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Characterization of potato potyvirus Y (PVY) isolates from seed potato batches. Situation of the NTN, Wilga and Z isolates

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Abstract

A collection of 38 PVY isolates from seed potato batches, originating from several Western European countries, was characterized by using current biological, serological and molecular tools differentiating PVY strains and groups. The correlation between the three kinds of tests was good but not absolute. No single serological or PCR method was able to discriminate among the five isolate groups found. Twenty-nine isolates belonged to the PVY^N strain and six to the PVY^O strain. No PVY^C was found. Two other isolates reacted serologically like PVY^O, but were unable to elicit a hypersensitive response from the Ny_{tbr} gene and probably represent the PVY^Z group. At the molecular level, these two isolates showed a combination of both PVY^O and PVY^N and could be recombinants of these strains. Another isolate reacted serologically like PVY^O, but induced vein necrosis in tobacco, like PVY^N-Wilga. Some PVY^N isolates caused tuber ring necrosis in glasshouse conditions. These might belong to the PVY^{NTN} group. The PVY^{NTN}, PVY^N-Wilga and PVY^Z groups probably represent pathotypes within strains PVY^N and PVY^O, respectively. The present study also confirms previous reports showing a high genetic variation at the 5′ end within the PVY^N strain.

Introduction

Potato potyvirus Y (PVY) has a worldwide distribution and is one of the most economically important virus in potato, together with potato leafroll luteovirus (Hooker, 1980). PVY is controlled, in the long term, by genetic resistance and, in the short term, by seed certification schemes (Kerlan et al., 1987). However, in recent years in the Basque Country and neighboring areas, extraordinarily high rates of PVY infection have been disrupting the seed potato industry. Tuber infection did increase from about 1% (basic seed grade) to well over 10%, (ware grade) in one single cycle of field multiplication (Legorburu et al., 1996). Such a phenomenon may be partially due to a shift in the proportions of the different PVY strains. Other reasons may be increased ware potato cropping and intense aphid flights. This strain shift is also important for the genetic control of the virus, by deploying new potato cultivars carrying the necessary resistance genes.

PVY isolates from potato have been traditionally classified into three strains: PVY^O, PVY^N and PVY^C (De Bokx and Huttinga, 1981), although these authors point out that some isolates cannot be classified into these groups. The last decade has seen the emergence of new particular isolates, inducing new diseases in this crop. Those called PVY^{NTN} (Le Romancer et al., 1994; van den Heuvel et al., 1994) constitute a sub-group of PVY^N responsible for potato tuber necrosis ringspot disease (PTNRD). This disease, firstly mentioned in Hungary at the beginning of the eighties (Beczner et al., 1984) is now widespread in the world (Weidemann and Maiss, 1996). In Spain, PTNRD has been observed in ware crops

in the Basque Country and Castile (Alonso, 1996). Another particular group, called PVY^Z, was proposed by Jones (1990) to classify some isolates found in Great Britain, which overcome the hypersensitive resistance to PVY^C and PVY^O conferred by the genes Nc and $Ny_{tbr.}$, respectively. This situation resulted in mosaic symptoms, previously unknown in the field, in varieties like Désirée and Pentland Crown. These mosaics have been observed in Basque seed potato fields since 1989 in the varieties Désirée and Kennebec. Another group of PVY^N isolates (Wilga-type PVY), which is more infectious and induces less severe symptoms in potato than the standard PVY^N, was reported in Poland (Chrzanowska, 1991; 1994), where it has become prevalent in the last years (Chrzanowska and Doroszewska, 1997).

In order to clarify PVY taxonomy, a molecular typing method for classifying the isolates in genetic strains has been developed by Blanco-Urgoiti et al. (1996b). It consists of an immunocapture (IC) of the particles, followed by reverse transcription (RT) of the viral RNA, amplification by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of the coat protein gene. This enables the calculation of genetic distances between isolates using their 'restrictotype' (the joint pattern obtained with the five restriction endonucleases used in RFLP analysis). In that work, three main clusters were obtained: potato PVY^N, potato PVY^O and non-potato PVY. Other methods of molecular characterization by RT-PCR (Weidemann and Maiss, 1996) or RT-PCR-RFLP (Glais et al., 1996) focused mainly on the PVY^N strain. In this latter case, the analysis of the 5′ terminal region of the genome made possible the separation of NTN from the rest of PVY^N in a set of 18 isolates. These are host-independent criteria to classify PVY isolates, which can be applied in variability and epidemiological studies.

The objective of the investigation presented was to characterize a collection of PVY isolates from seed potato batches originating in France, Luxemburg, Spain and The Netherlands. This collection is representative of the initial inoculum present in the Basque seed potato fields in 1994. All the tools available to study PVY variation have been used in two different laboratories. Firstly, biological (reaction on tobacco and cv. Désirée) and serological (monoclonal antibodies) criteria were used to differentiate between the PVY^O and PVY^N serotypes. Then, molecular characterization was carried out in the two regions of the viral genome mentioned above. The presence of possible PVY^{NTN} isolates was checked by observing the development of tuber lesions. Lastly, two suspect PVY^Z isolates were further characterized by transmission to potato cultivars carrying hypersensitivity genes. Some of the tests (inoculation into tobacco, ELISA with PVY^N monoclonal and PTNRD development) were repeated in two different laboratories. Preliminary results of the characterization of these isolates at CIMA have already been reported (Blanco-Urgoiti et al., 1996a).

Material and methods

Origin of virus isolates and working schedule

The field isolates originated in precontrol samples of Basic and A Certified potato seed, coming from several Western European countries and tested by the Basque Seed Inspectorate in the winter of 1993-94. The tubers were tested by ELISA, using a Gugerli extractor (Gugerli, 1979) and a mixed PVY+PLRV antiserum (BIOREBA AG, Basel, Switzerland). The positive ones were selected and planted in the glasshouse at CIMA. Foliage symptoms were observed on these plants and ELISA tests, inoculation on tobacco and Désirée and restrictotyping, were carried out. Daughter tubers from some of these plants were sent to INRA-Rennes, where a second generation of plants was grown. On these plants, ELISA tests, inoculations on tobacco and selected potato cultivars and 5' end molecular characterization were carried out between 1995–96. The standard isolates used were PVY^O-Sc from Scotland (Dr. R. Solomon, SCRI, Dundee), PVY^O-Irl from Northern Ireland (Dr. R.B. Copeland, DANI, Belfast), PVY^N-Nl605 from cultivar Gineke and PVY^C-Nl503 from cultivar Gelderse Rode from The Netherlands (Dr. JFJM van den Heuvel, IPO, Wageningen) and PVY^{NTN}-Lb94 from cultivar Lola from Lebanon (Dr. C. Kerlan, INRA, Rennes).

Serological reagents and ELISA tests

The antibodies used at CIMA were PVY (recognizing all potato isolates) and PVY^N monoclonals from BIOREBA (Gugerli and Fries, 1983); 1E10 (recognizing all PVY^C and some PVY^O isolates) and 10E3 (recognizing all PVY isolates, including non-potato ones) monoclonals from INGENASA (Sanz et al., 1990.) The BIOREBA antibodies were used in a DAS-ELISA format (Clark and Adams, 1977) and the INGENASA ones in an IDAS-ELISA format (Vela

et al., 1986), using polyclonal PVY antibodies from Boehringer (Mannheim, Germany) for trapping. Polyclonal PLRV antibodies from Boehringer were used in the DAS-ELISA format to discard samples positive to this virus. INRA-Rennes used its own monoclonals Y 123.1 (recognizing all PVY isolates) and Y 38.4 (recognizing most PVY^O) and the PVY^N monoclonal from BIOREBA. Locally produced polyclonal antibodies against PLRV, PVA and PVX were also used in order to discard isolates contaminated by these viruses. All these antibodies were used in DAS-ELISA format.

Test plant analysis

At CIMA, the tests were done in the glasshouse, with a day temperature of 20–25 °C, a night temperature of 15–20 °C and a natural photoperiod (spring). The isolates were mechanically inoculated into tobacco cultivar Xanthi (vein necrosis reaction to PVY^N) and potato cultivar Desirée (necrotic local lesions and top necrosis reaction to PVY^O; Jones, 1990). Daughter tubers of Désirée were inspected for PTNRD at harvest. At INRA-Rennes, the glasshouse was regulated at 20–22°C and had a natural photoperiod as well. There the isolates were transmitted from potato to Xanthi tobacco and from tobacco to potato cultivars King Edward, Eersteling (both hypersensitive to PVY^C), Foxton and Rosalie (both hypersensitive to both PVY^O and PVY^C; Jones, 1990). The originally planted potato plants were harvested in August and the daughter tubers scored for PTNRD before and during thick paper bag storage.

Molecular analysis of the coat protein gene

The isolates were characterized by IC-RT-PCR, according to Nolasco et al. (1993), followed by restriction analysis and clustering by genetic distance (Blanco-Urgoiti et al., 1996b). This analysis yields a phylogenetic tree with expression of the significance of the branch length. Isolates, whose sequences were obtained from molecular databases were included as a term of comparison (see Blanco-Urgoiti t al., 1996b for references). Sequences from two PVY^C isolates (Blanco-Urgoiti et al., 1998) were also included.

Molecular analysis of the 5′ genome end

The isolates were characterized by PCR amplification using different pairs of primers: c-d, amplifying all PVY; a-d, amplifying PVY^N but not PVY^O and b-d, amplifying PVY^O but not PVY^N. The c-d amplicon was digested by the Taq I restriction endonuclease, discriminating PVY^{NTN} from common PVY^N (Glais et al., 1996).

Results

Panel of field isolates

104 PVY+PLRV positive tubers were planted in the CIMA glasshouse in 1994. From these plants, 30 were found to be infected by PLRV only and 7 to be false positives. From the remaining 67, 13 were determined to be PVY-infected by serology and indicator hosts, 5 by PCR only and 49 by both methods. From these 67 isolates, 38 were further tested at INRA-Rennes and constitute the panel studied in the present work (Table 1). At the second laboratory, all of them were found to be PVY-infected by serology and tobacco indexing and free from PLRV, PVX and PVA by serology.

Reaction in tobacco and serology

Tobacco indexing and PVY^N-ELISA was done at both laboratories. Their results give a measure of the reliability of these tests and their reproducibility among laboratories. All the isolates reacted to monoclonal antibodies PVY, 10E3 and Y 123.1, except isolates 17 and 104, which failed to give any serological reaction in the first test at CIMA. No isolate reacted to monoclonal 1E10, which eliminates the presence of PVY^C in the panel. Two serotypes could be defined according to the reaction to the two other monoclonals: isolates reactive to PVY^N-BIOREBA and unreactive to Y 38.4 were called "N", while isolates unreactive to PVY^N-BIOREBA and reactive to Y 38.4 were called ‘O’. All the isolates serologically classified as ‘N’ induced vein necrosis in tobacco at INRA. Reference isolates PVY^N-Nl605 and PVY^{NTN}-Lb94 were necrotic while PVY^O-Sc was not. Isolate 17, however, induced vein necrosis, in spite of being serologically an ‘O’. Isolates 16, 45, 47, 111 and 138, although serologically ‘N", failed to induce vein necrosis in tobacco at CIMA. However, they resulted in being PVY^N’s following molecular analysis, indicating a failure of this biological test.

Table 1. Summary of isolates and results

Isolate	Cultivar	Origin	Molecular analysis			Serotype	test plants		
			CP	5′end PFLP	5′end PCR		Tobacco	Désirée	PTNRD
Reference									
PVY^{O}-Sc		Scotland		750	O	O	M	HR	–
PVY^{N}-Nl605	Gineke	Netherlands		400/450	N	N	VN	M	
PVY^{NTN}-Lb94	Lola	Lebanon		270/470	N	N	VN	M	Original
PVY^{N}									
1	Spunta	Spain	D16E1H15R222T172	270/470		N	VN	M	–
2	Spunta	Spain	D16E1H15R222T172	270/470	N	N	VN	M	Original
4	Spunta	Spain	D16E1H15R222T172	270/470		N	VN	M	–
9	Spunta	Spain	D16E1H15R222T172	270/470		N	VN	M	–
16	Jaerla	Spain	D16E1H16R222T172	270/470		N	VN	M	–
40	Jaerla	Spain	D16E1H15R222T172	270/470		N	VN	M	–
47	Spunta	Netherlands	D16E1H16R222T172	270/470		N	VN	M	–
58	Jaerla	Netherlands	D16E1H16R222T172	270/470		N	VN	M	–
62	Jaerla	Netherlands	D16E1H16R222T172	270/470		N	VN	M	–
71	Jaerla	Netherlands	D16E1H16R222T172	270/470		N	VN		Original
79	Jaerla	Netherlands	D16E1H16R222T172	270/470		N	VN		
95	Spunta	Netherlands	D16E1H16R222T172	270/470		N	VN	M	Original
102	Red Pontiac	Luxemburg	D16E1H15	270/470		N	VN	M	–
111	Spunta	Netherlands	D16E1H16R222T172	270/470		N	VN	M	–
122	Spunta	Netherlands	D16E1H16R254T172	270/470		N	VN	M	–
138	Jaerla	Netherlands	H16	270/470		N	VN	M	–
3	Spunta	Spain	D16E1H15R222T172	350/450	N	N	VN	M	–
50	Jaerla	Netherlands	D16E1H15R222T172	350/450		N	VN	M	–
20	Jaerla	Spain	D16E1H16R222T172	400/450		N	VN	?	Desirée
28	Jaerla	Spain	D16E1H16R222T172	400/450		N	VN	M	Original
45	Jaerla	Netherlands		400/450		N	VN	M	Original
59	Jaerla	Netherlands	D16E1H16R222T172	400/450		N	VN		
74	Claustar	France		400/450		N	VN		
80	Jaerla	Netherlands	D16E1H16R222T172	400/450		N	VN		
89	Spunta	Netherlands	D16E1H16R222T172	400/450		N	VN	M	–
130	Jaerla	Netherlands	D16E1H16R222T172	400/450		N	VN		
75	Claustar	France	D16E1R222T172			N	VN		Original
104	Jaerla	Netherlands	D16E1H16R222T172			N	VN	M	–
125	Jaerla	Netherlands	D16E1H16R222T172			N	VN		Original
PVY^{O}									
30	Jaerla	Spain	D29E1H7R112T254	750		O	M	HR	–
57	Jaerla	Netherlands	D29E1H7R112T254	750		O	M	HR	–
76	Baraka	Netherlands	D29E1H7R112T254	750	O	O	M	HR	–
85	Baraka	Netherlands	D29E1H7R112T254	750	O	O	M	HR	–
114	Baraka	Netherlands	D29E1H7R112T254	750		O	M	–	–
132	Jaerla	Netherlands		750		O	M	HR	–
Other									
17	Jaerla	Spain	D16E1H16R222T172	270/470		O	VN	M	–
18	Jaerla	Spain	D29E1H8R112T250	270/470	N	O	M	M	–
32	Jaerla	Spain	D29E1H8R112T250	270/470	N	O	M	M	–

5′ end and serotype: O = PVY^{O}-type, N = PVY^{N}-type. Tobacco and Désirée: M = mosaic, VN = vein necrosis, HR = hypersensitive reaction, – = no symptoms. PTRND: Original = symptoms developed on original cultivar, Désirée = symptoms developed on Cultivar Désirée, – = no symptoms on cultivar Désirée.

Indexing in Désirée potato and PTNRD expression

A hypersensitive reaction in Désirée foliage was always linked to mosaic symptoms in tobacco and an 'O' serotype, defining the PVY^O isolates. Mosaic symptoms in Désirée foliage were usually linked to vein necrosis in tobacco and an 'N' serotype, defining the PVY^N isolates. However, isolates 18 and 32 combined mosaic symptoms in Désirée with mosaic in tobacco as well and an 'O' serotype; which deserved further attention. PTNRD expression was only found in isolates serologically or biologically classified as PVY^N. These results, however, were not reproducible between the two laboratories: indeed isolate 20 induced PTNRD in the tubers of inoculated Désirée plants at CIMA but not in the progeny tubers of the original cultivar at INRA, while isolates 2, 28, 45, 71, 75, 95 and 125 behaved inversely.

Molecular analysis

Figure 1 shows the phylogenetic tree of the isolates according to their coat protein gene. There is a highly significant branch leading to the PVY^N strain, with little intra-branch variation. Another significant branch leads to the PVY^O strain, although, in this case, intra-branch variation is higher. The two reference PVY^C isolates appear separated from the two other branches, confirming that this strain is not present in the panel. Conversely to the coat protein gene analysis, the 5′ end analysis revealed more variation among the PVY^N isolates than among the PVY^O ones. Both the 400/450 (common PVY^N) and the 270/470 (PVY^{NTN}) RFLPs were found. PTNRD expression was, however, found in isolates with either pattern. A new pattern, 350/450, was also found. This analysis clearly separated isolates 18 and 32 from the typical PVY^O's, as these two isolates showed a 270–470 RFLP pattern (PVY^{NTN}). From the point of view of the coat protein gene, these two isolates branch apart from typical PVY^O isolates in the phylogenetic tree, but this does not reach statistical significance.

Further tests for isolates 18 and 32

Because of having shown a combination of PVY^O and PVY^N characteristics in several tests, these two isolates were inoculated in the potato cultivars King Edward, Eersteling, Foxton and Rosalie. Only systemic mosaics were obtained, demonstrating that they were neither PVY^O's nor PVY^C's. In the same experiment, the reference isolate PVY^C-Nl503 induced hypersensitive reactions in the four cultivars and isolate PVY^O-Irl did so in Foxton and Rosalie. From the molecular point of view, PCR amplifications were obtained for isolates 18 and 32 with primers a-d, but not with primers b-d, the same as with reference isolates PVY^N-NL605 and PVY^{NTN}-Lb94. Conversely, the reference isolate PVY^O-Sc, as the PVY^O isolates yielded amplification bands with primers b-d but not with primers a–d.

Discussion

Although not all isolates were subjected to all the tests, solid conclusions can be drawn for most of them. A few inconsistencies can be blamed on technical error (five PVY^N isolates failing to give necrosis in tobacco at CIMA).

The 29 isolates listed under PVY^N in Table 1 induced vein necrosis in tobacco and mosaic in Désirée, reacted to monoclonal antibody PVY^N but not to Y38.4, had D16-T172 coat protein restrictotypes and 270/470, 350/450 or 400/450 5′ end PCR-RFLPs. The induction of vein necrosis in tobacco is indicative of this strain, to the point that it was initially called tobacco veinal necrosis virus (Bawden and Kassanis, 1951). The tobacco bioassay gave false negatives at CIMA for five isolates. This is comparable to the results of a ring-test on the European scale (Dr. G. Adam, 1992; Final Report about the PVY-Ringtest 1991, unpublished), in which the bioassay failed in two of 19 isolates. The limited genetic variability of the PVY^N coat protein gene and its long genetic distance to other PVY strains (Figure 1 in this work, van der Vlugt et al., 1993 and Blanco-Urgoiti et al., 1996b) is in concordance with PVY^N being introduced from South America into Europe in the present century (see Klinkowski and Schmelzer, 1960 for references). Conversely, a high variation has been found at the 5′ end of the genome of this strain; a new RFLP pattern being described in the present work. Unfortunately, no correlation has been shown between the 270/470 RFLP and PTNRD expression, as previously reported (Glais et al., 1996). The expression of this disease in the glasshouse, however, is very variable, depending on the particular combination of virus isolate and potato cultivar (Kerlan and Tribodet, 1996). The conservative approach would be to consider as 'true' PVY^{NTN} only those isolates coming from field-grown tubers expressing symptoms. From this point of view, no PTNRD was observed in the 1994 harvest in the Basque

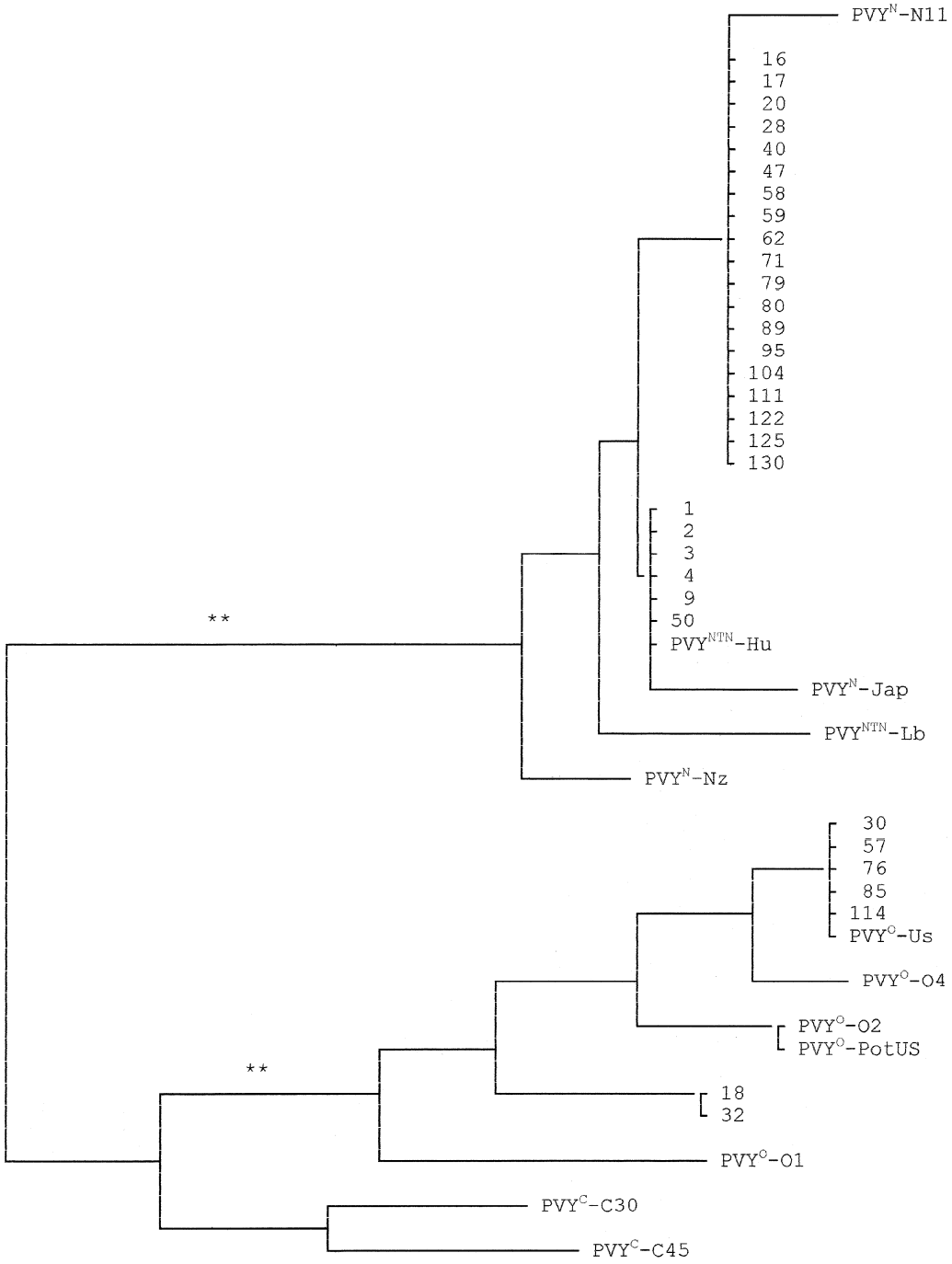

Figure 1. Phylogenetic tree derived from the RFLP analysis of the coat protein gene. Double asterisks indicate a significance over 99%.

crop of seed potato, although it was observed in the ware area nearby. Then, none of the PVY^N isolates in this work would be a true PVY^{NTN}. Nevertheless, the potato cultivars Spunta and Jaerla, common in the area, are known to express little PTNRD (Kus, 1992; Le Romancer et al., 1993). Since the isolates tested by Glais et al. (1996) were true PVY^{NTN}, from the point of view of having been isolated from field-grown tubers, an alternative explanation would be that all the 270/470 isolates in this work were true PVY^{NTN}, a part of them being in a latent stage.

The six isolates listed under PVY^O induced mosaic in tobacco, local lesions and top necrosis in Désirée, reacted to monoclonal 38.4 but not to PVY^N, had a D29H7R112T254 coat protein restrictotype and a 750 5' end RFLP. The ordinary strain of PVY is characterized by inducing a) more severe symptoms in potato than PVY^N does and b) a hypersensitive reaction in less European cultivars than PVY^C does (Beemster and de Bokx, 1987). The PVY^O hypersensitive reaction (acropetal necrosis) is histologically different to the one induced by PVY^C (acronecrosis; Quanjer, 1931). The potato gene Ny_{tbr}, responsible for hypersensitivity to PVY^O, has only recently been described (Jones, 1990). This gave us the opportunity of having a biological test positively identifying PVY^O (local lesions/systemic necrosis in potato cv. Désirée). Before Jones' report, inoculation into tobacco or *Physalis floridana* gave the same result for either PVY^O or PVY^C (de Bokx and Huttinga, 1981) and inoculation in other potato cultivars (Duke of York, synonymous with Eersteling; Beemster and de Bokx, 1987) gave a mosaic response, which is less clear-cut than a necrotic one.

These two groups of isolates showed a good correlation among biological, serological and molecular characteristics and behaved identically to the PVY^O and PVY^N or PVY^{NTN} reference isolates. Both PVY^O and PVY^N induced mild and severe mosaics at CIMA in the original cultivars (results not shown), in contrast to the generally accepted idea that PVY^N is milder (de Bokx and Huttinga, 1981). Some PVY^N isolates showed vein necrosis in the original potato cultivars at INRA, although this showed only partial correlation with PTNRD expression in tubers (results not shown). Even if a specific test for PVY^C (inoculation into King Edward or other cultivar carrying the N_c gene) was done only for isolates 18 and 32, two independent sources of evidence discard the presence of this strain among the isolates studied. One of them is serological (lack of reaction to monoclonal 1E10) and the other one molecular (branching apart from reference PVY^C isolates in the tree obtained from coat protein restrictotypes).

Three isolates showed mixed characteristics of PVY^O and PVY^N. Two of them, namely 18 and 32, induced mosaic in tobacco, reacted to monoclonal Y38.4 but not to monoclonal PVY^N and showed a D29...R112 coat protein restrictotype, like PVY^O, and induced mosaic in Désirée, amplified with primers a-d in PCR and showed a 270/470 5′ end RFLP, like PVY^N. These results were consistent between both laboratories, which eliminates technical or human error. Inoculation into potato cultivars carrying hypersensitivity genes Ny_{tbr} and N_c confirmed that they were neither PVY^O nor PVY^C. They are most probably PVY^Z isolates (Jones, 1990), although confirmatory evidence from inoculation into potato cultivars carrying the N_z gene and comparison with Jones' standard isolates is still lacking. This would be the first report of this group of isolates outside the United Kingdom. Isolate 17 behaved like a PVY^N except in serology, giving a PVY^O reaction in this test. This resembles the behaviour of the PVY^N-Wilga isolate from Poland (Chachulska et al., 1997) and the I-136 and I-L56 isolates from Canada (McDonald and Singh, 1996); this is the first report of such a phenomenon from Western Europe. Whether the abnormal serology of isolate 17 correlates with a deviant biology, like in Wilga, Y-136 and I-L56, deserves further investigation.

The coat protein gene is generally considered as a useful characteristic for potyvirus classification (Shukla and Ward, 1988). It has the advantage of being consistent with serological evidence. In addition to that, it has been shown that the variation of the central region of the potyvirus coat protein sequence parallels the one in the whole genome (Ward et al., 1992). From this point of view, PVY^{NTN}, PVY^N-Wilga and PVY^Z would not reach the status of biological strains, since they have coat protein genes similar to PVY^N and PVY^O, respectively (Figure 1). They would be better described as pathotypes within their strains. However, high variability has been found at the 5′ end of the PVY^N field isolates, the data obtained here confirming previous reports (Marie-Jeanne Tordo et al., 1995; Glais et al., 1996). Whole sequences from more PVY isolates are needed to check the potential of the coat protein gene as a genetic and evolutive indicator. Over 20 PVY coat protein gene sequences are available (Blanco-Urgoiti et al., 1996b), in comparison with only three whole genomes (Robaglia et

al., 1989; Thole et al., 1993; Singh and Singh, 1996). Isolates 18 and 32 show a combination of PVY^O-type 3′ ends and PVY^N-type 5′ ends. It is thus plausible that they evolved by intraspecific recombination of those strains; a phenomenon already known from plum pox potyvirus (Cervera et al., 1993) and PVY itself (Revers et al., 1996). The case of isolate 17 seems quite different, since it only deviates from PVY^N in the serological test; the coat protein restrictotype being typical from this strain. This would indicate a minor mutation, maybe a single amino acid substitution, altering the protein antigenicity.

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References

Alonso F (1996) El cultivo de la patata. Mundi-Prensa, Madrid

Bawden FC and Kassanis (1951) Serologically related strains of potato virus Y that are not mutually antagonistic in plants. Ann Appl Biol 38: 402–410

Beczner L, Horváth H, Romhanyi L and Forster H (1984) Etiology of tuber ringspot disease in potato. Potato Res 27: 339–351

Beemster ABR and de Bokx JA (1987) Survey of properties and symptoms. In: de Bokx JA and van der Want JPH (eds) Viruses of potato and seed potato production. Pudoc, Wageningen, The Netherlands

Blanco-Urgoiti B, Ponz F, Pérez de San Román C, Ritter E and Legorburu FJ (1996a) PCR-RFLP markers for studying PVY variability and epidemiology. Proc. 9th EAPR Virology Section Meeting, Bled (Slovenia), 1995: 89–93

Blanco-Urgoiti B, Sánchez F, Dopazo J and Ponz F (1996b) A strain type clustering of potato virus Y based on the genetic distance between isolates calculated by RFLP analysis of the amplified coat protein gene. Arch Virol 141: 2425–2442

Blanco-Urgoiti B, Sánchez F, Pérez de San Román C, Dopazo J and Ponz F (1998) Potato virus Y group C isolates are a homogeneous pathotype but two different genetic strains. J Gen Virol 79: 2037–2042

Cervera MT, Riechmann JL, Martín MT and García JA (1993) 3′ terminal sequence of the plum pox virus PS and d6 isolates: evidence of RNA recombination within the potyvirus group. J Gen Virol 74: 329–334

Chachulska AM, Chrzanowska M, Robaglia C and Zagorski W (1997) Tobacco veinal necrosis determinants are unlikely to be located within the 5′ and 3′ terminal sequences of the potato virus Y genome. Arch Virol 142: 765–779

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. Phytopathol Polonica 8 (XX): 15–20

Chrzanowska M and Doroszewska T (1997) Comparison between PVY isolates obtained from potato and tobacco plants grown in Poland. Phytopathol Polonica 13: 63–71

Clark MF and Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34: 475–483

de Bokx JA and Huttinga H (1981) Potato virus Y. CMI/AAB Descriptions of Plant Viruses No. 242

Glais L, Kerlan C, Tribodet M, Astier-Manifacier S and Robaglia C (1996) Molecular characterization of potato virus Y^N isolates by PCR-RFLP. Eur J Plant Pathology 102: 655–662

Gugerli P (1979) Le test immunoenzymatique (ELISA) et son application pour le diagnostic rapide des viroses de la pomme de terre. Revue Suisse Agricole 11: 253–260

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

Hooker WJ (1980) Compendium of potato diseases. APS Press, St Paul, Minnesota, USA

Jones RAC (1990) Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. Ann Appl Biol 117: 93–105

Kerlan C, Robert Y, Perennec P and Guillery E (1987) Mise au point sur l'incidence du virus Y^O et des méthodes de lutte mises en oeuvre en France pour la production de semences de pomme de terre. Potato Res 30: 651–667

Kerlan C and Tribodet M (1996) Are all PVY^N isolates able to induce potato tuber necrosis ringspot disease? Abstr 13th Triennial Conf EAPR, Veldhoven, The Netherlands: 65–66

Klinkowski M and Schmelzer K (1960) A necrotic type of potato virus Y. Am Pot J 37: 221–229

Kus M (1992) Potato tuber necrotic ringspot disease. Varietal differences in appearance of ringspot necrosis symptoms on tubers. Proc EAPR Meeting Vitoria-Gasteiz, 29th June-3rd July 1992, 81–83

Le Romancer M, Kerlan C and Nedellec M (1993) Potato tuber necrotic ringspot disease (PTNRD): a comprehensive study of genetic basis of the necrosis phenomenon. Abstr 12th Triennial Conference EAPR, Paris: 351–352

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

Legorburu FJ, Marquínez R and Ruiz de Gauna JA (1996) Spatio-temporal gradients of PVY infection from an external source. Abstr 13th Triennial Conf. EAPR, Veldhoven, The Netherlands: 120–121

Marie-Jeanne Tordo V, Chachulska AM, Fakhfakh H, Le Romancer M, Robaglia C and Astier-Manifacier S (1995) Sequence poly-

morphism in the 5′NTR and in the P1 coding region of Potato virus Y genomic RNA. J Gen Virol 76: 939–949

McDonald JG and Singh RP (1996) Host range, symptomatology, and serology of isolates of potato virus Y (PVY) that share properties with both the PVY^{N} and PVY^{O} strain groups. Amer Potato J 73: 309–315

Nolasco G, de Blas C, Torres V and Ponz F (1993) A method combining immunocapture and PCR amplification in a microtiter plate for the detection of plant viruses and subviral pathogens. J Virol Methods 45: 201–218

Quanjer HM (1931) The methods of classification of plant viruses, and an attempt to classify and name potato viruses. Phytopathol 21: 577–613

Revers F, Le Gall O, Candresse T, Le Romancer M and Dunez J (1996) Frequent occurrence of recombinant potyvirus isolates. J Gen Virol 77: 1953–1965

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

Sanz A, Cambra M, Pérez de San Román C, Miguet JG, Cortes 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 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 M and Singh PP (1996) Nucleotide sequence and genome organization of a Canadian isolate of the common strain of potato virus Y (PVY^{O}). Can J Plant Pathol. 18: 209–224

Thole V, Dalmay T, Burgyán J and Balázs E (1993) Cloning and sequencing of potato virus Y (Hungarian isolate) genomic RNA. Gene 123: 149–156

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 Necrosis 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 comparisons of the viral coat proteins and 3′ non-translated regions. Arch Virol 131: 361–375

Vela C, Cambra M, Cortés E, Moreno P, Miguet JG, Pérez de San Román C and Sanz A (1986) Production and characterization of monoclonal antibodies specific for citrus tristeza virus and their use for diagnosis. J Gen Virol 67: 91–96

Ward CW, McKern NM, Frenkel MJ and Shukla DD (1992) Sequence data as the major criterion for potyvirus classification. Arch Virol, Suppl 5: 283–297

Weidemann HL and Maiss E (1996) Detection of the potato tuber necrotic ringspot strain of potato virus Y (PVY^{NTN}) by reverse transcription and immunocapture polymerase chain reaction. J Plant Dis Prot 103(4): 337–345