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RECOMBINANTS OF PVY STRAINS PREDOMINATE AMONG ISOLATES FROM POTATO CROP IN POLAND

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Abstract: 282 *Potato virus Y* (PVY) isolates collected from potato crops in northern and central Poland from 1995 to 2009 were characterized by serological and biological assays. From these, 112 isolates collected from 2006 to 2009 were additionally analyzed by one-step triplex Reverse Transcription-Polymeraze Chain Reaction (RT-PCR). Recombinants of PVY strains predominate among the isolates tested. Using one-step triplex RT-PCR most PVY^{N-Wi} isolates were classified as subgroup PVY^{N}Wi-P and most PVY^{N} and/or PVY^{NTN} isolates as the recombinant PVY^{NTN} strain. A recombinant PVY^{NTN} isolate (12/94) and two additional PVY^{N} and/or PVY^{NTN} isolates were not detected by one-step triplex RT-PCR. Twelve isolates were identified as the PVY^{O} strain but PVY^{N}, non-recombinant PVY^{NTN} and PVY^{C} strains were not found. Serological and biological assays of 144 isolates of PVY^{N-Wi} strain showed that 100 isolates were the expected PVY^{O} serotype with vein necrosis (VN) symptoms on tobacco. However, 10 isolates of the PVY^{N}Wi-P subgroup exhibited vein clearing (VCl) on tobacco and 2 isolates of the PVY^{N}N242 subgroup unexpectedly exhibited as the PVY^{N} serotype. All the isolates of PVY^{N-Wi} strain induced severe local lesions (LL) on *Chenopodium amaranticolor*. Out of 126 isolates of PVY^{NTN} strain tested, 76 were typical PVY^{N} serotype with VN on tobacco, but their reactions on *C. amaranticolor* were different: 13 isolates did not show symptoms, 23 isolates induced weak, and 40 isolates induced severe LL. The remaining isolates of PVY^{N-Wi}, PVY^{N} and/or PVY^{NTN} or PVY^{O} were serologically PVY^{N} and PVY^{O} positive or exhibited unpredictable serological and biological reactions.

Key words: PVY, Recombinant, One-step triplex RT-PCR, *Chenopodium amaranticolor*, potato, Poland

INTRODUCTION

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae*, with a single-stranded positive-sense genomic RNA of approximately 9.7 kb (Hull 2009). Based on symptoms in tobacco and differential potato cultivars carrying specific resistance genes (*Ny*, *Nc*, *Nz*) PVY isolates from potato (*Solanum tuberosum* L.) can be classified into seven strain groups, with the strain group PVY^{N} capable of inducing vein necrosis (VN) and the strain group PVY^{O} unable to induce VN but causing vein clearing (VCl) on tobacco (Kerlan 2006; Singh *et al.* 2008). Since the 1980s, variant isolates within the PVY^{N} strain group had been reported from different geographic regions, including PVY^{NTN} that causes potato tuber necrotic ringspot disease (PTNRD) and PVY^{N-Wi} which is not detected using PVY^{N} specific monoclonal antibodies (mAb) and is a PVY^{O} serotype (Beczner *et al.* 1984; McDonald and Singh 1996a, b; Nie and Singh 2002; Ramírez-Rodríguez *et al.* 2009). In Poland, the first PVY^{N-Wi} isolate (named Wi) was identified on the potato cv. Wilga collected from west Poland in 1984 (Chrzanowska 1991) and the first PVY^{NTN} isolate (named 12/94) was detected on a tobacco bait plant grown in a potato field at Młochów in 1994 (Chrzanowska and Doroszewska 1997). Both PVY^{N-Wi} and PVY^{NTN} are recombinants between the PVY^{O} and PVY^{N} strains (Glais *et al.* 2002; Crosslin *et al.* 2006; Schubert *et al.* 2007; Visser and Bellstedt 2009; Boukhris-Bouhachem *et al.* 2010). Recently, a novel PVY strain variant NE-11 has been shown to be a recombinant between PVY^{N} and North American type PVY^{NTN} (Lorenzen *et al.* 2008) and the variant PVY^{NTN-NW} a recombinant between PVY^{NTN} and PVY^{N-Wi} (Chikh Ali *et al.* 2010). In contrast, evolution of a North American isolate (Tu660) of PVY^{NTN} strain was reported to originate from PVY^{N} by mutation rather than recombination (Nie and Singh 2003).

The characterization of molecular determinants of PVY^{N} isolates involved with VN in tobacco suggests that several genomic regions are likely to be involved. Two single-nucleotide polymorphisms A/G_{2213} and A/C_{2271} that lead to modification of residues K_{400}/E_{419} (in the necrotic PVY genotype) to residues R_{400}/D_{419} (in the non-necrotic PVY genotypes) within the HC-Pro protein were initially identified (Tribodet *et al.* 2005). Recently another viral genetic determinant of VN in tobacco has been found; a single nucleotide change (A-1627 to G-1627) resulting in the single amino acid change (D-205 to G-205) in the HC-Pro cistron that correlates with the loss of VN phe-

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notype of an isolate named L26 (Hu *et al.* 2009b). However, the viral sequences or domains responsible for PTNRD have not yet been determined (Glais *et al.* 2002; Schubert *et al.* 2007; Singh *et al.* 2008). The finding that non-recombinant PVY^{NTN} induces PTNRD means that the recombinant structure of the genome is not necessary for the isolate to cause PTNRD (Nie and Singh 2003; Ogawa *et al.* 2008). Moreover, isolate L26's ability to cause PTNRD but not VN in tobacco, provides further evidence that necrosis in potato tubers and tobacco are controlled in the virus by different genetic determinants (Hu et al. 2009b). In addition, point mutation in a Syrian PVY isolate (PVY-12) was reported as changing the PVY^{N} serotype into PVY^{O} serotype (Chikh Ali *et al.* 2007) and an American PVY^{O} isolate (PVY^{O}-O5) was recognized by a PVY^{N}-specific monoclonal antibody, 1F5 (Karasev *et al.* 2010).

The aim of the present study was to identify the main PVY strains/subgroups infecting ware potato crops grown in Poland by characterizing selected isolates from the Młochów Research Center of the Plant Breeding and Acclimatization Institute – National Research Institute [Instytut Hodowli i Aklimatyzacji Roślin – Państwowy Instytut Badawczy (IHAR-PIB/Młochów)] potato virus collection using a one-step triplex RT-PCR (Rigotti and Gugerli 2007) and serological and biological methods. The naming of PVY strains was done according to Singh *et al.* (2008) and the naming of two subgroups (PVY^{N}Wi-P and PVY^{N}N242) within the PVY^{N-Wi} strain according to Rigotti and Gugerli (2007).

MATERIALS AND METHODS

PVY isolates

A total of 282 PVY isolates from the Młochów Research Center of the Plant Breeding and Acclimatization Institute – National Research Institute virus collection, collected from potato fields between 1995 and 2009, representing 26 locations of northern and central Poland and 64 host potato cultivars were studied. Isolates were maintained on their host cultivars by replanting infected potato tubers yearly in an insect-free greenhouse (20–26°C/14–16°C, day length 16 h). In addition, some isolates were collected from the tobacco bait plants grown in potato fields and inoculated to susceptible potato cultivars *e.g.* Vital, Jagoda, Igor or Perkoz. Four previously sequenced isolates were used for reference: LW (PVY^{O}, accession number AJ890349); Ny (PVY^{N}, FJ666337); Wi (PVY^{N-Wi}, EF558545) and 12/94 (PVY^{NTN}, AJ889866) together with isolate 605 (PVY^{N} from Switzerland, X97895) and H (PVY^{NTN} from Hungary, M95491) obtained from a previous international research project (Browning *et al.* 2004). These reference isolates were maintained on potato host cultivars as described above.

Nicotiana tabacum, Chenopodium amaranticolor and potato bioassay

N. tabacum (cv. Samsun) and *C. amaranticolor* were used as test plants. In *C. amaranticolor*, PVY^{O} and PVY^{C} induce local lesions (LL) and PVY^{N} is asymptomatic (Kerlan 2006; Blanchard *et al.* 2008). For inoculation to tobacco, leaf sap from PVY infected potato cultivars was applied to leaves (3–4 leaf stage) lightly sprinkled with carborundum powder. Subsequently, *C. amaranticolor* plants (5–6 leaf stage) were inoculated with leaf sap from the PVY-infected tobacco. Foliar symptoms were recorded two to four weeks post-inoculation. For each isolate, two indicator plants were used. The inoculation was conducted once between spring and summer of 2009 and 2010. All plants were grown in an insect-free greenhouse at Młochów (20–26°C/14–16°C, day length 16 h).

Occasionally tubers of the PVY host cultivars were inspected visually for symptoms.

Serological identification of PVY isolates

Double antibody sandwich (DAS) Enzyme-Linked Immunosorbent Assay (ELISA) was used as described by Syller (2001) with the following antibodies: 1) monoclonal cocktail antibody (Bioreba 112911) that recognizes all known isolates of all groups/subgroups of PVY; 2) PVY^{N} specific mAb (Bioreba 112712) that recognizes the necrotic strain group PVY^{N}, except the Wilga type (PVY^{N-Wi}); 3) PVY^{O} and PVY^{C} specific mAbs (SASA) where the coating antibody binds all PVY strain groups and the conjugate antibody specifically identifies PVY^{O} and PVY^{C}. DAS-ELISA was conducted using PVY infected potato and tobacco leaf tissue. A sample was considered positive if the absorbance value was more than twice the healthy control.

Total RNA extraction

Total RNA was extracted from potato leaf tissue with RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Total RNA was eluted in 50 µl of RNase-free water and stored at –80°C.

One-step triplex Reverse Transcription – Polimerase Chain Reaction (RT-PCR)

In order to differentiate the PVY isolates into strains/subgroups, a one-step triplex RT-PCR method was used (Rigotti and Gugerli 2007), with the addition of an internal control primer. The primers used were 1) PVYc3/f that targets the 5'NTR and P1 genomic region of PVY^{O} isolate named 139; 2) PVY3+/3 – that targets the CI and 6K2 genes of PVY^{N} isolate 605; 3) CP2+/1 – that targets the CP gene of PVY^{O} isolate 803; 4) the internal control primer pair Nad5-F/Nad5-R (5'-gat gct tct tgg ggc ttc ttg tt-3' / 5'-ctc cag tca cca aca ttg gca taa-3'), that targets the apple mitochondrial *nad5* gene encoding NADH dehydrogenase subunit 5 and generates a band of 181 bp in length (Menzel *et al.* 2002). The one-step triplex RT-PCR allows identification and classification of PVY into: strains PVY^{N}, PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, and PVY^{C} and; subgroups PVY^{N}Wi-P and PVY^{N}N242 within the PVY^{N-Wi} strain and non-recombinant PVY^{NTN} and recombinant PVY^{NTN} within the PVY^{NTN} strain. However, the great similarity between the genomes of PVY^{N} and non-recombinant PVY^{NTN} prevents their differentiation using this method (Rigotti and Gugerli 2007). Additionally variants of PVY^{NTN} such as NE-11 and North American PVY^{NTN} cannot be clearly diagnosed (Rigotti *et al.* 2011). RT-PCR was performed using Superscript III one step RT-PCR with platinum *Taq* DNA polymerase (Invitrogen) according to the manufacturer's instructions. The RT-PCR products were separated on 1% agarose gel and visualized by ethidium bromide staining.

RESULTS

Viral isolate detection by one-step triplex RT-PCR

A total of 112 PVY isolates collected from 2006 to 2009 were analyzed by one-step triplex RT-PCR. The PCR product (181 bp) amplified by the internal control primer pair was present in every test (Fig. 1a and b), confirming the validity of the RT-PCR reaction. The PVY specific primers generated no PCR product for the healthy control (Fig. 1a, lane 7). The reference isolates 605 (PVY^{N}), H (PVY^{NTN}) and Wi (PVY^{N-Wi}) generated the expected RT-PCR products of 440 bp and 1110 bp (isolate 605), 440 bp (isolate H) and 530 bp (isolate Wi) (Fig. 1a, lanes 1, 4 and 6). However, the reference isolates 12/94 (PVY^{NTN}) and Ny (PVY^{N}) were not detected by this method (no band in Fig. 1a, lane 5 and Fig. 1a, lane 2 respectively). For the reference isolate LW (PVY^{O}) only the 530 bp band was present (*i.e.* the band for PVY^{N}Wi-P subgroup of PVY^{N-Wi} strain) and the expected 660 bp band was not present (Fig. 1a, lane 3). PVY^{N}, non-recombinant PVY^{NTN} and the PVY^{C} strains were not detected (Table 1). Of the PVY^{NTN} strain 26 isolates were identified as recombinant PVY^{NTN} (Table 1, Fig. 1b, lane 7) and three isolates amplified no band. Of the PVY^{N-Wi} strain 69 isolates were classified as PVY^{N}Wi-P subgroup (Table 1, Fig. 1b, lanes 2, 4, 5 and 6) and two as PVY^{N}N242 subgroup (Table 1, Fig. 1b, lane 3). Twelve isolates were confirmed as PVY^{O} strain (Table 1, Fig. 1b, lane 1).

Serological and biological characteristics of the isolates

Serological and biological characterization was conducted on the 112 isolates analyzed using the one-step triplex RT-PCR, together with another 170 isolates collected from 1995 to 2005. Previously, 73 out of these 170 isolates had been identified as PVY^{N-Wi} and 97 isolates as PVY^{N} or PVY^{NTN} based on ELISA and symptoms on tobacco. The serological and biological results are summarized for PVY^{N-Wi}, PVY^{N} and/or PVY^{NTN}, and PVY^{O} respectively (Tables 2–4).

Table 1. Identification by one-step triplex RT-PCR of PVY isolates collected from 2006 to 2009[a]

PVY strain (subgroup)	Amplified bands (bp)	Number of isolates[b]
PVY^{N}	440, 1110	0
Non-recombinant PVY^{NTN}	440, 1110	0
Recombinant PVY^{NTN}	440	26
Recombinant PVY^{NTN}	no band[b]	3
PVY^{O}	660, 530	12
PVY^{N}W (PVY^{N}Wi-P)	530	69
PVY^{N}W (PVY^{N}N242)	440, 530	2
PVY^{C}	660	0
Sum		112

[a]the one-step triplex RT-PCR was conducted essentially according to Rigotti and Gugerli (2007)
[b]in this work; bp – base pair

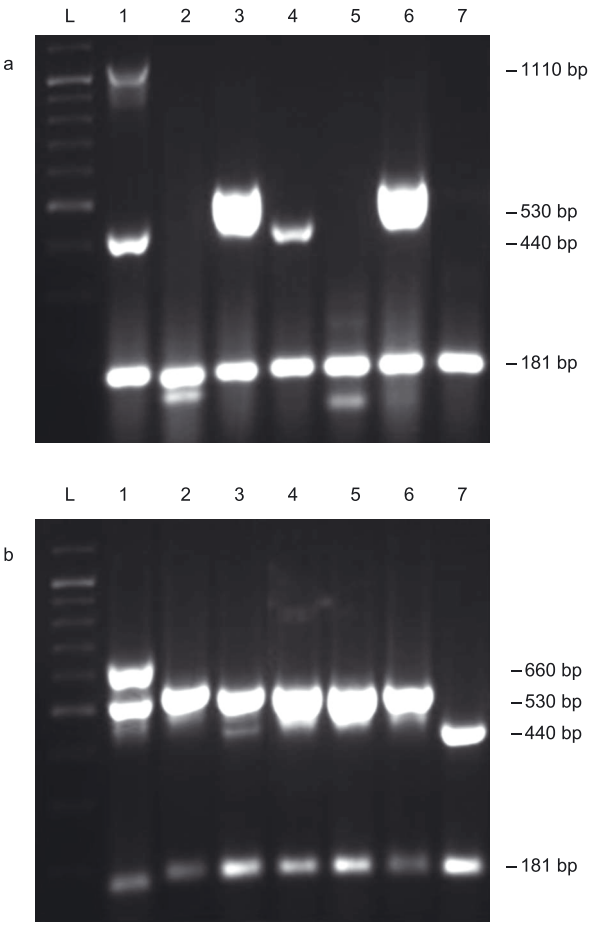

Fig. 1. Viral isolates detection by one-step triplex RT-PCR. a: Reference isolates. 1 – isolate 605 (PVY^{N} from Switzerland, X97895) (Browning *et al.* 2004); 2 – Ny (PVY^{N}, FJ666337); 3 – LW (PVY^{O}, AJ890349); 4 – H (PVY^{NTN} from Hungary, M95491) (Browning *et al.* 2004); 5 – 12/94 (PVY^{NTN}, AJ889866); 6 – Wi (PVY^{N}W, EF558545); 7 – healthy control (potato cv. Ursus) b: Isolates collected from 2006 to 2009. 1 – PVY^{O} isolate; 2, 4, 5, 6 – PVY^{N}W (PVY^{N}Wi-P) subgroup isolates; 3 – PVY^{N}W (PVY^{N}N242) subgroup isolate; 7 – recombinant PVY^{NTN} isolate; L – DNA molecular marker

Biological and serological diversity was found within strains and subgroups. As shown in table 2, of the 144 PVY^{N-Wi} isolates, 100 (69.4%) were as expected the PVY^{O} serotype with VN on tobacco. However, 10 (7.0%) isolates of PVY^{N}Wi-P subgroup exhibited VCl on tobacco instead of VN and 2 (1.4%) isolates of PVY^{N}N242 subgroup were unexpectedly the PVY^{N} serotype. All isolates of the PVY^{N-Wi} strain induced severe LL on *C. amaranticolor*.

Of the 126 isolates of PVY^{N} and/or PVY^{NTN}, 76 were typical PVY^{N} serotype with VN on tobacco, but their reactions on *C. amaranticolor* were different: 13 (10.3%) isolates did not show symptoms, 23 (18.3%) isolates induced weak local lesions (LL), and 40 (31.7%) isolates induced severe LL (Table 3). Three isolates which were not detected by one-step triplex RT-PCR were PVY^{N} serotype with VN on tobacco and induced severe or weak LL on *C. amaranticolor*. Furthermore, visual inspection of PTNRD symptoms showed that 7 isolates of recombinant PVY^{NTN} and three isolates which were not detected by one-step triplex RT-PCR induced PTNRD on the host

cultivars Bryza, Cekin, Ditta and Satina. The PVY^{NTN} reference isolates H and 12/94 and the PVY^{N} reference isolates 605 and Ny, induced PTNRD on the cultivars Nicola and Igor. One recombinant PVY^{NTN} isolate named Gr99 induced unexpected VCl on tobacco and weak LL on *C. amaranticolor*, but it did not cause PTNRD on potato.

Of 12 PVY^{O} isolates, 4 showed expected PVY^{O} serotype, VCl on tobacco and severe LL on *C. amaranticolor* (Table 4). Four isolates induced VN on tobacco instead of VCl.

Some isolates, of PVY^{N-Wi}, PVY^{O} or PVY^{N} and/or PVY^{NTN}, were serologically PVY^{N} and PVY^{O} positive (Tables 2–4). Unexpected serological and biological reactions were found for 21 (14.6%) isolates of PVY^{N-Wi}, 13 (10.3%) of PVY^{N} and/or PVY^{NTN} and one of PVY^{O}.

Table 2. Serological and biological characteristics of virus isolates of PVY^{N-Wi} strain

Strain/ subgroup	Characteristics		Number of isolates					
			expected type	unexpected serological or biological type				
	serotype by	ELISA	[a]All + [b]N – [c]O +	All + N + O +	All + N + O –	All + N – O +	unpredictable	sum
	symptoms on	tobacco	VN	VN	VN	VCl		
		C. amaranticolor	severe LL	severe LL	severe LL	severe LL		
[d]PVY^{N-Wi}			61	1	0	0	11	73
[e]PVY^{N}Wi-P			39	10	0	10	10	69
[e]PVY^{N}N242			0	0	2	0	0	2
Sum			100	11	2	10	21	144
Percent total			69.4%	7.6%	1.4%	7.0%	14.6%	100%

[a]monoclonal cocktail antibody for all PVY strains (Bioreba 112911, Switzerland)
[b]monoclonal antibody (mAb) for PVY^{N} except PVY^{N-Wi} (Bioreba 112712, Switzerland)
[c]mAb for PVY^{O} and PVY^{C} (PVY^{all} mix mAb for PVY^{O} and PVY^{C}, SASA, UK)
[d]isolates collected from 1995 to 2005 classified based on ELISA and tobacco symptoms
[e]isolates collected from 2006 to 2009 identified by one-step triplex RT-PCR (Rigotti and Gugerli 2007)
VN – vein necrosis; VCl – vein clearing; LL – local lesions; + positive; – negative

Table 3. Serological and biological characteristics of virus isolates of PVY^{N} and/or PVY^{NTN} strain

Strain/ subgroup	Characteristics		Number of isolates						
			expected type	unexpected serological or biological type					
	serotype by	ELISA	[a]All + [b]N + [c]O -	All + N + O +	All + N + O –	All + N + O –	All + N + O –	unpredictable	sum
	symptoms on	tobacco	VN	VN	VN	VN	VCl		
		C. amaranticolor	no symptom	severe LL	weakLL	severe LL	weak LL		
[d]PVY^{N} or PVY^{NTN}			8	36	14	29	0	10	97
[e]Recombinant PVY^{NTN}			5	0	8	9	[f]1	3	26
[e]Recombinant PVY^{NTN} (no band)			0	0	1	2	0	0	3
Sum			13	36	23	40	1	13	126
Percent total			10.3%	28.6%	18.3%	31.7%	0.8%	10.3%	100%

Note – see table 2.
[f]isolate named Gr99

Table 4. Serological and biological characteristics of virus isolates of PVY^{O} strain

Strain / subgroup	Characteristics		Number of isolates				
			expected type	unexpected serological or biological type			
	serotype by	ELISA	[a]All + [b]N – [c]O +	All + N + O +	All + N – O +	unpredictable	total
	symptoms on	tobacco	VCl	VN	VN		
		C. amaranticolor	severe LL	severe LL	severe LL		
[e]PVY^{O}			4	3	4	1	12

Note – see table 2.

DISCUSSION

Use of one-step triplex RT-PCR has shown that the recombinant isolates belonging to the PVY^{N}Wi-P subgroup of the PVY^{N-Wi} strain and the recombinant PVY^{NTN} strain are currently the predominant forms of PVY infecting ware potato crops in Poland which is similar to reports from other countries (*e.g.* Kerlan 2003/4; Crosslin *et al.* 2006; Schubert *et al.* 2007; Visser and Bellstedt 2009; Nie

2010). Some PVY isolates from the IHAR-PIB/Młochów virus collection have been sequenced elsewhere (Glais *et al.* 2002; Schubert *et al.* 2007), and represent recombination pattern #2 (e.g. isolate LW) within PVY^{N-Wi} and pattern #4 (Ditta), #6 (Gr99) and #7 (12/94, 34/01) within PVY^{NTN} (Hu *et al.* 2009a). The worldwide spread of PVY^{NTN} and PVY^{N-Wi} has been explained by their selective advantage over the parent strains (Kerlan 2003/4) and in Poland, transmissibility by *Myzus persicae* of PVY^{NTN} and PVY^{N-Wi} isolates has been found to be higher than that of PVY^{O} and PVY^{N} isolates (Kaliciak and Syller 2009). Moreover, PVY^{NTN} and PVY^{N-Wi} can frequently escape detection by visual inspection in seed potato certification schemes because of mild symptoms (Kerlan 2003/4). This is especially true for Polish isolates of PVY^{N-Wi} which seem to be more infective to most potato cultivars and causes mild mosaic symptoms, making negative selection in growing seed crops difficult (Chrzanowska 1994).

Another main finding of this study was the large biological and serological diversity within the recombinant PVY^{NTN} strain and within the PVY^{N}Wi-P subgroup of the PVY^{N-Wi} strain. On *C. amaranticolor*, isolates belonging to recombinant PVY^{NTN} strain caused three types of symptoms: expected no symptoms, unexpected weak LL or severe LL. Previously, it had been reported that eight tuber-necrosing isolates of PVY^{NN} strain (*i.e.* currently named PVY^{NTN}) were unable to infect *C. amaranticolor*, but the standard isolate PVY^{O}-R and PVY^{N}-R used had induced necrotic local lesions (Le Romancer *et al.* 1994). However, others had found that whereas PVY^{N} and PVY^{NTN} did not induce symptoms on *C. amaranticolor*, PVY^{O} caused LL (McDonald and Singh 1996a; Blanchard *et al.* 2008). In this study, all isolates of PVY^{N-Wi} caused severe LL on *C. amaranticolor*, consistent with those reported for isolates I-136 and I-L56 which share properties with both PVY^{O} and PVY^{N} (McDonald and Singh 1996 a). On tobacco, isolates belonging to the PVY^{N}-Wi-P subgroup of the PVY^{N-Wi} strain caused two types of symptoms, expected VN and unexpected VCl. The PVY^{N-Wi} isolates not able to induce VN in tobacco were also reported among a new group of isolates $PVY^{N-Wi\ minus}$ variant collected in the United States and Canada, which have a PVY^{O} serotype and a recombinant genome organization, but do not induce vein necrosis on tobacco (Gray *et al.* 2010). All isolates belonging to the PNY^{N} and/or PVY^{NTN} strains induced expected VN, except that a recombinant PVY^{NTN} isolate named Gr99 caused VCl on tobacco. In addition, isolate Gr99 did not induce PTNRD on potato. Serologically, two isolates belonging to the PVY^{N}N242 subgroup of the PVY^{N-Wi} strain exhibited unexpected PVY^{N} serotype. Overall, our data confirms the large diversity of PVY species that exists, even within the isolates collected from potato, and is similar to the diversity reported by others (Piche *et al.* 2004; Baldauf *et al.* 2006; Barker *et al.* 2009; Nie *et al.* 2011). The occurrence of biologically diverse isolates with their highly homologous genomes brings into question the exact nature of gene structure in relation to function (Barker *et al.* 2009). Point mutation has been shown to be responsible for serotype variation of certain isolates (Chikh Ali *et al.* 2007; Karasev *et al.* 2010). For the recombinant PVY^{NTN} isolate Gr99, two amino acid changes in the viral genetic determinants (K-400 to R-400, E-419 to D-419 in the HC-Pro protein) may be responsible for the loss of VN in tobacco (Hu *et al.* 2009b).

In conclusion, the present study demonstrates that the recombinant isolates belonging to the PVY^{N}Wi-P subgroup of the PVY^{N-Wi} strain and the recombinant PVY^{NTN} strain are currently the predominant forms of PVY infecting ware potato crops in Poland. Some tested isolates of the PVY^{N}-Wi-P subgroup of the PVY^{N-Wi} strain did not induce VN on tobacco, confirming the recent results reported by others (Gray *et al.* 2010). Some tested isolates of recombinant PVY^{NTN} strain did not induce any symptoms on *C. amaranticolor*, consistent with previous findings (Le Romancer *et al.* 1994; McDonald and Singh 1996a; Blanchard *et al.* 2008). However, the majority of tested isolates of recombinant PVY^{NTN} strain indeed caused weak or severe LL on *C. amaranticolor*, what was not reported elsewhere so far.

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