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Molecular and Biological Characterization of *Potato virus Y* Isolates from Vietnam

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Abstract

Eight isolates from different potato growing regions in Vietnam were characterized. All were highly pathogenic in some potato cultivars, but did not overcome the extreme resistance of *Solanum stoloniferum* and *Solanum demissum*. RT-PCR analysis revealed that all of these isolates are recombinants. Sequence data for 4 isolates were obtained, and their reaction in potato cultivars harbouring specific *N* genes was determined. Different phylogenetic analyses of viral sequences confirmed previous results that the recombinant isolates evolved from different parental sequences. One of the Vietnamese isolates investigated had a specific structure. The need for a clear classification of PVY^{N}Wi isolates is discussed.

Introduction

In Vietnam, potato is rapidly becoming a staple source of food. Mainly non-adapted foreign varieties of potato are cultivated as a second crop after rice between October and March, in the Red River Delta and northern highlands. To meet the demands for food from a growing population, the government of Vietnam supports activities aimed at enhancing potato production, including a national potato breeding programme. The aim of the latter is to breed high-yielding cultivars with resistance to important pests and diseases (Giang et al. 2013). Among the pathogens and pests, *Potato virus Y* (PVY) causes serious phytosanitary problems in Vietnam. As a consequence, a breeding programme to increase the resistance of potato to this virus was set-up. In Europe, PVY is transmitted mainly by aphids, especially those species that do not colonize potato. The situation in Vietnam cannot be judged as there are currently no specific analyses of this problem. There is only a limited amount of information on the molecular characteristics of PVY isolates from Vietnam (Ha et al. 2008), and it is unknown whether the commonly exploited extreme resistance from the highly polymorphic Mexican wild species *Solanum stoloniferum*, present in a number of potato varieties (Cockerham 1970; Lindner et al. 2011), will be stable and resistant to local isolates. The same is true of the newly exploited extreme resistance from *S. tarnii* (Thieme et al. 2008). For this reason, we investigated the ability of several isolates from potato to overcome extreme resistance and four of them in detail at the molecular level.

Potato virus Y (PVY) is divided into different strains based on the resistance of particular cultivars of potato carrying specific resistance genes that control their hypersensitive reaction (HR; PVY^{O}, PVY^{C}, PVY^{Z}, PVY^{E}; Jones 1990; Le Romancer et al. 1994). PVY^{C} induces HR in the potato cv. King Edward (carries the *Nc* gene), while PVY^{O} induces HR in cvs. Desirée, Maris Bard, and Pentland Crown (*Ny* gene). Isolates of the strain group PVY^{Z} induce HR in cvs. Maris Bard or Pentland Ivory (*Nz* gene), but not in the presence of *Ny* or *Nc* genes. This new strain group PVY^{Z} is clearly

distinct from PVY^N, because unlike the latter it does not induce vein necrosis in tobacco (Jones 1990; Singh et al. 2008). The PVY^N strain does not produce HR in the presence of all three known HR genes in potato (Cockerham 1970; de Bokx and Huttinga 1981). In tobacco, PVY^C, PVY^O and PVY^Z only induce mosaic and vein clearing, whereas most isolates of PVY^N cause vein necrosis and stunting (Jones 1990). Strain PVY^E (previously PVY^{ZE}) like PVY^N produces no HR in the presence of any potato HR gene, but induces only mosaic and vein clearing in tobacco (Kerlan et al. 1999; Singh et al. 2008; Kerlan and Karasev 2011). The genomes of PVY^C, PVY^O and PVY^N strains are non-recombinant. PVY^Z and PVY^E strains are recombinant, composed of segments of parental PVY^O, PVY^N and other genomes (Hu et al. 2009; Kerlan et al. 2011; Galvino-Costa et al. 2012; Quintero-Ferrer et al. 2014).

The designation tuber necrosis strain was introduced for isolates of PVY^N that cause tuber necrosis – PVY^{NTN}. Molecular investigations revealed the recombinant nature of PVY^{NTN} (Singh et al. 2008). Later, it was shown that isolates of PVY^N can also cause tuber necrosis (Fomitcheva et al. 2009). Isolates causing veinal necrosis in tobacco were further divided on the basis of their serological reactions (Chrzanowska 1994). They were designated as PVY^NWi, because this type was first detected in cultivar Wilga. They react with the SASA O-/C-specific monoclonal antibody (MAb), but not the Bioreba N-specific MAb. The recognition sequence of this N-specific MAb is a linear epitope consisting of 17 amino acids GNDTIDAGGSTKKDAKQ (PVY^N-specific amino acids underlined; Nikolaeva et al. 2012). The recognition motif of the O-/C-specific MAb has yet to be determined. This molecular/serological feature is not related to any known interaction with resistance genes.

Based on molecular investigations, it became obvious that several other sequence variants exist, like the North American N strain (NA-N; Lorenzen et al. 2006) or NE-11 (Piche et al. 2004; Lorenzen et al. 2008). Recently, based on more sequences of PVY^O, it was shown that isolates of this strain can be subdivided into several lineages (Karasev et al. 2011; Ogawa et al. 2012). Some of them are specific to particular geographical regions. A comprehensive overview of strain classification is presented by Karasev and Gray (2013).

Materials and methods

Sampling of plant material

Sampling of potato material was carried out during excursions to potato growing areas in Vietnam in late

Table 1 Origins of the PVY isolates from Vietnam and their characteristics compared to standard European isolates of PVY^{NTN} and PVY^NWi

Strain	Geographical origin	Short designation	Molecular struct.	Sequence accession number	Reaction on potato cultivars (13/15/20 dpi):					Resulting genetic type
					Delikat (*Ny:nc:nz*)	Desirée (*Ny:nc:nz*)	King Edward (*ny:Nc:nz*)	Maris Bard (*Ny:Nc:Nz*)	Pentland Ivory (*Ny:Nc:Nz*)	Tobacco	
VNP411	Tien Lang Hai Phong, Vietnam	2	PVY^{NTN}	HG810949	sm/sm,cr/vm,cr, VN	m/m/sm	vm/vm/vm,cr	sm/vm, cr/vm,cr	wm/wm/wm	VN (14)*	PVY^N
VNP413	Tien Lang Hai Phong, Vietnam	4	PVY^{NTN}	HG810950	wm,cr/wm, cr/vm,cr,VN	sm/sm/sm	sm/vm, cr/vm,cr	sm/vm, cr/vm,cr	wm/wm/wm	VN (14)	PVY^N
VNP415	Den Do – Bac Ninh, Vietnam	6	PVY^NWi	HG810951	sm, cr/vm, cr/vm,cr	wm/wm/m	sm/sm, cr/vm,cr	sm/vm, cr/vm,cr	ns/ns/m	VN (15)	PVY^N
VNP417	Den Do – Bac Ninh, Vietnam	8	PVY^NWi	HG810952	vm, cr/vm, cr/vm,cr	m/m/sm	vm/vm/vm	sm/sm/sm	ns/ns/ns	VN (14)	PVY^N
Wilga 261-4	Germany	Wilga	PVY^NWi	AM113988	ns/ns/m	wm/wm/m	wm/sm/vm	wm/m/sm	ns/ns/wm	VN (>35[+])	PVY^N
NTN-Gr99	Poland	NTN	PVY^{NTN}	AJ890343	ns/ns/m	ns/ns/sm,cr	wm/wm/sm	ns/ns/sm	ns/ns/wm	VN (>35[+])	PVY^N

ns, no symptoms; wm, weak mosaic; m, mosaic; sm, strong mosaic; vm, very strong mosaic; cr, crinckling; VN, veinal necrosis; *, in brackets, dpi, when first veinal necrosis was observed; [+], veinal necrosis developed 18 dpi in a climate chamber at a temperature of 20°C.
Inoculation date of plants propagated *in vitro*: 12.08.2014. All plants were positive in DAS-ELISA 35 dpi.

autumn 2012. In the fields, leaves and tubers from plants showing severe symptoms of PVY-infection were collected. The symptoms were leaf and shoot tip necrosis as well as leaf dropping. The farmers did not know what cultivars of potato they were growing. In the laboratory, rapid tests (serological pouch-type test kits; Agdia Inc., Elkhart, IN, USA) were used to verify that the plants were infected with PVY.

Inoculation techniques and purification of RNA

Freeze-dried material from infected potato plants collected at Tun Lang (one isolate) and Tien Lang (four isolates), Hai Phong province and Den Do (three isolates), and Bac Ninh province was inoculated in spring 2013 into tobacco plants ('Samsun' NN) growing in a glasshouse in Europe. Dried leaf samples were homogenized in a mortar with 20 vol (w:v) of 50 mM sodium phosphate buffer at a pH of 7.0. The homogenate was rubbed on leaves dusted with carborundum. Three weeks after inoculation plants were tested for the presence of PVY using ELISA (DAS-ELISA kit from Bioreba, Reinach, Switzerland MAb coctail 112911). Potato plants of the cultivars Delikat (*Ny*:*nc*:*nz*), Desirée (*Ny*:*nc*:*nz*), King Edward (*ny*:*Nc*:*nz*), Maris Bard (*Ny*:*Nc*:*Nz*) and Pentland Ivory (*Ny*:*Nc*:*Nz*) were inoculated in a similar way using material from freshly infected tobacco leaves. Symptoms were scored every second day after the first appearance of symptoms. Some of the results of scoring are given in Table 1. Symptoms are shown in Fig. 1.

Breeding material with resistance genes from *S. tarnii* (Thieme et al. 2008) and wild *S. stoloniferum* (accession GLKS 30071, The Groß Lüsewitz Potato Collections, IPK Genebank External Branch 'North', Groß Lüsewitz) was grafted onto infected rootstocks of cv. Hermes.

Total RNA was isolated using an RNA extraction kit (Machery-Nagel; NucleoSpin® RNA Plant, Düren, Germany) following the manufacturer's instructions. RNA was eluted from the columns with 50 μl of the water provided. For cloning purposes, cDNA was synthesized using the different primers listed in Fomitcheva et al. (2009) and MMLV reverse transcriptase (Thermo Fisher Scientific Inc., Waltham, MA, USA) in 25 μl of reaction volume with 3 μl of RNA elute, again following the manufacturer's instructions.

Cloning and sequence analysis

PCR fragments were generated by means of a self-made Taq polymerase (Ferralli et al. 2007) in 50-μl reaction volume containing 3 μl of the cDNA reaction mix, 200 nM of each primer (Fomitcheva et al. 2009), 0.5 mM dNTP's, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, pH 8.3 @ 25°C. PCR conditions were 2 min at 96°C followed by 32 cycles of 20 s/96°C, 20 s/58°C and 3 min/72°C. Fragments were analysed by running them on a 1% agarose gel in TAE buffer, isolated, purified (GeneJET Gel Extraction and DNA Cleanup Micro Kit; Thermo Fisher Scientific Inc.) and ligated in the vector pGEM-T (Promega, Madison, WI, USA). For transformation, *E. coli* NEB-turbo cells were used.

Clones containing desired fragments of DNA were purified (GeneJET Plasmid Miniprep Kit; Thermo Fisher Scientific Inc.) and sequenced using a CEQ-DTCS Quick starter sequencing kit and a Beckman-Coulter CEQ 8800 sequencer (Beckman–Coulter, Brea, CA, USA). For each amplified fragment, at least two clones were sequenced in parallel. Base calling,

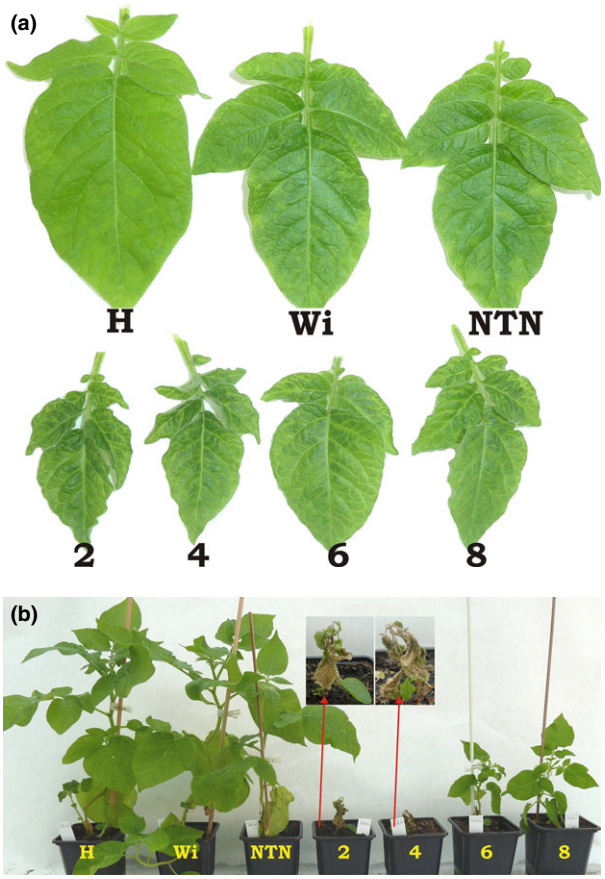

Fig. 1 Symptoms associated with infection by the different PVY isolates. (a) Cultivar King Edward, 2nd leaf from top of infected plant, 20 dpi; (b) cultivar Delikat, 28 dpi. H-healthy control; Wi-PVY^{N}Wi, isolate 261-4; NTN-PVY^{NTN}, isolate Gr99; 2, 4-Vietnamese isolates 2 and 4, PVY^{NTN}; 6, 8-Vietnamese isolates 6 and 8, PVY^{N}Wi.

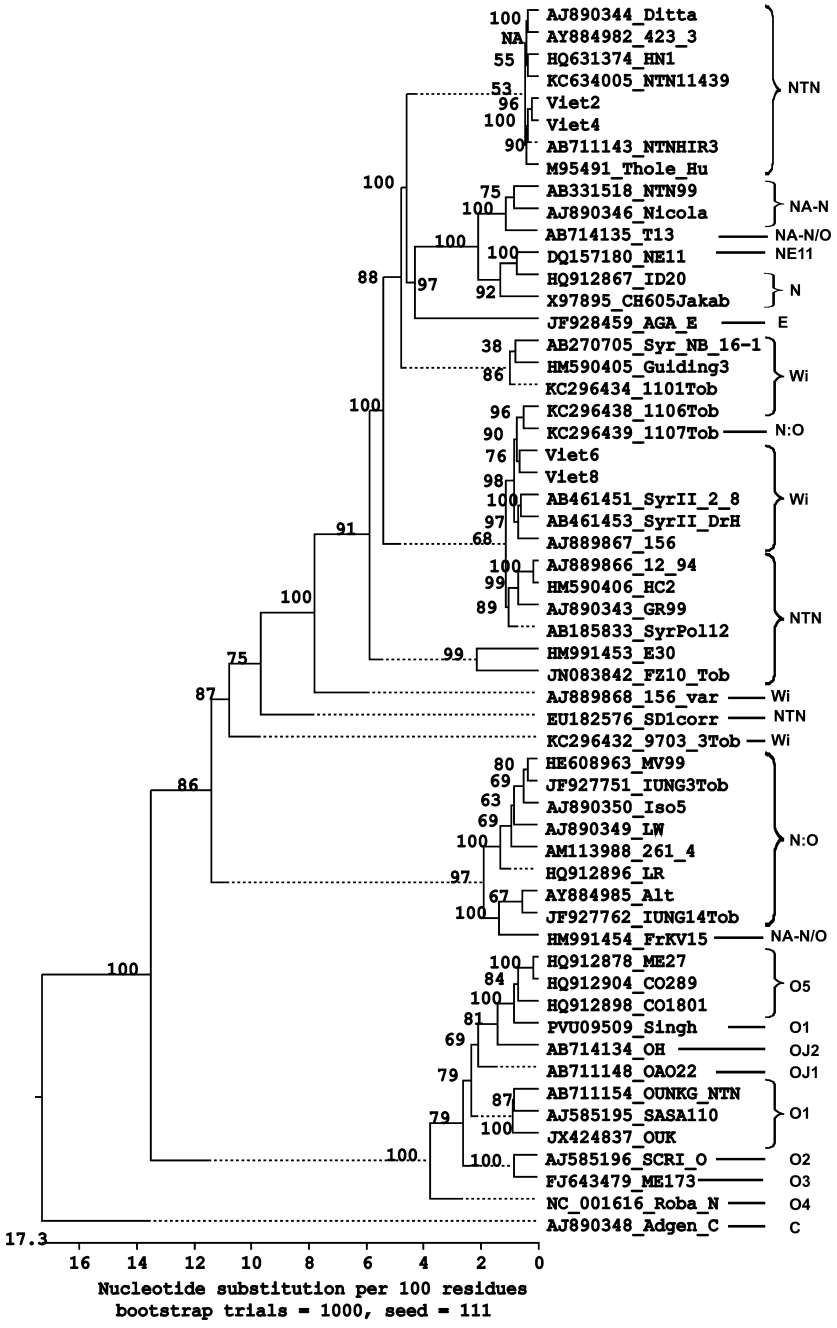

Fig. 2 Phylogenetic analysis based on a set of complete PVY sequences. The O strain is designated based on Ogawa et al. (2012).

Table 2 Designation rules for recombinant isolates of the strain group PVY^N used in the current publication

Strain	Veinal necrosis in tobacco	Molecular characteristics	Proposed reaction with Bioreba N-MAb (*in silico*)
$PVY^{N}Wi$	+	Recombinant N:O	
PVY^{NTN}	+	Recombinant N:O, downstream from position ~2.400 further N:O recombination event(s)	+
$PVY^{N:O}$	+	Recombinant N:O, downstream from position ~2.400 no further recombination event	+

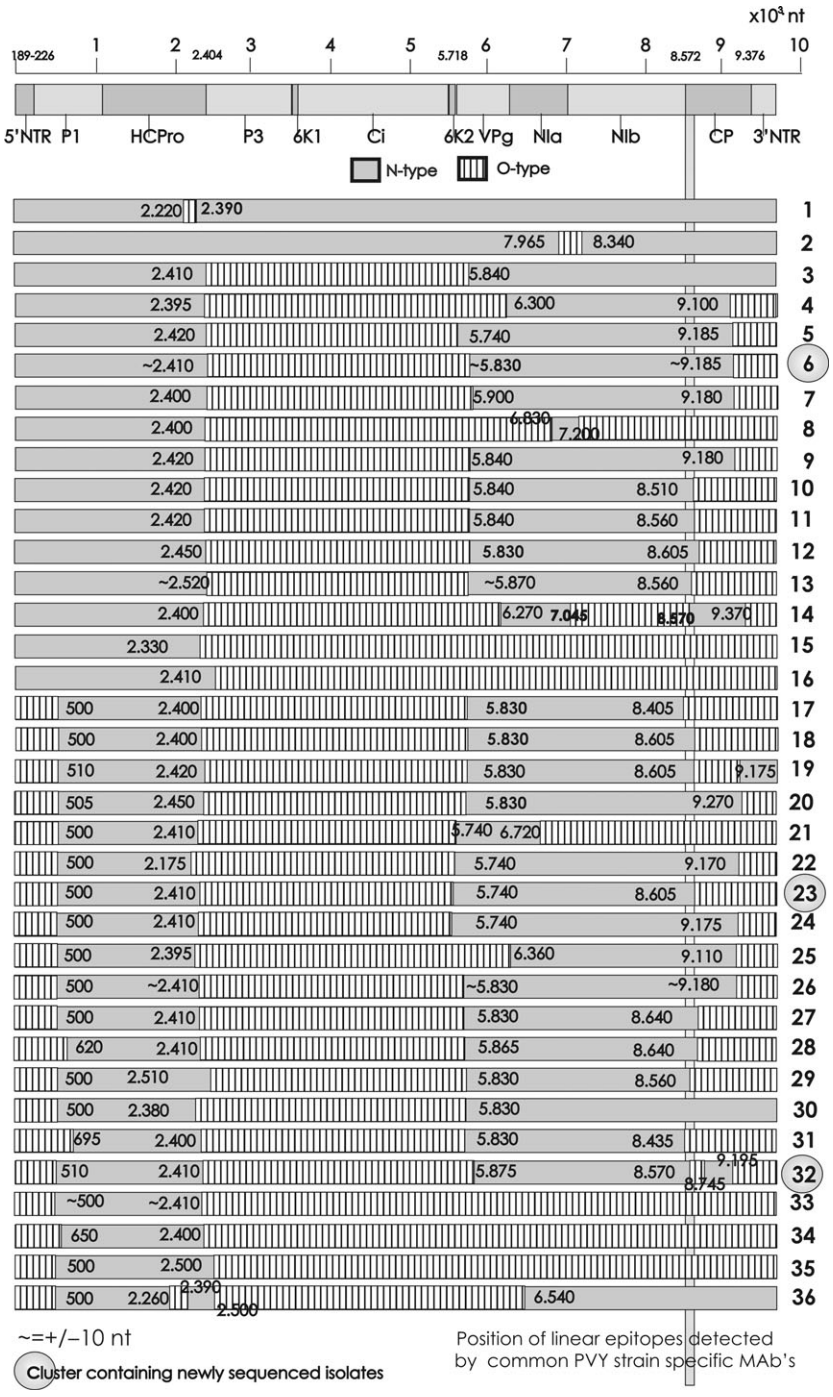

Fig. 3 Structure of PVY N:O recombinant isolates. Information on the sequences in the different clusters is given in Table 3.

alignments and the phylogenetic analysis were performed using the Lasergene 11 software package (DNA Star, Madison, WI, USA). For recombination analyses, we used the software package RDP 4.2.2 (beta, Martin et al. 2010). For identifying the strains, the primer system designed by Hühnlein et al. (2013) was used.

Results

First visible symptoms of infection of tobacco with PVY appeared approximately 10 days after inoculation and took the form of faint chloroses and vein clearing. Later, the tobacco plants exhibited severe leaf and veinal necrosis. These symptoms were caused

Table 3 Isolates of PVY and accession numbers of their sequences belonging to the different clusters shown in Fig. 3

Cluster	Isolate name	Corresponding accession number
1	ID20	HQ912867
2	SCRI-N	AJ585197
3	Y27	AB461450
4	Nysa	FJ666337
5	NIBNTN, Satina	AJ585342, AJ890347
6	HN1, A95,ID155, NNTK1, GAM2, Ditta, Linda, L26 (PVY^Z), N4, HR1, Pb312, V942490, Thole, Viet2, Viet4, M3 (PVY^Z)	HQ631374, HQ912866, HQ912869, AB711146, AB711145, AJ890344, AJ890345, FJ204165, FJ204164, FJ204166, EF026075, EF016294, M95491, HG810949, HG810950, KF850513
7	423-3	AY884982
8	9703-3	KC296432
9	IUNG1,-4,-8,-9, -11, -13, -15, NTN-UK, 11627-12, 11227-2NTN, 1105Tob, NTN11439, 11627-10, 9703_4, NTN11629_9, N20917029, NTN HIR3	JF927749, JF927752, JF927757, JF927756, JF927759, JF927761, JF927763, KC614702, KC634007, KC634004, KC296437, KC63400, KC634006, KC296441, KC634008, KC634009, AB711143
10	1101	KC296434
11	1103	KC296435
12	SyrNb-16-1	AB270705
13	HN2, Guiding3, 9703_5Tob	GQ200836, HM590405, KC296433
14	SD1 (corrected)	EU182576
15	IUNG14	JF927762
16	OR1-Wi, ID1-Wi, Alt, L56, ID431, Pb209, LR, SA207, ME142, Me162, Mb112, ID14_2_14a, IUNG2,-5, -6, -10, 09_3a, ID431,	DQ157179, DQ157178, AY884985, AY745492, EF026076, HQ912896, AJ584851, HQ912871, HQ912872, HQ912872, AY745491, HQ912870, JF927750, JF927753, JF927754, JF927758, JF795485, HQ912862
17	SyrII DrH	AB461453
18	SyrII BE1	AB461452
19	SyrII-2-8	AB461451
20	OKU M4,ONGI3, OHO37, SyrPol12	AB711150, AB711151, AB711149, AB185833
21	156var	AJ889868
22	Gr99	AJ890343
23	156, Viet6	AJ889867, HG810951
24	12-94, 34-1	AJ889866, AJ890342
25	E30	HM991453
26	HC2, N3	HM590406, HQ912868,
27	1106Tob	KC296438
28	1107Tob	KC296439
29	1108Tob	KC296440
30	1104Tob	KC296436
31	SyrIII L4	AB461454
32	Viet8	HG810952
33	1107Tob	KC296439
34	09-3a, Del66, IUNG3, MV99, MV175, N1, LW, Iso5, NWilga, PN10a	JF795485, JN034046, JF927751, HE608963, HE608964, HQ912863, AJ890349, AJ890350, EF558545, DQ008213
35	261-4	AM113988
36	AQ4, IUNG12	JN083841, JF927760
37	IUNG7Tob	JF927755
38	LR	HQ912896
39	FZ10Tob	JN083842

by all the isolates investigated. Vietnamese isolates caused symptoms much faster than both of the European isolates used for comparison (Table 1). Infected plants of the tested potato cultivars did not show a hypersensitive reaction. Vietnamese isolates induced symptoms much faster and more strongly than any of the European isolates used. These symptoms were especially pronounced for isolates 2 and 4. Most European isolates of PVY^NWi cause only mild symptoms in potato (Chrzanowska 1991; Yin et al. 2012). Very severe symptoms of virus infection of locally grown susceptible cultivars were also observed in the field in Vietnam: necrosis of veins, leaf fall and dark spots on leaves ('ink spots' or oak leaf necrotic pattern). Of the cultivars tested that lacked PVY HR genes, only Hermes (results not shown) survived. It was used for grafting experiments. Four weeks after grafting onto PVY inoculated cv. Hermes the scions and rootstocks were tested for the presence of virus using ELISA. While the ELISA of susceptible controls (Hermes) and rootstocks revealed high concentrations of PVY ($OD_{405\ nm} > 0.5$),the material with ER genes originating from *S. tarnii* and *S. stoloniferum* remained free of virus (data not shown). Consequently, both sources of PVY resistance can be used in programmes breeding for resistance. On the other hand, the severe symptoms of virus infection and death of the susceptible cultivar emphasize the importance of developing resistant potato cultivars not only for growing in Vietnam but also in Europe. It cannot be excluded that these highly pathogenic isolates will eventually arrive in Europe, for example in tobacco leaves.

Using specific primers (Hühnlein et al. 2013), isolates of the strains PVY^{NTN} and $PVY^{N}Wi$ were identified. The strains NA-N, N, O and C were not detected. Four isolates were chosen for further sequence analysis. Complete genomic sequences were obtained for all four of these isolates (Table 1). A phylogenetic analysis based on a MUSCLE alignment of all the complete PVY sequences published was performed (results not shown). Based on this analysis, typical isolates from the main branches supported by bootstrap values of above 65 were chosen and included in a further phylogenetic analysis. Results are shown in Fig. 2. For designating the recombinant isolates, we followed the rules cited in Table 2. The Vietnamese isolates group in typical NTN or Wilga clusters. A correlation with geographical origin is not obvious.

Recombination analysis with RDRP 4 is based on aligning completely sequenced isolates using MUSCLE. The structure of the recombinant isolates is given in Fig. 3 (Table 3). For grouping the Vietnamese isolates either into PVY^{NTN} or $PVY^{N}Wi/PVY^{N:O}$ (Table 3), an *in silico* analysis of the possible reactions with Bioreba's N-specific MAb was performed. Based on this analysis, isolates 2 and 4 belong to the NTN strain and 6 and 8 to $PVY^{N}Wi$. Biological data (Table 1) indicate that the isolates do not belong to

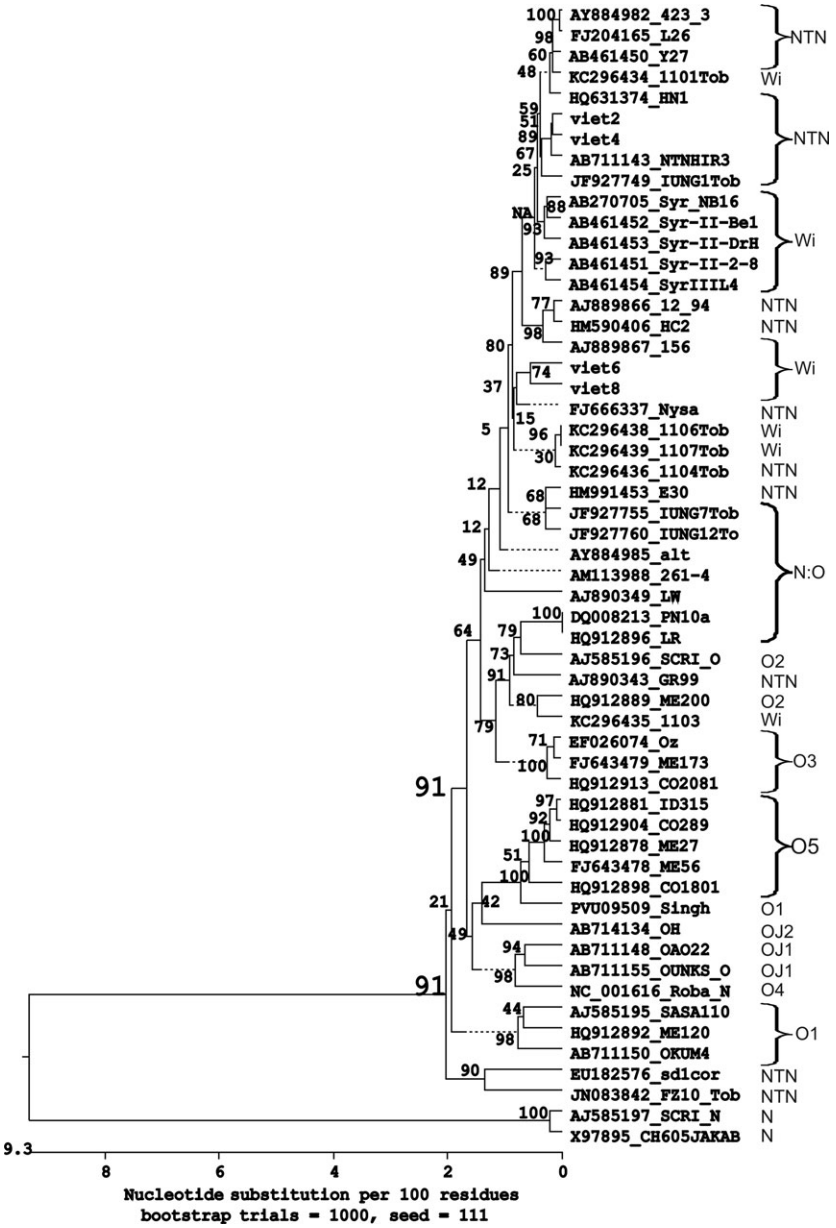

Fig. 4 Phylogenetic analysis based on the internal PVY^{O}-specific part of recombinant N:O isolates. The O strain is designated based on Ogawa et al. (2012).

strain group E as all induce veinal necrosis in tobacco and do not cause HR in tested cultivars.

Except for isolate 8, all other Vietnamese isolates analysed resemble previously described recombinant structures. In the case of isolate 8, the basic coat protein (CP) sequence is of O-type interrupted by a stretch of N-type (Fig. 3). Consequently, the high virulence of these isolates is not related to a specific recombinant structure.

Discussion

Four highly pathogenic PVY isolates from Vietnam were characterized at a molecular level. Their pathogenicity in some cultivars cannot be explained by a novel type of recombination.

The occurrence of the Vietnamese isolate 8 originating from potato and Chinese isolate 1107 (accession number KC296439) originating from tobacco indicates that for a clear identification of isolates of type $PVY^{N}Wi$ by means of RT-PCR, it will be necessary to position strain-specific primers on the first 90 nt of the CP gene region, which is where the main linear epitopes differing between N and O strains and recognized by MAb's are located (Keller et al. 2005; Chikh Ali et al. 2007; Ranki et al. 2008; Nikolaeva et al. 2012).

The biological features of the Vietnamese isolates and the new structure of isolate 8 raise the question whether the differentiation between NTN and Wilga/N:O strains should be maintained: similar to NTN strains, the Vietnamese Wilga isolates 6 and 8 cause severe symptoms in several potato cultivars, whereas the first described isolates of the Wilga strain were characterized as causing mild symptoms in potato (Chrzanowska 1994). Both strains cause veinal necrosis in tobacco. It is reported that some amino acid motifs of the CP may play a role in symptom expression in *Physalis floridana* (Hu et al. 2011). Genetic determinants of PVY involved in the induction of veinal necrosis in tobacco reside in the HC-Pro, CI and NIa cistrons (Tribodet et al. 2005; Faurez et al. 2012). There are no indications that the two amino acids of the N-terminus of the CP, which according to the MAb-based classification identify these isolates as strains of Wilga, play any role in symptom expression

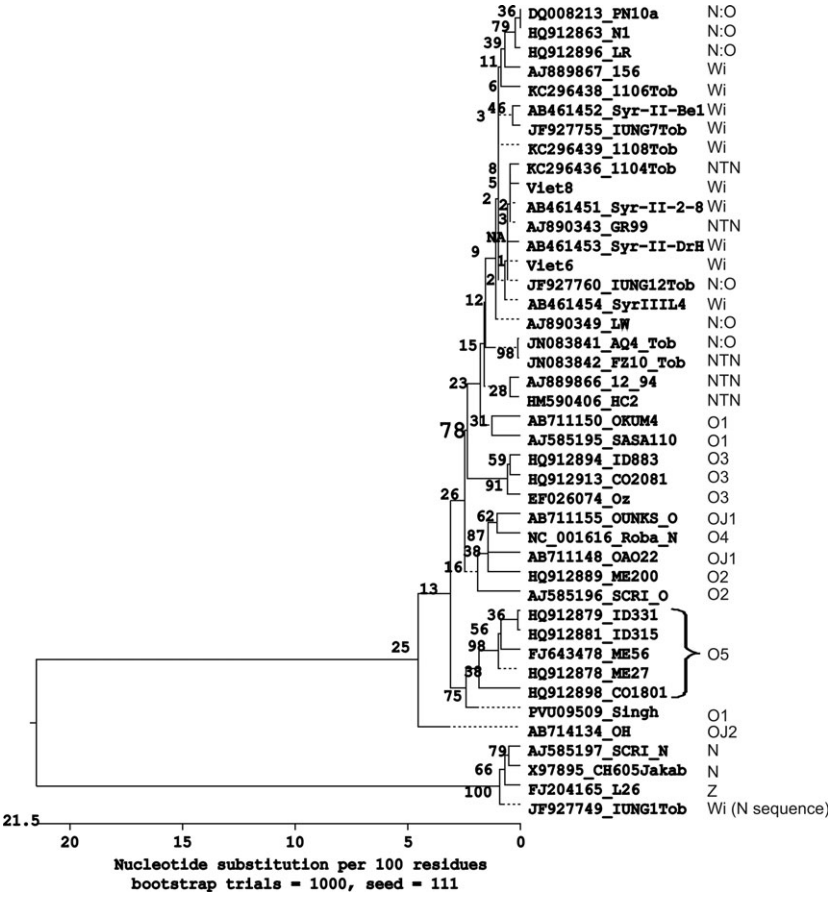

Fig. 5 Phylogenetic analysis based on the 5′-terminal PVY^{O}-specific part of recombinant N:O isolates. The O strain is designated based on Ogawa et al. (2012).

in potato. Based on a limited number of isolates, it is suggested that all PVY^{NTN} isolates trigger a HR in potato carrying the Nz_{tbr} gene, whereas isolates of strain PVY^{N}-Wi/$PVY^{N:O}$ do not (Rowley et al. 2014). Of course, if this biological difference is confirmed for a larger number of isolates with different molecular structures, the division between conventional PVY^{NTN} and PVY^{N}Wi (sub)strains needs to be retained.

There are several different MAb's with recognition motifs different from those of Bioreba's N-MAb that can be used to differentiate between NTN and Wilga isolates (Ounouna et al. 2002; Chikh Ali et al. 2007; Ranki et al. 2008; Nikolaeva et al. 2012). This might cause confusion as a single point mutation in the recognition motifs in the CP gene would change the classification of the strains but leave the biological features of the isolates unchanged. This is the case for the isolate Syr-12 (Chikh Ali et al. 2007), in which a point mutation induced reactivity of the CP with the PVY^{O}-specific Agdia PVY^{O}-MAb2: AA29 changed from Glu (E; GAA) to Gly (G; GGA).

All the recombinant isolates so far sequenced share a common feature: the genomic region of nt 700–2260 is always of N-type, while the region 2500–5740 is of O-type. The biological meaning of this feature is unknown. Using a phylogenetic analysis, Karasev et al. (2011) demonstrated that the sequences of the central PVY^{O}-specific part of recombinant isolates

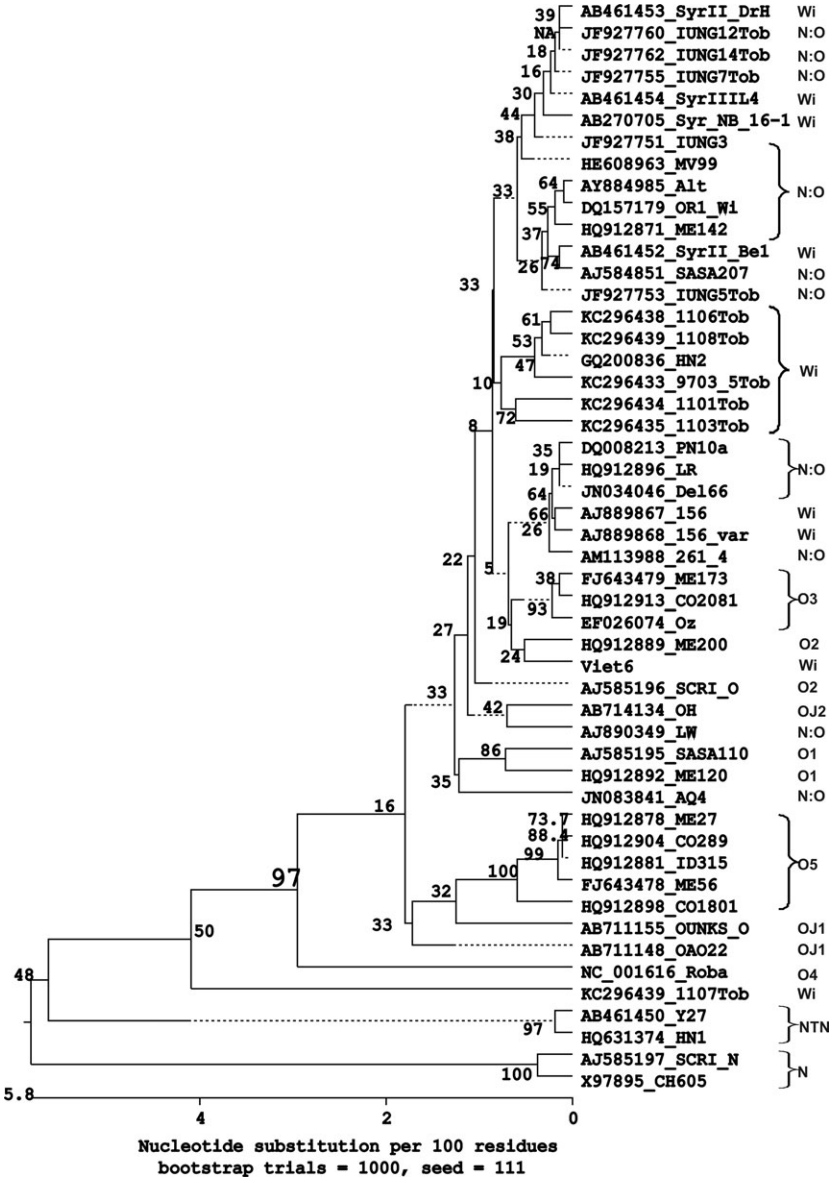

Fig. 6 Phylogenetic analysis based on the 3′-terminal PVY^{O}-specific part of recombinant N:O isolates. The O strain is designated based on Ogawa et al. (2012).

(pos. 2406–5821) originated from different parental sequences. The sequence described by them of the new PVY^{O}-O5 type was never an integral part of the recombinant isolates analysed. A similar phylogentic analysis based on this region and including the Vietnamese isolates is presented in Fig. 4. Clustering suggests that the recombinant isolates evolved from PVY^{O} types 2 and 3. The O-type sequences typical of some Japanese isolates (O-J1, -J2) are not an integral part of the Vietnamese isolates.

A similar analysis was performed using a selected number of PVY N:O isolates from two other genomic regions of O-type sequences. The first region spanned nucleotides 34–491 and the second 8.640–9.682 (positions according to accession number X97895). Phylogenetic trees are given in Figs 5 and 6. In the case of the 5′ end sequences, the separation of the clusters is only poorly supported by bootstrap values. In contrast, the central part of the O-specific sequences seems to originate from types 1 or 3. In the case of the 3′ end sequences analysed, which include parts of CP and 5′-NTR, the clusters are poorly supported by bootstrap values. The specific origin of the N:O sequences is unknown. Only isolate KC296493_1107 originating from tobacco is different from all the other PVY^{O} sequences investigated.

Phylogenetic analysis based on the region of recombinant isolates spanning nucleotides 500–2.400 is

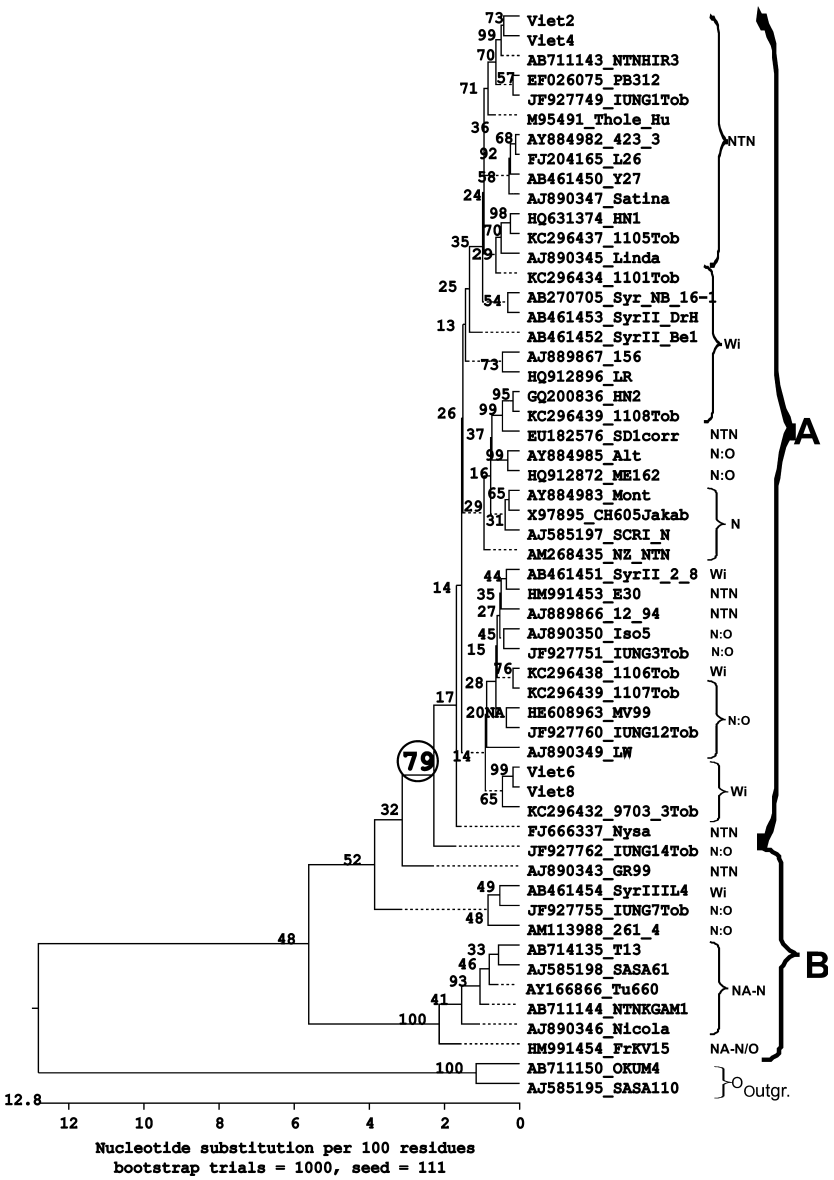

Fig. 7 Phylogenetic analysis based on the 5′-terminal PVY^{N}-specific part of recombinant N:O isolates.

shown in Fig. 7. As a rule, the presence of this region in recombinant isolates is indicated by a N-type sequence (Fig. 3). Again, in most cases the clusters are only poorly supported by bootstrap values. The tree can be divided into two clusters, A and B, specific for PVY^{NTN}/PVY^{N}Wi/$PVY^{N:O}$ or NA-N isolates, respectively. Interestingly, some isolates of PVY^{NTN}, PVY^{N}Wi and $PVY^{N:O}$ also occur in cluster B. This indicates that the origin of this region is different.

Taken together, the phylogenetic analyses of the different regions of recombinant genomes indicate that they evolved in different ways. Different parts seem to originate from different sources. For a clear definition of the recombinant isolates, it will be necessary to agree on specific epitopes that are recognized by commercially available MAb's.

The recently published results of Rowley et al. (2014) demonstrate that conventional potato cultivars may contain several undiscovered HR genes. It is possible that the strong reaction of the cultivar Delikat to the Vietnamese isolates 2 und 4 is based on such gene (s). Crossing experiments are under way to test this hypothesis.

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