	+	–
14.	Y^N 602	m	m	+	–
15.	Y^N 605	m	m	+	–
16.	Y^N 607	m	m	+	–
17.	Y^N C3VN	s	s	+	–
18.	Y^N Nysa	m	s	+	–

VN, veinal necrosis; LD, leaf distortion; m, mild; s, severe.
+ and – were defined as higher and lower than $A_{405} \times 2$ ELISA value given by healthy control (mean of three samples), respectively.

Manifacier et al., unpublished results), some of them inducing vein necrosis on tobacco and the others mottle symptoms.

RT-PCR amplification

All isolates were tested with the 5′ primers *a* or *b* or *c* along with the 3′ primer *d*. According to the already known sequences, we obtained a fragment 974 bp long with primers *c* plus *d* and 931 bp long with primers *a* plus *d* (Figure 2). Subsequently, for simplification, we have extrapolated these different sizes at 1 Kb. In order to confirm that the amplicon *c-d* was specific for PVY genome, we performed an hemi-nested amplification reaction with the internal primers *a* or *b* (Figure 1) corresponding to known PVY sequences (Marie-Jeanne Tordo et al., 1995). In the set of isolates tested in this study, all reacted positively with the primer *a*, but no PCR-product was obtained with the primer *b*. The line T (Figure 2) represents the negative control, i.e. healthy plant extract, amplified with primers *c* and *d*. This control was also negative with the other primers (result not shown).

Restriction analysis

We noticed that the primer *a* was useful as an internal control, but cannot discriminate NTN isolates from the others. We performed RFLP analysis of the amplicon *c-d*. Electrophoretic patterns were obtained after the digestion of this amplicon by *Taq I, Ava II* and *Hinc II* (Figure 3).

Taq I restriction analysis yielded two main distinct patterns. The first one, obtained with the NTN isolates, showed a typical band of 270 bp size and another one of about 470 bp long (Figure 3A, lines 1 at 13) not present in two Austrian isolates (Figure 3A, lines 7–8). The pattern of the other five isolates comprised two fragments of about 400 and 450 bp long (Figure 3A, lines 14 at 18). The amplicon *c-d* from NTN isolates was restricted with *Ava II* generating a fragment of approximately 800 bp; the amplification products of other isolates were not cut (Figure 3B). With *Hinc II*, the NTN pattern consisted of a specific band of about 660 bp and a band of 300 bp common in all isolates; four bands were obtained from the other isolates (Figure 3C).

Discussion

In the present study, we used primers selected in the 5′ NTR region where significant sequence differences between PVY isolates have been described (Marie-Jeanne Tordo et al., 1995). However, despite this polymorphism, RT-PCR with primer *a* did not separate NTN from the other isolates. In our experiment, all tested isolates had the same behaviour; they were amplified with the primers *a-d* and *c-d* but not with *b-d*. A second experiment with isolates belonging to the PVY^O group showed that they were amplified with primers *b-d* (results not shown here).

Restriction analysis of the PCR products gave two major electrophoretic patterns showing that the 18 isolates were separated into two groups, one of them at least being easily identified on the basis of characteristic bands (about 270 bp, 800 bp and 660 bp with *Taq I, Ava II* and *Hinc II*, respectively). This cleavage pattern corresponds to the prediction deduced from the nucleotide sequence of an isolate of the NTN subgroup, PVY^{NTN}-H (Thole et al., 1993), i.e. 270 bp, 808 bp and 662 bp with *Taq I, Ava II* and *Hinc II*, respectively. The *Taq I* restriction profile of two Austrian

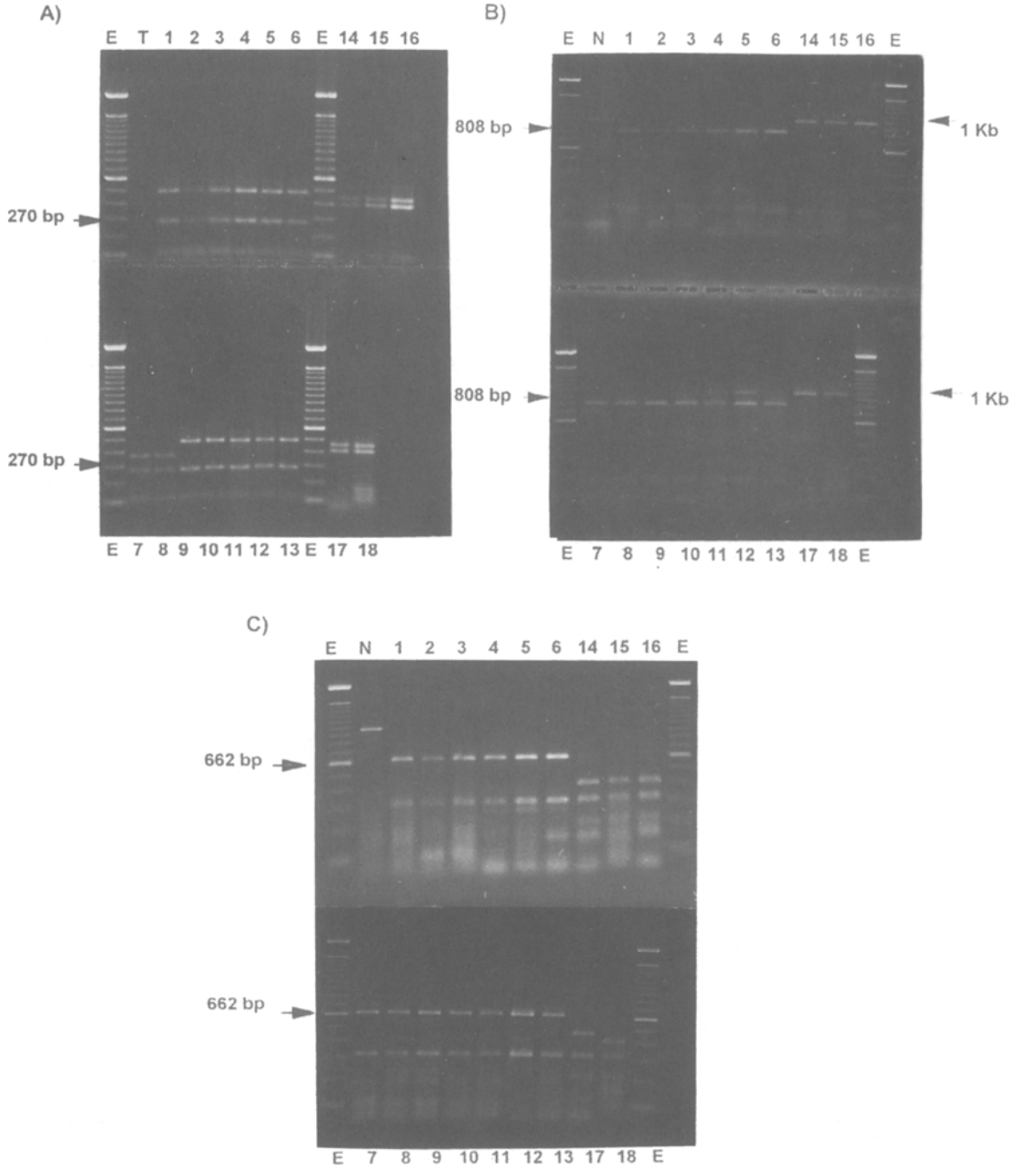

Figure 3. 1.5% agarose gel electrophoresis analysis of *Taq I* (A), *Ava II* (B) and *Hinc II* (C) restriction endonuclease digestion of RT-PCR products amplified with primers *c-d*. Lines E, 100 bp DNA Ladder; line T, total RNA from an healthy tobacco plant amplified and restricted by Taq I (A); lines N, isolate NTN non digested; lines 1 to 18, total RNA from tobacco plants infected with isolates 1 to 18 listed in the Table 1 respectively amplified and restricted by *Taq I* (A), *Ava II* (B) and *Hinc II* (C).

isolates originating from the cv. Hermes suggests that minor variations can occur in this part of the genome between the NTN isolates.This clustering may reflect biological features since all NTN isolates, both reference NTN isolates and 11 isolates originating from tubers showing PTNRD symptoms, are gathered in the same group. Conversely, all isolates of the second group were known not to induce necrosis on tubers. The three Dutch isolates, Y^N 602, Y^N 605, Y^N 607, were shown to be non-tuber necrosing in the same conditions in which an Austrian isolate (not tested here) evoked necrosis on tubers (Van Den Heuvel et al., 1994). These three isolates, like the Scottish isolate Y^N C3VN and the Polish isolate Y^N Nysa, were never found associated to PTNRD in the field.

In conclusion, the present study confirms the diversity inside the PVY^N group observed in the 5' terminal region of the viral RNA (Marie-Jeanne Tordo et al., 1995). RT-PCR followed by RFLP of the PCR products allows clear and reproducible discrimination between two groups of isolates, all NTN isolates being clustered in one of these groups. We have now to confirm on a larger range of isolates that this molecular differentiation is totally correlated with the biological separation between tuber necrosing and non-necrosing PVY isolates.

The application of PCR to potato virus detection has already been reported (Barker et al., 1993; Hataya et al., 1994). This technique can be used for virus detection in leaves as well as in tubers. This method, followed by an additional restriction enzyme digestion of the amplification product, has potential for rapid identification of PVY^{NTN} isolates. It could be empoyed for preliminary screening before confirmation by indexing on a highly susceptible PTNRD potato cultivar. In this way, it could be applied, in the future, to the domain of seed potato certification and potato breeding.

Acknowledgements

The authors wish to thank Dr Ruth Solomon-Blackburn, Dr M Chrzanowska, Dr E Schiessendoppler, Dr C Chatot, Dr P Dedic, Dr JFJM Van Den Heuvel, Dr H Weidemann, Dr JM Gravoueille, who kindly supplied either pure PVY isolates or PTNRD infected samples, together with Ms Jane Hall for her careful revision of the English.

References

Barker H, Webster KD and Reavy B (1993) Detection of potato virus Y in potato tubers: a comparison of polymerase chain reaction and enzyme-linked immunosorbent assay. Potato Res 36: 13–20

Barnett OW (1992) A summary of potyvirus taxonomy and definitions. Arch Virol (Suppl) 5: 435–444

Beemster ABR and De Bokx JA (1987) In: De Bokx JA and Van der Want JPH (ed) Survey of properties and symptoms. 2nd ed (84–114) Pudoc, Wageningen

Chrzanowska M (1991) New isolates of the necrotic strain of potato virus Y (PVY^N) found recently in Poland. Potato Res 34: 179–182

Chrzanowska M (1994) Differentiation of potato virus Y (PVY) isolates. Phytopath Polonica 8 (XX): 15–20

Clark MF and Adams AM (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34: 475–83

De Bokx JA and Huttinga H (1981) Potato Virus Y. CMI/AAB Descriptions of plant viruses No. 242

Dougherty WG and Carrington JC (1988) Expression and function of potyviral gene products. Ann Rev Phytopathol 26: 123–143

Frankel MJ, Ward CW and Shukla DD (1989) The use of 3′ noncoding nucleotide sequences in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus-N. J Gen Virol 70: 2775–2783

Gugerli P and Fries P (1983) Characterization of monoclonal antibodies to potato virus Y and their use for virus detection. J Gen Virol 64: 2471–2477

Hari V (1981) The RNA of tobacco etch virus: further characterization and detection of protein linked to RNA. Virology 112: 391–399

Hataya T, Kazuko Inoue A and Shikata E (1994) A PCR-microplate hybridization method for plant virus detection. J Virol Methods 46: 223–236

Hollings M and Brunt AA (1981) Potyvirus group. CMI/AAB Descriptions of plant viruses No. 245

Lain S, Riechmann JL, Mendez E and Garcia JA (1988) Nucleotide sequence of the 3′ terminal region of plum pox potyvirus RNA. Virus Res 10: 325–342

Le Romancer M (1993) La maladie des nécroses annulaires superficielles des tubercules de la pomme de terre. Identification et caractérisation de l'agent pathogène: le variant Y^{NTN} du virus Y de la pomme de terre. Thesis, Univ Rennes I, 146 p

Le Romancer M, Kerlan C and Nedellec M (1994) Biological characterization of various geographical isolates of potato virus Y inducing superficial necrosis on potato tubers. Plant Pathol 43: 138–144

Marie-Jeanne Tordo V, Chachulska AM, Fakhfakh H, Le Romancer M, Robaglia C and Astier-Manifacier S (1995) Sequence polymorphism in the 5′NTR and in the P1 coding region of Potato virus Y genomic RNA. J Gen Virol 76: 939–949

Mc Donald JG and Kristjansson GT (1993) Properties of strains of potato virus Y^N in North America. Plant Dis 77: 87–89

Oshima K, Inoue AK, Ishikawa Y, Shikata E and Takashi H (1990) Production and application of monoclonal antibodies specific to ordinary strain and necrotic strain of potato virus Y. Ann Phytopathol Soc Jpn 56: 508–514

Riechmann JL, Lain S and Garcia JA (1992) Highlights and prospects of potyvirus molecular biology. J Gen Virol 73: 1–16

Robaglia C, Durand-Tardif M, Tronchet M, Boudazin G, Astier-Manifacier S and Casse-Delbart F (1989) Nucleotide sequence of potato virus Y (N strain) genomic RNA. J Gen Virol 70: 935–947

Robaglia C, Bruening G, Haseloff J and Gerlach GL (1993) Evolution and replication of tobacco ringspot virus satellite RNA mutants. EMBO Journal 12: 2969–2976

Sanz A, Cambra M, Perez de San Roman C, Miguet JG, Cortés E, Gorris MT and Vela C (1990) Preparation of additional monoclonal antibodies for detection and discrimination of potato virus Y isolates infecting potato. Potato Res 33: 365–375

Shukla DD, Strike PM, Tracy SL, Gough KH and Ward CW (1988) The N and C termini of the coat proteins of potyviruses are surface-located and the N terminus contains the major virus-specific epitopes. J Gen Virol 69: 1497–1508

Shukla DD and Ward CW (1989) Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Arch Virol 106: 171–200

Singh RP (1992) Incidence of the tobacco veinal necrotic strain of potato virus Y (PVY^N) in Canada in 1990 and 1991 and scientific basis for eradication of the disease. Can Plant Dis Surv 72: 113–119

Singh RP, Boucher A, Somerville TH and Dhar AK (1993) Selection of a monoclonal antibody to detect PVY^N and its use in ELISA and DIBA assays. Can J Plant Pathol 15: 293–300

Thole V, Dalmay T, Burgyan J and Balazs E (1993) Cloning and sequencing of Potato virus Y (Hungarian isolate) genomic RNA. Genes 123: 149–156

Thompson GJ, Hofman DCA and Prins PJ (1987). A deviant strain of potato virus Y infecting potatoes in South Africa. Potato Res 30: 219–228

Van den Heuvel JFJM, Van der Vlugt RAA, Verbeek M, De Haan PT and Huttinga H (1994) Characteristics of a resistance-breaking isolate of potato virus Y causing potato tuber necrotic ringspot disease. Eur J Plant Pathol 100: 347–356

Van der Vlugt RAA, Leunissen J and Goldbach R (1993) Taxonomic relationships between distinct potato virus Y isolates based on detailed comparaisons of the viral coat proteins and 3′ non-translated regions. Arch Virol 131: 361–375

Watson MA (1956) The effect of different host plants of potato virus C in determining its transmission by aphids. Ann Appl Biol 44: 599–607

Weidemann HL (1988) Importance and control of potato virus Y^N (PVY^N) in seed potato production. Potato Res 31: 85–94