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Potato virus Y (PVY) strains in Belgian seed potatoes and first molecular detection of the N-Wi strain

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Abstract

Potato virus Y (PVY), one of the most important agents causing potato crop losses worldwide, is transmitted by a variety of aphid species in a non-persistent manner. Several PVY strains have been differentiated, all of them causing different symptoms and symptom expression levels on numerous commercial potato cultivars. In Belgium, strains belonging to the N group have been reported as the most prevalent, but no detailed information on the relative importance of the PVY strains in Belgium have been published to date.

We report here on a survey performed on Belgian seed potatoes harvested in 2010 in which 2700 individual tubers from 54 seed potato lots originating from 54 farms were screened for presence of PVY. The results revealed a high PVY incidence and substantial strain diversity in some farms. The dominance of the N group in Belgian seed potatoes was confirmed, while the O strain was only found in a few locations. Further characterization using multiplex PCR identified 75% of the isolates as NTN strains and 7.5% as Wilga strain (N-Wi). The presence of the N-Wi strain was confirmed and characterized for the first time in Belgian seed potato production.

Key words: PVY^{NTN}, $PVY^{N\text{-}Wi}$, *Solanum tuberosum*, strain variability, survey

Introduction

Potato virus Y (PVY) is a major pathogen in potato production worldwide. The two main strain groups O and N are responsible for most of the damage in potato growing areas (Kerlan 2006, Rolot 2009). Apart from O and N, PVY^{C}, PVY^{Z} and PVY^{E} are also recognized as PVY strain groups (Karasev & Gray 2013). Obvious mosaic, stunting and leaf drop are reported as the main symptoms of the O strain, whereas the N strain causes mild mosaic and occasional leaf necrosis (Singh et al. 2008). Over the past years, a lot of progress has been made in understanding the molecular properties of PVY strains. PVY^{O} and PVY^{N} are considered to be the two starting groups from which one to three recombinations resulted in several subgroups (Nie et al. 2013). Within the N strain group, the NTN strain (PVY^{NTN}) is the causal agent of the potato tuber necrotic ringspot disease (PTNRD) (Beczner et al. 1984, Le Romancer et al. 1994). In some potato cultivars, this strain can cause severe damage, both on leaves and tubers, whereas recombinants belonging to another N-like subgroup containing the Wilga strain ($PVY^{N\text{-}Wi}$) (Chrzanowska 1991, Glais et al. 2001) and $PVY^{N:O}$ (Singh et al. 2003) mainly produce mild symptoms and can even remain latent in potato. However, symptoms caused by the same PVY strains can vary a lot depending on the potato cultivar (Nie et al. 2012). Molecular studies have revealed that even within the subgroups considerable variations in the PVY genome evolved differently, e.g. in North America and Europe, resulting in a very complex situation of strains, strain groups and variants (Karasev & Gray 2013). However, these variations are mainly introduced by recombination in different parts of the genome (Glais et al. 2002, Nie & Singh 2003a, Lorenzen et al. 2006a, Chikh Ali et al. 2007a, b, 2010b). Three to four recombinant junctions in NTN strains and only one or two recombinant junctions in $PVY^{N\text{-}Wi}$ and $PVY^{N:O}$ have been reported (Glais et al. 2002, Nie & Singh 2003a, Lorenzen et al. 2006a, b, Schubert et al. 2007, Visser et al. 2012, Karasev & Gray 2013). Position and length of these recombinant segments, resulting in variations in recombinant junctions or breakpoints, have led to several publications on the existence of NTN variants (a and b) and on differentiating N-Wi & N:O subgroups, which have been summarized in the recent reviews of Visser et al. (2012) and Karasev & Gray (2013). Yet, fitness pressure selects and boosts the relative importance of some populations that have been created by point mutations and/or recombination. Geographic isolation could conduct different fitness pressure and natural selection to select and boost various recombinations, resulting in separate lineages, such as some O and Wilga strains in North America (the so called PVY^{O}-O5 subgroup and $PVY^{N:O}$) (Singh et al. 2008) and NA-PVY^{NTN} that has evolved from NA-PVY^{N} by mutation rather than recombination (Nie & Singh 2003a).

Available data indicate that the N strain group (including PVY^{N}, PVY^{NTN} and $PVY^{N\text{-}Wi}$ strains) is the most common strain group in Europe (Weidemann 1988, Glais et al. 1998, Glais et al. 2002, Nie & Singh 2003b), while in North America, the ordinary strain group (PVY^{O}) still has a predominant place (Crosslin et al. 2006, Lorenzen et al. 2006a, Nanayakkara et al. 2012). Both in Germany (Lindner 2008) and Switzerland (Rigotti et al. 2011), the NTN-strain has been shown to be the most prevalent PVY strain, although both authors report a gradual shift over the years toward prevalence of the $PVY^{N\text{-}Wi}$ strain. This shift towards the

Wilga strain was also reported in the Netherlands (Van der Vlugt et al. 2008, Verbeek 2009) and France (Kerlan et al. 1999). In France, the presence of Wilga, N, C, Z and O strains were reported, with O as the most dominant strain (Kerlan et al. 1999), but the spread of NTN in France was also reported recently (Blanchard et al. 2008).

The importance of the N strain for Belgium has also been demonstrated by Rolot & Steyer (2008). In a more recent study, Rolot (2009) confirmed an increase in importance of the N strain group. Note that all of these records are based on serological tests that cannot differentiate between N and NTN. Additionally, $PVY^{N\text{-}Wi}$ is serologically identified as an O strain (Singh et al. 2008).

PVY clearly has a large economic impact on potato seed production, but the various strains have a different impact depending on the symptoms they cause. Obtaining accurate data on the occurrence and prevalence of the different strains is thus important. The overall objective of this study is to support the seed potato certification scheme by means of a detailed characterization of the variability in PVY strains in Belgium and their relative importance.

Materials and methods

PVY screening and collection of PVY isolates

The survey was initiated with seed potatoes sampled on farms during the official certification scheme in 2010. Each sample consisted of 100 tubers. PVY incidence was screened using the grow-out method followed by the DAS-ELISA (Clark & Adams 1977) test by polyclonal antibody (DSMZ, Germany) on the leaves. In each lot, the chosen plants were also assessed for symptom development. Individual PVY-infected plant samples were stored at −70°C for further analysis.

Strain determination

Total plant RNA was extracted from maximum 100 mg potato fresh leaf material using the Spectrum Plant Total RNA extraction Kit (Sigma, Bornem, Belgium). RNA yield and quality were checked spectrophotometrically (ND-1000 Spectrophotometer, NanoDrop, Isogen, Temse, Belgium).

First strand DNA was synthesized by adding 1 µl of RNA template to 19 µl of a mixture containing 1 µl RevertAid Premium Reverse Transcriptase, 4 µl 5x RT buffer, 1 µl dNTP Mix (10 mM), 0.5 µl Ribolock RNase inhibitor and Oligo-dT primer at a final concentration of 2.5 µM (RT, Thermo Fisher Scientific, Waltham, MA, USA). The RT reaction was done at 42°C for 60 min followed by inactivation of the RT by heating at 95°C for 5 min.

Multiplex PCR for strain differentiation was carried out according to Chikh Ali et al. (2010a) (primers listed in Table 1) with some modifications. PCR was conducted using OneTaq™ DNA Polymerase (Bioké, Leiden, the Netherlands) according to the following program: denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 64°C, 62°C and 60°C in the first, second and third 10 cycles for 30 s, respectively, and extension at 68°C for 5 min. This was followed by a final 5 min extension step at 72°C. PCR products were visualized using Qiaxel capillary electrophoresis (QIAxel Advanced System). The obtained amplicons were compared to the expected size (Chikh Ali et al. 2010a) and those of the PVY reference isolates that were included in the experiment.

The whole genome of the nine isolates was compiled (assembled using Codoncode and MEGA5 software) after amplifying nine segments and gaps using the primer pairs described by Nie & Singh (2003a) and also designed in this study. Extra primer pairs were developed for additional sequencing of the overlapping regions of the nine segments (Table 1). PCR product purification and sequencing with the corresponding forward and reverse primers were performed by Macrogen Inc. (Amsterdam, the Netherlands). Consensus sequences of sense and antisense strands of all nine selected isolates were aligned and were deposited in GenBank and the accession numbers were linked to the isolate overview in Table 2. A phylogenetic dendrogram was compiled by means of UPGMA (Partial Deletion Gaps, Bootstrap test). The selected PVY isolates of this study were compared with a representative number of PVY isolates of the respective strain (sub)groups that were retrieved from the GenBank database (Table 3). A schematic diagram of the recombinant junctions (breakpoints) was drafted based on the RDP4 multiple recombination detection software (Martin et al. 2010).

Results

Prevalence of PVY types in Belgium

An overall general PVY infection rate of 3.6%, ranging from 0% to a maximum of 42%, was recorded in the 54 seed potato lots that were included in the survey. PVY infected tubers were identified in 42.5% (23/54) of the seed lots. A geographical distribution of samples positive for PVY, covering 23 administrative divisions in Belgium, is presented in Table 2.

Strain Determination

Forty PVY isolates were selected from PVY infected samples in ELISA tested from 21 locations in Belgium. They were subjected to strain determination using the multiplex RT-PCR protocol described by Chikh Ali et al. (2010a). As shown in Figures 1 and 2, analysis of the obtained fragments confirmed the presence of four strains and a variation (a and b) in NTN strains. These isolates were identified as NTN, O, N-Wi, and N with a relative abundance of 75%, 7.5%, 7.5%, and 2.5%, respectively. A minor percentage (2.5%) of the infected plants revealed mixed infections with two or three strains (NTN and N-Wi and/or O), and 2.5% of the isolates could not be identified, probably also due to mixed infections (Fig. 1).

Table 1: Primers used in this study

Primer name	Polarity	Sequence (5'–3')	Location	Reference	Application
n156	+	GGGCAAACTCTCGTAAATTGCAG	160–179	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
o514	+	GATCCTCCATCAAAGTCTGAGC	515–536	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
n787	–	GTCCACTCTCTTTCGTAAACCTC	770–792	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
n2258	+	GTCGATCACGAAACGCAGACAT	2260–2281	Lorenzen et al. (2006a)	Multiplex RT-PCR – characterization
o2172	+	CAACTATGATGGATTTGGCGACC	2169–2191	Lorenzen et al. (2006a)	Multiplex RT-PCR – characterization
n2650c	–	TGATCCACAACTTCACCGCTAACT	2627–2650	Lorenzen et al. (2006a)	Multiplex RT-PCR – characterization
o2700	–	CGTAGGGCTAAAGCTGATAGTAG	2678–2700	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
S5585m	+	GGATCTCAAGTTGAAGGGGAC	5578–5598	Lorenzen et al. (2006a)	Multiplex RT-PCR – characterization
o6400	–	GTAACTCCTAAACAAATGGTGGTTCG	6405–6430	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
n7577	+	ACTGCTGCACCTTTAGATACTCTA	7582–7605	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
YO3-8648	–	CTTTTCCTTTGTTCGGGTTTGAC	8635–8657	Schubert et al. (2007)	Multiplex RT-PCR – characterization
SeroN	–	GTTTCTCCTATGTCGTATGCAAGTT	8864–8888	Chikh Ali et al. (2010a)	Multiplex RT-PCR – characterization
1	+	GGATCCAATTAAAACAACTCAATA	5' end	Nie & Singh 2003a	Sequencing
	–	CATTTGTGCCCAATTGCC	1091–1073	Nie & Singh 2003a	Sequencing
2	+	TTCAGTTCTCAAGCGCTGAA	1033–1052	Nie & Singh 2003a	Sequencing
	–	TCTTAGTGAAATCCTTTGCAT	2133–2113	Nie & Singh 2003a	Sequencing
3	+	GCGATGTTGATTAACATT	2085–2102	Nie & Singh 2003a	Sequencing
	–	TTATTGCCTGACACACTGC	3294–3276	Nie & Singh 2003a	Sequencing
4	+	TCTTCAGGCGTTTGCCAACTTT	3203–3224	Nie & Singh 2003a	Sequencing
	–	TTGCGCATCAACAAATGATTGG	4367–4346	Nie & Singh 2003a	Sequencing
5	+	TGTCAGCTACTCCAGTGGGAAG	4266–4285	Nie & Singh 2003a	Sequencing
	–	TCAGTGCGCAATGTGTATGC	5268–5250	Nie & Singh 2003a	Sequencing
6	+	CACGAAATGCTTTGGGAAAC	5169–5185	Nie & Singh 2003a	Sequencing
	–	CAGGAAACTTGGCAATGCCATT	6198–6177	Nie & Singh 2003a	Sequencing
7	+	GCCACACAACCCACTCAAA	6143–6161	Nie & Singh 2003a	Sequencing
	–	TCCGACTGCAGCTTTCAT	7400–7383	Nie & Singh 2003a	Sequencing
8	+	GGCTATCAATAGGGTTATCAT	7289–7309	Nie & Singh 2003a	Sequencing
	–	CTTGATGGTGCACTTCATAAG	8571–8551	Nie & Singh 2003a	Sequencing
9	+	GCTTTCACTGAAATGATGGT	8502–8521	Nie & Singh 2003a	Sequencing
	–	GTTTTCCCAGTCACGACTTTTTTTTTTT	3´ end	Nie & Singh 2003a	Sequencing
5Pr	–	ATCTGGACATCAGTCTTGTATC		This study	Sequencing
Gap 1	+	TGTCAACCAAAGGAGGGTCTG		This study	Sequencing
	–	ACCGGTTCAGTTAAGTGCTCT			
Gap 2	+	AAATTGATCCAGCGAAGGGC		This study	Sequencing
	–	CTCCTCCTTCTCTGAAAGGTGA			
Gap3	+	ATGGTGTTGCAAGTTGTTAAGAA		This study	Sequencing
	–	CAGCATCGAACACCATGATGA			
Gap 4	+	TTCAAGAAGCCAACACTGCG		This study	Sequencing
	–	GCAATGCTCCTATTGTCAATGTC			
Gap 5	+	AACTTGCCAGTGATGACAGG		This study	Sequencing
	–	GCGCCTGCTATGATCAAGTC			
Gap 6	+	GGTATGGGCAAGTCAAGCAG		This study	Sequencing
	–	ACAGGGAAATCTTTCGGCAT			
Gap 7	+	ACAGGGAATTTGCAAGCTGT		This study	Sequencing
	–	TCTAAAGGTGCAGCAGTGAA			
Gap 8	+	GACAGCACGTGTGTATTCTTTG		This study	Sequencing
	–	CTGTGATTGAGTTGCTCGAGT			
3Pr	+	GACAGCACGTGTGTATTCTTTG		This study	Sequencing

Table 2: Sampling location, infection rate and PVY strain type for different potato cultivars to PVY in Belgian potato seed farms based on ELISA screening and RT-PCR strain typing. For nine selected isolates, the isolate ID and GenBank accession numbers are added

Location	No. of farms tested (total 54)	% Infected seed lots (ELISA)	Cultivar	Strain (RT-PCR)	Isolate ID and GenBank Accession No.
Adinkerke	1	16	Eersteling	NTN Mix (NTN & O & N-Wi)	
Amberloup	2	6	Rode eersteling	Unknown	
		4	Spunta	NTN	
Assenede	3	0	Draga	---	
		0	Draga	---	
		0	Draga	---	
Boekhoute	3	0	Marfona	---	
		0	Marfona	---	
		8	Paramount	NTN x2	
Brielen	1	0	Granola	---	
Ehein-Neupré	1	2	Spunta	NTN	
Gistel	1	0	Spunta	---	
Grimbergen	1	0	---	---	
Hoeleden	3	0	Diamant	---	
		0	Diamant	---	
		0	Diamant	---	
Hompré	2	4	Kennebec	NTN	
		10	Spunta	NTN x2	JQ969033
Kieldrecht	1	0	Granola	---	
Koekelare	1	0	Desiree	---	
Langemark-Poelkapelle	2	4	Spunta	NTN	
		0	Marfona	---	
Lo-Reninge	3	0	Diamant	---	
		0	Cara	---	
		2	Cara	NTN	
Méeffe	1	0	Diamant	---	
Milmort	4	8	Diamant	N	JQ969036
		2	Diamant	NTN	JQ969037
		2	Spunta	NTN	
		4	Spunta	NTN	
Moerbeke-Waas	6	0	Spunta	---	
		0	Spunta	---	
		0	Draga	---	
		0	Draga	---	
		0	Draga	---	
		0	Draga	---	
Roeselare	8	0	Marfona	---	
		0	Marfona	---	
		0	Spunta	---	
		10	Spunta	NTN x2	JQ969035
		14	Spunta	NTN x2	
		18	Anosta	NTN x2 & N-Wi*	JQ969040*
		10	Anosta	N-Wi	JQ969039
		2	Spunta	NTN x2	
Sommière	1	0	Spunta	---	

Table 2: (Continued)

Location	No. of farms tested (total 54)	% Infected seed lots (ELISA)	Cultivar	Strain (RT-PCR)	Isolate ID and GenBank Accession No.
Tinlot	2	14	Lady Rosetta	O x3	JQ969038
		0	Hermes	---	
Veurne	5	0	Nicola	---	
		0	Diamant	---	
		0	Charlotte	---	
		4	Nicola	N-Wi	JQ969041
		2	Charlotte	Unknown	
Vlissegem	1	6	Spunta	NTN x2	
Wulpen	1	42	Bintje	NTN x7	JQ969034

Table 3: Sequences retrieved from GenBank and used in phylogenetic analysis

GenBank Accession No.	Strains	Country
JQ969033, JQ969035, JQ969037, JQ969034, JQ969039, JQ969040, JQ969041, JQ969036, JQ969038	NTN, N-Wi, N, O	Belgium
JQ924285, JF928458, JF928459, JF928460	O, E, NTN	Brazil
AY166867, AY166866, U09509	O, N, NTN	Canada
HM590407	O	China
HM991454, AJ890348	C, N-Wi	France
HE608964, AJ889868, HE608963, AJ890346, AJ890347, AJ890350	NTN, N-Wi	Germany
JF927749, JF927761, JF927756, JF927752	NTN	Hungary
AB711154, AB711150, AB711149, AB714135	O, N	Japan
AM268435	N	New Zealand
EU563512	C	the Netherlands
JF795485, AJ890343, AJ890342, AJ889866	NTN, N-Wi, N	Poland
X97895	N	Switzerland
AB270705	N	Syria
AJ585195, AJ585198, AJ585197, JX424837	O, N	United Kingdom
HQ912892, HQ912891, HQ912897, HQ912914, HQ912913, HQ912884, HQ912883, HQ912909, HQ912879, HQ912876, HQ912878, HQ912875, HQ912874, HQ912868, HQ912896, HQ912863, HQ912871, HQ912862, HQ912872, HQ912870, HQ912869, HQ912890, HQ912893, HQ912864, FJ643478, EF026074, EF026076, FJ204166, FJ204165, FJ204164, EF026075, EF026074, AY884983, AY884984	O, O5, N-Wi, N:O, NTN, NA_PVY-N	United States of America
NC_004039	Potato virus A	Hungary
NC_004010	Potato virus V	United Kingdom
M96425, NC_001517	Pepper Mottle virus	United States of America

The obtained virtual banding patterns of the PCR products of a selection of representative PVY isolates and reference isolates (JKI, Germany) produced with the six primer pairs of the multiplex RT-PCR is presented in Fig. 2. Phylogenetic analysis of the sequences (Fig. 3) was in a good correspondence with the multiplex banding pattern, except for GBVC_PVY_15 NTN (JQ969034).

Based on the banding patterns in the multiplex PCR, three isolates in this study were identified as Wilga strains (GBVC_PVY_26 N-Wi (JQ969039), GBVC_PVY_23 N-Wi (JQ969040) and GBVC_PVY_34 N-Wi (JQ969041)). The phylogenetic analysis confirmed the attribution to the Wilga subgroup and revealed that all three isolates cluster in the N-Wi subgroup, (Nie & Singh 2003b, Karasev et al. 2011, Karasev & Gray 2013, Visser et al. 2012). However, the variability between the Wilga strain (GBVC_PVY_23 N-Wi (JQ969040) and GBVC_PVY_26 N-Wi (JQ969039)) from two infected Anosta lots from the same location (Roeselare)

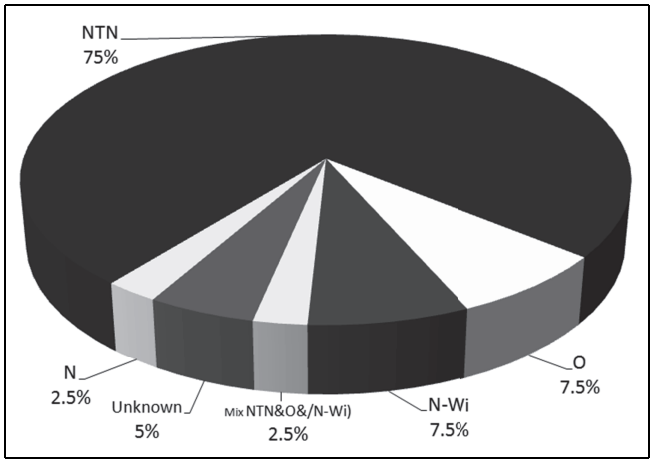

Fig. 1: Prevalence of the different PVY strains on naturally infected seed potato cultivars in Belgium as determined by multiplex RT-PCR.

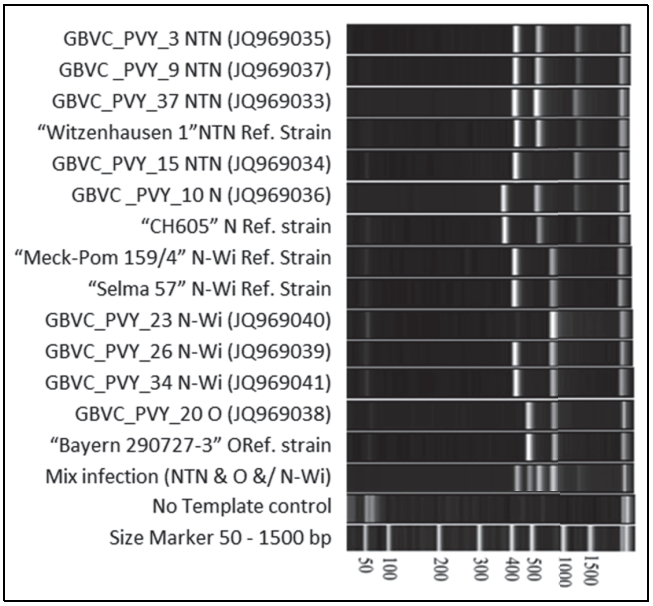

Fig. 2: Virtual banding patterns of PCR products of representative PVY isolates and standards, produced with the six primer pairs of Chikh Ali et al. (2010a). NTC: no template control. The JKI (Julius Kuhn Institute, Braunschweig, Germany) reference ID for the reference isolates is indicated between quotation marks: N-Wi2 = "Selma 57"; N-Wi3 = "Meck-Pom 159/4"; O = "Bayern 290727-3"; N = "CH605"; NTN = "Witzenhausen 1".

was also significant with both isolates clustering in different clades (Fig. 3). Additionally, the recombination pattern of all three Wilga strains was similar showing two breakpoints at RJ1 and RJ2 which is corresponding to the other N-Wi strains of which the complete sequence is available in GenBank (Fig. 4).

Four isolates that were identified as NTN strains by means of the multiplex PCR were also fully characterized. The multiplex PCR banding pattern (Fig. 2) produced two bands (441 and 1307 bp) for GBVC_PVY_15 NTN (JQ969034) and three for other NTN isolates. Based on this difference, Chikh Ali et al. (2010a) differentiated the isolates into type A (GBVC_PVY_3 NTN (JQ969035), GBVC_PVY_9 NTN (JQ969037) and GBVC_PVY_37 N_NTN (JQ969033)) and type B (GBVC_PVY_15 NTN (JQ969034)). However, based on the full genome sequence analysis (Fig. 3), the Belgian isolates were all identified as NTNa (type A) as described in the review of Karasev & Gray (2013), clustering in a major clade with other NTNa strains. The NTNb reference isolates which were retrieved from GenBank cluster in a separate small clade, also contained the NTN strain AJ889866 (Schubert et al. 2007) from Poland. Additionally, this was also confirmed by the recombination map (Fig. 4) of the NTN isolates in this study that showed three breakpoints, corresponding to RJ2, RJ3 and RJ4, and identified all of them as NTN type A after Karasev & Gray (2013).

The alleged O strain (GBVC_PVY_20 O (JQ969038)) also clustered in the same group together with O strains from US, EU and Japan that were deposited in GenBank (Fig. 3). Additionally, the suspected N (GBVC_PVY_10 N (JQ969036)) clustered with the corresponding N strains that are available in GenBank, including N Mont (AY884983), N 605 (X97895) and N New Zealand (Fig. 3). PVY^{E}, PVY^{C} strains and other potyviruses (*Potato virus A* and V and *Pepper Mottle virus*) were also included in the phylogenetic analysis and clustered significantly different from all isolates (Fig. 3).

Symptoms, strain and potato cultivar relation

The seed potato samples infected with the Wilga strain belonged to the cultivars Anosta and Nicola were collected in Veurne and Roeselare (Table 2). The most prevalent strain (NTN) was detected in the following cultivars: Spunta, Diamant, Bintje, Eersteling, Anosta, Paramount, Cara and Kennebec.

PVY-infected plants ranged from symptomless to mottling, mild and severe mosaic and leaf malformation. Tissue necrosis and stunting were only occasionally observed. Cultivar 'Spunta', produced on 21 farms, was found to be mainly infected with NTN and showed symptoms ranging from mild mosaic to leaf necrosis. N mainly caused mosaic, while O and N-Wi symptom development was more variable, ranging from symptomless to mottling, mild to severe mosaic and growth reduction on several cultivars. No clear correlation between the symptoms and the cultivars could be recorded.

Discussion

Based on the ELISA screening results, the average of PVY infection rate of 3.6% in Belgian seed potato farms in our study was slightly higher than the rate (2.4%) obtained in 2007 (Rolot 2009). In agreement with the report of Rolot (2009), our results also confirmed that the N strain group (NTN, N-Wi and N strains) is the most prevalent (89.5%) in Belgium. In accordance with published data from other

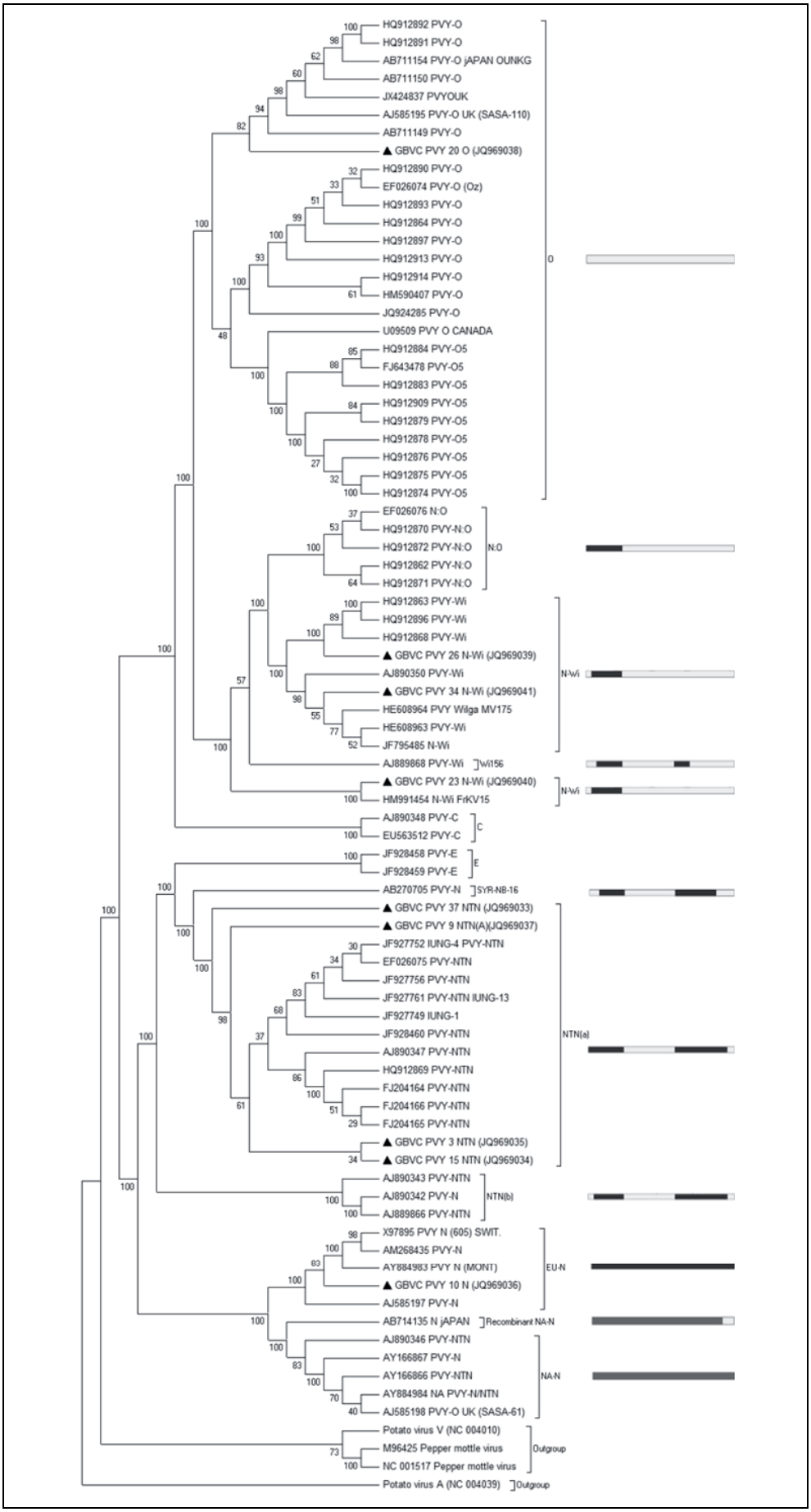

Fig. 3: Phylogenetic analysis of PVY isolates from Belgian seed potato production (this study) (▲). All reference sequences are retrieved from GenBank (http://www.ncbi.nlm.nih.gov). Statistical Methods: Neighbour-joining test, test of phylogeny (bootstrap values are indicated on the nodes), evolutionary analyses were conducted with MEGA5. The bars represent schematic recombination maps of the respective strain groups.

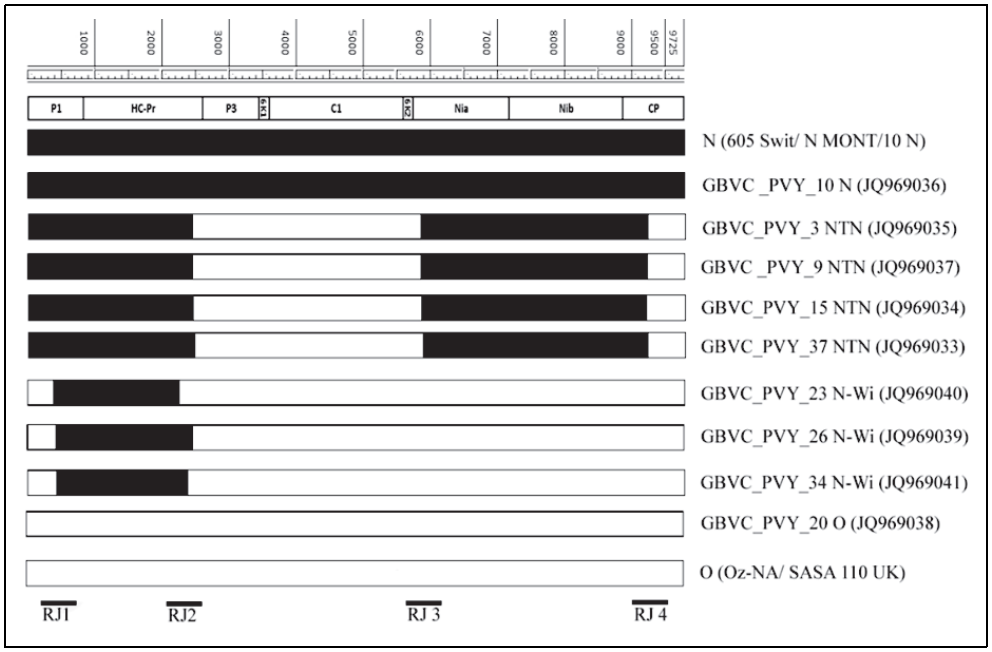

Fig. 4: Schematic recombination map of nine selected PVY isolates compiled by the RDP 4 multiple recombination detection software. RJ: recombination junction.

Fig. 5: Comparison of sequence of annealing sites of the NTN strains with relevant forward primer (n156) (Chikh Ali et al. 2010a). Base variations are indicated in black squares.

important West European potato growing areas, this study confirmed the relative importance of the NTN strain compared to the specific N strain. However, the percentage NTN strains that was detected (75%) was lower than what was recorded in Germany (Lindner 2008), the Netherlands (Verbeek 2009) and Switzerland (Rigotti et al. 2011).

The NTN population collected in Belgium did not consist of homogenous isolates based on analysis using the PCR method of Chikh Ali et al. (2010a). Presence or absence of the 633 bp amplicon is discriminative between NTN variants (A and B) and indicates the presence or absence of an extra recombinant junction (RJ1) at the 5´end. NTNb therefore starts with a small segment of the O strain (Chikh Ali et al. 2010a, Karasev & Gray 2013). GBVC_PVY_15 NTN (JQ969034) lacked this 633 bp amplicon, but sequence analysis revealed that this was rather due to a base replacement at a critical position (Fig. 5) at the annealing site with the relevant forward primer (n156) (Chikh Ali et al. 2010a), rather than a recombination on which the actual A and B typing is based (Karasev & Gray 2013). We recommend that future sequence analysis on sufficiently large fragments of the genome in the RJ regions allowing to reconstitute recombination maps, or ideally, full genome sequencing is necessary to accurately type PVY isolates.

Our results confirmed the presence of N-Wi strain in Belgian seed potatoes for the first time. In addition, the phylogenetic tree also showed that the Belgian Wilga isolates clustered in the different interior branch of the European and American Wilga isolates (Fig. 3) (Glais et al. 2002, Nie & Singh 2003b). Additional pathological and biological characterization of these isolates is necessary to further type the NTN and N-Wi populations. This would also allow researchers to further categorize the NTN strain into PVY^{NTN}-Hun (European PVY^{NTN}) and PVY^{NTN}-Tu 660 (North American PVY^{NTN}), the two NTN types identified and discussed by several research groups (Thole et al. 1993, Nie & Singh 2002, 2003a, b, Piche et al. 2004, Chikh Ali et al. 2007b, 2010b, Hu et al. 2009, 2011).

In summary, this study presented the relative distribution of the PVY strains across seed potato lots in Belgium. We could conclude that NTN is the most dominant strain in all parts of Belgium and that the NTN isolates belong to the NTNa type. Additionally, the presence of the Wilga strain was confirmed and genetically fully characterized for the first time in Belgium. All Wilga strains belong to the N-Wi subgroup and no N:O variants were identified. The other strains, O, and PVY-N, appeared less important and were also more restricted in their geographical distribution. No PVY^{C}, PVY^{E} or PVY^{Z} was put in evidence.

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