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Incidence of potato viruses and characterisation of *Potato virus Y* variability in late season planted potato crops in Northern Tunisia

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Abstract Surveys were conducted of symptomatic potato plants in late season crops, from the major potato production regions in Northern Tunisia, for infection with six common potato viruses. The presence of *Potato leafroll virus* (PLRV), *Potato virus Y* (PVY), *Potato virus X* (PVX), *Potato virus A* (PVA), *Potato virus S* (PVS) and *Potato virus M* (PVM) was confirmed serologically with virus infection levels up to 5.4, 90.2, 4.3, 3.8, 7.1 and 4.8%, respectively. As PVY was prevalent in all seven surveyed regions, further biological, serological and molecular typing of 32 PVY isolates was undertaken. Only one isolate was shown to induce PVY^{O}-type symptoms following transmission to tobacco and to react only against anti-PVY^{O-C} antibodies. Typical vein necrosis symptoms were obtained from 31 samples, six of which reacted against both anti-PVY^{N} and anti-PVY^{O-C} antibodies showing they contained mixed isolates, while 25 of them reacted only with anti-PVY^{N} antibodies. An immunocapture RT-PCR molecular test using a PVY^{NTN} specific primer pair set in the 5'NTR/P1 genomic region and examination of recombinant points in three genomic regions (HC-Pro/P3, CI/NIa and CP/3'NTR) showed that all 25 serotype-N PVY isolates were PVY^{NTN} variants with similar recombinations to the standard PVYNTN-H isolate. This is the first report of the occurrence of the PVY^{NTN} variant and its high incidence in late season potatoes in Tunisia.

Keywords Late season crop · Potato viruses · PVY variability · Recombinant PVY^{NTN} · Tunisia

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Introduction

In Tunisia, potato is one of the most important food crops with 365,000 tons produced in 2006–2007 (Economic balance 2008). It is grown as three distinct, though partly overlapping, seasonal crops

(Azzouz 1996). The early season crop is harvested in February–March and is located in frost-free coastal regions. The main season crop harvested in May–June and the late autumn season crop (36% of the total production) harvested in December–January, both grown on similar acreages in coastal and irrigated areas throughout the country (Khamassy 1999). About 46000 tons of potato seed is used per year of which almost the half is imported from Europe. Around 20% of late potatoes are planted from local certified seed potatoes provided by GIL (Groupement Interprofessionnel des Légumes: Vegetable Interprofessional Group) and around 80% are produced from mother tubers saved by farmers from tubers harvested in the previous main-season. These farm-saved potato seeds are known to have significant levels of virus. Spunta is the prevalent cultivar with 75 % of the planted acreage. Other cultivars such as Nicola, Liseta, Atlas, Safrane and Pamina occupy 25% of the planted acreage (Khamassy 1999).

Potato is susceptible to 36 viral species (Kerlan 2008) of which 25 occur more commonly, and cause reduction of tuber yield and number of quality tubers (Valkonen 2007). Among the most common viruses affecting potatoes, *Potato leafroll virus* (PLRV, genus *Polerovirus*), *Potato virus X* (PVX, genus *Potexvirus*), *Potato virus A* and *Potato virus Y* (PVA, PVY, both genus *Potyvirus*), *Potato virus S* and *Potato virus M* (PVS, PVM, both genus *Carlavirus*) are known to be the most economically damaging to potato crops worldwide (Kerlan 2008). In Tunisia, these viruses cause yield losses ranging from 8 to 80% (Azzouz 1996). They are transmitted either by aphids in a non-persistent manner (PVY, PVA, PVM and PVS), or in a persistent manner (PLRV), or by plant-to-plant contact (PVX and PVS).

Among potato viruses, PVY is one of the most important causing serious losses in potato and other susceptible crop species including other *Solanaceae* family members (tomato, pepper and tobacco) worldwide (Valkonen 2007). In Tunisia, it was identified as a major problem in seed potato multiplication and production causing approximately 35% yield losses in crops planted from local self-produced potato seed (Khamassy 1999). PVY has a large diversity of strains and variants, linked to its biological, serological and molecular properties. Three main PVY potato strain groups have been identified according to their ability to induce (i) systemic veinal necrosis symptoms on tobacco (*Nicotiana tabacum*) leaves and very mild mottling with occasional necrotic leaves on potato (PVY^N strain group) or (ii) mottling and mosaic symptoms on tobacco plants and mild to severe mosaic and leaf drop on potato (PVY^O strain group) or (iii) mottling and mosaic symptoms on tobacco plants and stipple streak symptoms on some potato cultivars (PVY^C strain group) (de Bokx and Huttinga 1981). Over the last two decades, the emergence and the spread of the PVY^N-W, PVY^{NTN} and $PVY^{N:O}$ variants have caused serious concerns for potato growers (Kerlan 2008). PVY^N-W and $PVY^{N:O}$ variants have biological properties close to those of PVY^N, while PVY^{NTN} variants are responsible for the potato tuber necrotic ringspot disease (PTNRD) (Le Romancer et al. 1994). This disease is now well-known in most countries cultivating potatoes (Kerlan 2008). Recent surveys for PVY in potato in Germany (Lindner and Billenkamp 2005) showed that almost 90% of infections were caused by PVY^{NTN} or PVY^N-W variants. In 2005, PTNRD symptoms were observed in late season potatoes in Southern Tunisia (Boukhris-Bouhachem et al. 2008).

Emergence of these variants has highlighted the limitation of the available serological tools since they are unable to distinguish between PVY^N-W and PVY^O isolates (serotype O) and between PVY^{NTN} and PVY^N isolates (serotype N) (Singh et al. 2008). As an alternative to serological assays, numerous specific and sensitive molecular approaches were developed for PVY characterisation group and variant identification such as RFLP mapping (Glais et al. 2002), immunocapture RT-PCR (Glais et al. 2005), uniplex and multiplex RT-PCR (Nie and Singh 2002). Most PVY^{NTN} and PVY^N-W isolates have a recombinant genome with exchange of sequences in the HC-Pro (PVYNTN and PVYN-W), NIa and CP genes (PVY^{NTN}) between PVYN and PVYO sequence types (Glais et al. 2002). Such sequence exchanges were used for specific detection of PVY^N-W and PVY^{NTN} isolates by specific RT-PCR (Glais et al. 2001; Glais et al. 2005) and they were used in this study.

In this study we surveyed potato plants and identified the common viruses from seven growing areas in Northern Tunisia during autumn (late season) 2005. We then characterised the diversity of PVY in late season- potato crops in Tunisia.

Materials and methods

Plant material

One hundred and eighty four leaf samples were collected from potato plants showing symptoms associated with virus infection (leaf mosaic, mottle, necrosis, rugosity and/or plant dwarfing). Samples were collected from seven regions: Bizerte (49 samples), Cap Bon (32 samples), Manouba (22 samples), Jendouba (19 samples), Kairouan (12 samples), Monastir (23 samples) and Mahdia (27 samples) (Fig. 1) during the late season crop in autumn 2005. Totals of 125, 51, 4 and 4 samples were collected from potato cultivars Spunta, Nicola, Atlas and Pamina, respectively. The presence of aphids and weeds was especially noted in Bizerte and Monastir.

Standard PVY isolates

The Tunisian PVY isolates were compared to a collection of five well characterised isolates representative of PVY strains and variants: PVY^{N} (B203), PVY^{NTN} (-H and NZ), PVY^{N}-W (-iP) and PVY^{O} (Irl). All these isolates were previously described (Table 1) and were maintained during the experiment period on *Nicotiana tabacum* cv. Xanthi.

Serological testing

Each isolate was tested by Double Antibody Sandwich-ELISA (DAS ELISA) (Clark and Adams 1977) using anti-PVY, anti-PVX (INRA-FNPPPT, Le Rheu, France), anti-PLRV, anti-PVA and anti-PVS (Bioreba, Basel, Switzerland) polyclonal antisera. PVY isolates

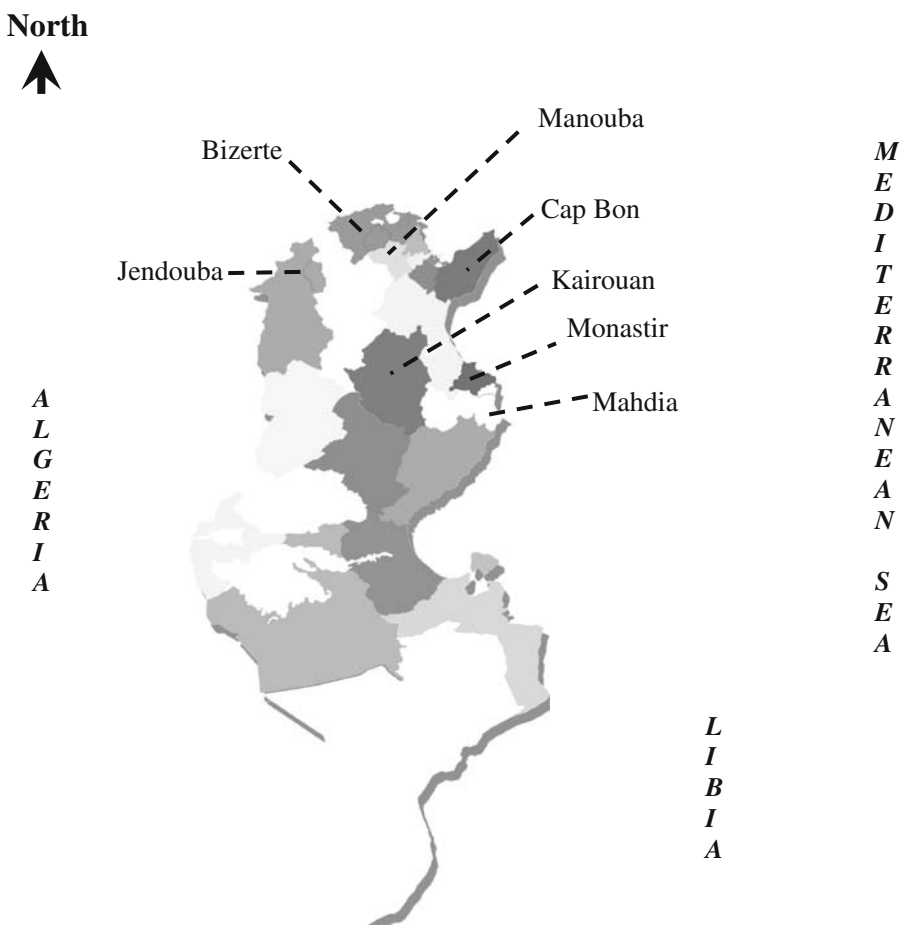

Fig. 1 Geographic origin of the 184 collected leaf potato samples

Table 1 Characteristics of the five standard PVY isolates

Isolate		Origin	Pathotype[a]	Serotype[b]	Source	Recombination sites
Type	Reference					
PVY^{O}	Irl	Ireland	N	N	Glais et al. 1998	
PVY^{N}	B203	France	N	N	Glais et al. 1998	
PVY^{NTN}	-H	Hungary	N	N	Gibbs et al. 2000	HC-Pro/P3, CI/NIa CP/3'Ntr
PVY^{NTN}	NZ	New-Zealand	N	N	Glais et al. 2005	
PVY^{N}-W	i-P	Poland	N	O-C	Glais et al. 1998	HC-Pro/P3

[a] Type of symptoms induced in *Nicotiana tabacum*

[b] Serotype N or O-C determined by using specific antibodies

N veinal necrosis, *O* mosaic

were further tested using anti-PVY^{N} (Bioreba and INRA-FNPPPT) and anti-PVY^{O-C} (Adjen, Ayr, UK) specific monoclonal antibodies.

Microtitre plates (Nunc-immuno plate MaxiSorp surface Polylabo) were used. Absorbance values of alkaline phosphatase-substrate reactions were read at 405 nm using a microplate reader (Molecular Devices, Palo Alto, USA in the Tunisian laboratory and Multiskan in the French laboratory).

Biological typing

Seedlings (three-leaf stage) of *N. tabacum* grown were mechanically inoculated with PVY extracts as previously described (Le Romancer et al. 1994). PVY^{N} is known to induce a veinal necrosis with necrosis with smaller distorted leaves and dwarfing of the whole plant in tobacco cv Xanthi; whereas PVY^{O} and PVY^{C} induce only vein banding or interveinal clearing without any necrosis, crinkling or dwarfing (Kerlan 2008). The inoculated plants were kept in a temperature regulated (20°C±2°C) insect-proof greenhouse. Symptoms were recorded weekly for 5 weeks.

Molecular typing

Primer sequences and their location are described in Table 2. The specific detection of both recombinant

Table 2 Sequence and genomic location of primer pairs used

primers		Sequence 5'–3'	Genomic location[a]	PCR fragment length (bp)	References
RT	d	TG(CT)GA(CTA)CCACGCACTATGAA	955-974		Glais et al. 1996
	$3'^{NTR}C$	GTCTCCTGATTGAAGTTTAC	9684-9703		Glais et al. 1998
PCR	FR_{2000}/F_2	TCAAACTCTCGTAAATTGCAGA	159-180		Glais et al. 2001
	d	TG(CT)GA(CTA)CCACGCACTATGAA	955-974	815	Glais et al. 1996
	$_{1227/46}Y^{N}F$	CTTGCCAGCCAGTGACTTAC	1227-1246		Glais et al. 2001
	$_{2547/68}Y^{O}R$	CAACA(GA)GTAAGGCTCATCTAAC	2547-2568	1341	Glais et al. 2001
	$_{5606/27}Y^{O}F$	TGGAA(GC)AA(AG)TCATTAGTGGC(CT)A	5606-5627	761	Glais et al. 2001
	$_{6343/67}Y^{N}R$	TCCATATTCA(AG)CAGACACTTTTACT	6343-6367		Glais et al. 2001
	$_{9132/51}Y^{N}F$	TCGTAATCTGCGCGATGGAA	9132-9151	368	Glais et al. 2001
	$_{9481/00}Y^{O}R$	CCCTGCCACCTCTATCTATT	9481-9500		Glais et al. 2001

[a] Location relative to the sequence of PVY^{NTN}-H (Thole et al. 1993)

and non-recombinant PVY^{NTN} variants was obtained by using the primer pair FR_{2000}/F_2-d based on the nucleotide sequence polymorphism observed in the 5'NTR/P1 region (Glais et al. 1996). The expected amplification fragment from this primer combination will be 815 bp in length. Specific detection of recombinant PVY^{NTN} isolates was obtained by using three primer pairs targeting the three recombination sites observed in the NTN genome within the HC-Pro/P3 region (primer pair $_{1227/46}Y^NF/_{2547/68}Y^OR$), CI/NIa region (primer pair $_{5606/27}Y^OF/_{6343/67}Y^NR$) and CP/3'NTR region (primer pair $_{9132/51}Y^NF/_{9481/00}Y^OR$) (Table 3) (Glais et al. 2002). The expected amplification products for these primer combinations are 1341, 761 and 369 bp, respectively (Table 2). The primers $_{1227/46}Y^NF$, $_{6343/67}Y^NR$ and $_{9132/51}Y^NF$ have an N-type nucleotide sequence; the primers $_{2547/68}Y^OR$, $_{5606/27}Y^OF$ and $_{9481/00}Y^OR$ have an O-type nucleotide sequence.

Immunocapture RT-PCR was used in this study as modified by Nolasco et al. (1993). Plant tissues were ground (1: 10 w/v) in 1 ml PBS-0.05% Tween using a roller press. About 50 µl of this homogenate were then added to each well of a plate previously coated with 50 µl of PVY polyclonal antibody diluted 1/1000 in carbonate buffer pH 9.6. After overnight incubation at 4°C, the plate was washed three times with PBS-T. Reverse transcription was carried out in the plate by adding 20 µl of the reverse transcription mixture containing 1X reverse transcriptase first-strand buffer (50 mM Tris-HCl pH 8.3, 30 mM KCl, 6 mM MgCl2 and 10 mM DTT) (Promega), 1 mM of each dNTPs (Promega), 20 units of RNasin Ribonuclease Inhibitor (Promega), 2.5 mM MgCl2, 1 µm of the $3'^{NTR}C$ or d primers and 2 units of AMV reverse transcriptase (Promega) to each well. After 1 h incubation at 42°C, first cDNA strands of the potentially complete (with $3'^{NTR}C$ primer) or partial (with d primer) genome of PVY were generated. About 5 µl of the reverse transcription product was added to 25 µl PCR mixture containing 1X PCR buffer (Promega) (10 mM Tris-HCl (pH 8.3), 50 mM KCl and 0.5 MgCl2), 0.2 mM each dNTPs, 0.4 µM of each primer, 2 mM MgCl2 and 1.25 units of the DNA polymerase Go Taq (Promega). For all primer pairs, the amplification reactions in a Perkin Elmer Gene-Amp PCR system 9600 were carried out using the following program: 3 min initial denaturation at 94°C, 30 cycles of 1 min denaturation at 94°C; 1 min annealing at 57°C, 1 min extension at 72°C and 10 min final extention at 72°C. PCR products were analysed using electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Results

Serological testing

One hundred and eighty four potato leaf samples were tested. One hundred and sixty six (90.2 %), 8 (4.3 %), 7 (3.8 %), 9 (4.8 %), 13 (7.1 %) and 10 (5.4 %) reacted with polyclonal antibodies anti-PVY, -PVX, -PVA, -PVM, -PVS and -PLRV, respectively (Table 3). Multiple infections of two viruses, three, four, five or six viruses were found in 41 samples (22 % co-infection). Twenty-three out of 166 samples infected by PVY were found to be co-infected with other viruses, especially with the PVX. Only one case each of coinfection by 5 viruses (PVY+X+S+M+PLRV) and by all six viruses was found.

Characterisation of a PVY isolates

Successful infection occurred for only 32 of the 55 PVY isolates inoculated to tobacco plantlets (Table 4).

Table 3 Incidence of common potato viruses in the late planted potatoes in Northern Tunisia

	Bizerte	Cap Bon	Mannouba	Jendouba	Kairouan	Monastir	Mahdia	Total Nb	%
PVY	39	32	19	18	12	23	23	166	90.2
PVX	2	0	0	2	3	1	0	8	4.3
PVA	3	1	0	0	0	3	0	7	3.8
PVM	4	0	0	0	0	5	0	9	4.8
PVS	2	7	2	0	1	1	0	13	7.1
PLRV	1	5	1	0	0	3	0	10	5.4

1. Biological typing Thirty one out of 32 inoculated plantlets of *Nicotiana tabacum* cv. Xanthi displayed vein necrosis, interveinal chlorosis and leaf distortion associated with plant dwarfing, as did standard PVY^{N} and PVY^{NTN} isolates. PVY^{O}-type symptoms, i.e. typical mottle without distortion of the tobacco leaves, were obtained after inoculation from only one potato sample.
2. Serological typing Positive reactions with both anti-PVY^{O-C} and PVY^{N} antibodies were obtained from six PVY isolates that induced necrotic symptoms on tobacco, whereas the other 25 isolates reacted against anti-PVY^{N} antibodies only. Plantlets showing PVY^{O}-type symptoms inoculated from one potato sample reacted against anti-PVY^{O-C} antibodies but not against anti-PVY^{N} antibodies.

Molecular typing

Combination of both biological and serological testing showed that 6/32 isolates were infected by a mixture of PVY^{O} (or $PVY^{N}W$) and PVY^{N} (or PVY^{NTN}) isolates, 1/32 samples was infected by PVY^{O} isolates, while 25/32 were infected by isolates being either PVY^{N} or PVY^{NTN} isolates. Molecular typing was performed on these 25 isolates in order to check the presence of PVY^{N} isolates and/or recombinant and non-recombinant PVY^{NTN} isolates (Table 4).

Based on the polymorphism in the 5'NTR/P1 region, the selected primer pair (FR_{2000}/F_2-d) gave an amplification product of the expected size (around 800 bp) from all 25 Tunisian isolates tested as well as from the PVY^{NTN}-H and the PVY^{NTN}-NZ reference

Table 4 Combination of biological, serological and molecular typing of the first set of the 55 PVY isolates

Origin	Number of isolates	Potato cv	PVY pathotype[a]	PVY serotype	IC-RT-PCR amplification of the 5'NTR/P1 polymorphic region	IC-RT-PCR amplification of the HC-Pro/P3, CI/NIa and CP/3'NTR recombination sites
Jendouba	4	Pamina	N	N	+	+
	5	Spunta	N	N	+	+
	2	Nicola	N	N	+	+
	1	Spunta	NI	−	NT	NT
	3	Nicola	NI	−	NT	NT
	1	Nicola	O	O	NT	NT
Kairouan	2	Nicola	N	N	+	+
	1	Atlas	N	N	+	+
	2	Spunta	N	N	+	+
	3	Nicola	NI	−	NT	NT
Manouba	3	Spunta	N	N	+	+
	1	Nicola	N	N	+	+
	6	Spunta	NI	−	NT	NT
	1	Nicola	NI	−	NT	NT
	2	Spunta	N	N/O	NT	NT
Monastir	1	Spunta	N	N	+	+
	1	Spunta	NI	−	NT	NT
Mahdia	1	spunta	N	N	+	+
	2	Spunta	N	N/O	NT	NT
Cap bon	1	Nicola	N	N	+	+
	5	Nicola	NI	−	NT	NT
Bizerte	2	Spunta	N	N	+	+
	3	Spunta	NI	−	NT	NT
	2	spunta	N	N/O	NT	NT

[a] Type of symptoms induced in *Nicotiana tabacum*

N pathotype or serotype of PVY^{N}-type (veinal necrosis), *O* pathotype or serotype of PVY^{O}-type (mosaic), *NI* non infection, *N/O* coinfection with both Y^{O} and Y^{N} serotypes, + positive test, −: negative test, *NT* not tested

Fig. 2 1.5 % agarose gel electrophoresis analysis of the 5'NTR/P1 region amplified by immunocapture RT-PCR using primer pairs "d" and "FR_{2000}-F_2" for the specific PVY^{NTN} detection. *1*: healthy tobacco sample, *2*: PVY^{O} Irl, *3*: PVY^{N}B203; *4*: PVY^{NTN}-H, *5* PVY^{NTN}-NZ; *6*: PVY^{N}Wi-P, *L* Smart Ladder (Eurogentec), *7–13*: Tunisian PVY^{N} isolates

isolates (Fig. 2). No amplification was obtained, either from the negative control sample, or from the PVY^{O}Irl, PVY^{N}B203 and PVY^{N}Wi-P standard isolates.

Secondly, evidence for recombination events typical of numerous PVY^{NTN} isolates was sought in the genome of these 25 PVY^{NTN} isolates, by using specific primers flanking the three HC-Pro/P3, CI/NIa and CP/3'NTR recombination sites (Fig. 3). About the HC-Pro/ P3 recombination region, the selected primer pairs gave an amplification product of the expected size (roughly 1400 bp) from all Tunisian PVY^{N} isolates as well as from the PVY^{NTN}-H and PVY^{N}W-iP standard isolates (Fig. 3a). No amplification was obtained, either from the negative control sample, or from the PVY^{O}Irl, the PVY^{N}B203 and the PVY^{NTN}-NZ standard isolates. Similarly, the specific primer pairs for the CI/NIa and the CP/3'NTR recombination sites gave the amplification products of the expected sizes (approximately 760 bp and 360 bp, respectively) from all Tunisian PVY^{N} isolates and from only the PVY^{NTN}-H standard isolate (Fig. 3b and c). No amplification was obtained either with the negative control sample, or with the other standard PVY isolates.

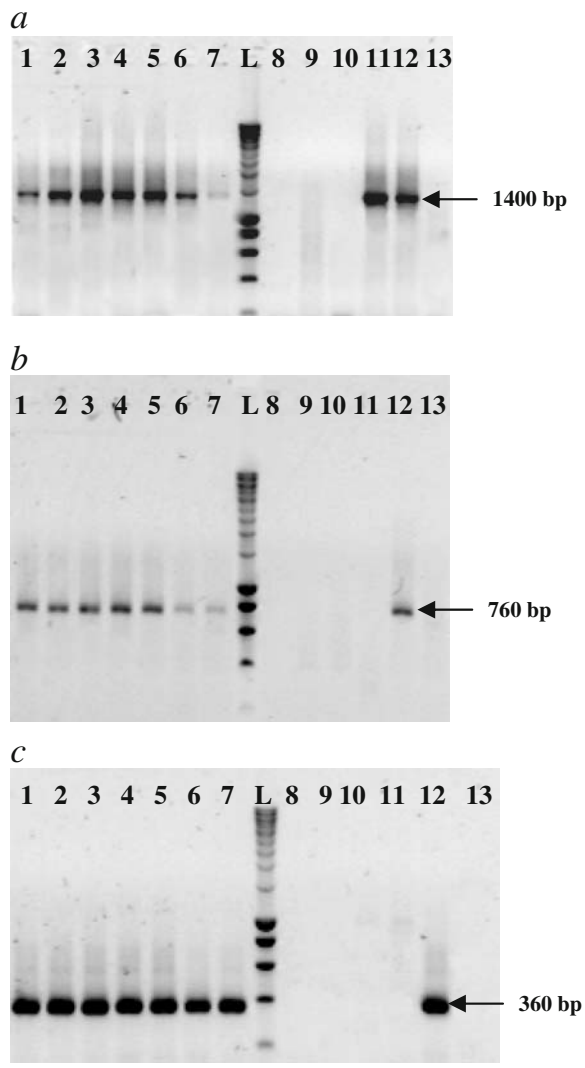

Fig. 3 1.5 % agarose gel electrophoresis analysis of the HC-Pro/ P3 (**a**); the CI/NIa (**b**) and the CP/3'NTR (**c**) recombination sites amplified by immunocapture RT-PCR using "$_{1227/46}Y^{N}F$" / "$_{2547/68}Y^{O}R$", "$_{5606/27}Y^{O}F$" / "$_{6343/67}Y^{N}R$" and $_{9132/51}Y^{N}F$" / "$_{9481/00}Y^{O}R$" primer pairs, respectively. *1–7*: Tunisian PVY^{NTN} isolates; *L*: Smart Ladder (Eurogentec); *8*: healthy tobacco control; *9*: PVY^{O} Irl; *10*: PVY^{N}B203; *11*: PVY^{N}Wi-P; *12*: PVY^{NTN}-H; *13*: PVY^{NTN}-NZ

Discussion

Virus occurrence

A limited detection of virus other than PVY was noted. This could be attributed to a limited spread of these other viruses, due to either the lack of vectors, i.e. of aphids, or the lack of winter alternate hosts. However such an hypothesis seems unfounded given the presence of various weeds around and even frequently within potato plots and mild conditions that may have allowed aphids to survive during winter on various alternative hosts.

Results of this study regarding incidence of the six main potato viruses have been more or less in agreement with those of similar surveys previously

conducted in Tunisia (Jendoubi 2001, personal communication). These previous studies showed virus infection levels of 50, 8, 3 and 3 % with PVY, PVS, PVA and PVM, respectively. However, greater infection levels with PLRV (20%) and PVX (26 %) were reported in 2001 (Jendoubi, personal communication). The apparent decrease in incidence of PVX and PLRV warrants further investigation. Such a trend, leading to high prevalence of PVY and low levels of other viruses, is in close agreement with the situation reported in numerous potato growing areas in the world, especially in western Europe (Valkonen 2007) and southern America (Salazar et al. 2000). The frequency of co-infections by many viral species in Tunisia (22% in our study; 26% found by Jendoubi in 2001) could be due to the fact that some potato varieties have been cultivated for many successive generations, which create conditions favouring virus buildup.

Several factors may have contributed to the widespread distribution of PVY in Tunisia. The large range of aphid species present in Tunisia able to transmit this virus (Boukhris-Bouhachem et al. 2007) and also the presence of various PVY host plants, either weeds or cultivated, most of which are overwintering under Tunisian climatic conditions. It may also be explained by the absence of well-established systems for multiplication and distribution of high quality seed potatoes. Indeed the farmers frequently resort to locally-produced seeds selected from tubers of ware potatoes, either the smallest ones or those without any marketable value. Such a practice inevitably results in spread of viral infections within a potato growing area and even from one region to another.

The high prevalence of PVY, in all surveyed fields in Tunisia and in all collected potato cultivars, is of great concern. Indeed PVY is ranked among the six most important potato viruses in terms of worldwide yield reduction and is currently listed as the most economicallysignificant potato virus (Valkonen 2007). Furthermore, PVY appears as a major threat in Tunisia, since it was recently found in Southern Tunisia to be associated with PTNRD, i.e. to the presence of necrotic lesions on tubers making them unmarketable for export, fortunately only on cv Monalisa which is not widely cultivated (Boukhris-Bouhachem et al. 2008). Recent outbreaks of this disease in the last twelve years have occurred in France (Crouau and Gokelaere 1997), Italy (Tomassoli and Lumia 1998), Greece (Bem and Varveri 1999), Japan (Ohshima et al. 2000), Peru (Salazar et al. 2000), USA and Canada (Crosslin et al. 2002).

Characterisation of PVY isolates

The characterisation of our PVY isolates was focused on the detection of the PVY^{NTN} variant responsible of PTNRD (Le Romancer et al. 1994). This variant cannot be detected by simple serological or indexing tests, but numerous molecular detection assays have been reported, often based either on polymorphism in one protein (Glais et al. 1996; Boonham et al. 2002), or on sequence exchanges in the whole genome (Glais et al. 2002; Schubert et al. 2007). In this way, this study explored the polymorphism and recombinant nature in four genomic regions (5'NTR-P1; HC-Pro/P3, CI/NIa and CP/3'NTR) by using the immunocapture RT-PCR diagnostic method (Glais et al. 2001) to allow a specific detection of the PVY^{NTN} variant and to check the presence of the three recombination points. Our results showed the high incidence, in all regions examined and for all tested cultivars (Nicola, Pamina, Atlas and Spunta as well), of PVY isolates with recombination patterns typical of the recombinant PVY^{NTN} strain (Table 4). All the 25 Tunisian isolates tested gave PCR products with sizes identical to those produced by the reference PVY^{NTN}-H isolate (Thole et al. 1993) which displayed a recombinant genome with exchanges of sequences in the HC-Pro, NIa and CP genes between PVY^{N} and PVY^{O} (Glais et al. 2002). Indeed non-recombinant PVY^{NTN} isolates such as the PVY^{NTN}-NZ isolate found in New-Zealand are not frequent (Boonham et al. 2002).

Very few of our isolates belonged to the PVY^{O} group and this finding is in agreement with other studies showing the spread of the PVY^{N} group during the last decade such as in Poland (Chrzanowska 1991), Spain (Blanco-Urgoiti et al. 1998), France (Kerlan et al. 1999), United States (Crosslin et al. 2002), Canada (Nie and Singh 2002) and Germany (Lindner and Billenkamp 2005).

It should be stressed that the $PVY^{N}W$ variant has never been reported in late potato crops in Tunisia, but is of concern in seed potato production, inducing more or less symptomless infections and being not readily detectable in field inspections (Chrzanowska 1991; Lindner and Billenkamp 2005). Thus, a specific

detection of this variant must be assessed in Tunisia using specific primers (Glais et al. 2005).

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