Journal of Research in Biological Sciences, 03 (2018) 63-69
p- ISSN: 2356-573X / e-ISSN: 2356-5748

www.knowledgejournals.com

Research article

NGS Data screening of potato virus Y infection: The near-complete sequence of Tunisian PVY isolate characterized from potato leaves sample

Ahmed Ben Hafsa[a,b], Nesrine Nabi [a,b], Mohammed Salem Zellama [a,b] and Maher Chaouachi [2]

[a]*Laboratoire de Génétique, Biodiversité et valorisation des Bio-ressources, Institut Supérieur de Biotechnologie de Monastir, Université de Monastir, 5000 Monastir, Tunisie*
[b]*Laboratoire de Recherche "Bioressources": Biologie Intégrative & Valorisation, Institut Supérieur de Biotechnologie de Monastir, Université de Monastir, 5000 Monastir, Tunisie*
**Corresponding authorEmail: benhafsa.ahmed@gmail.com; Phone number: +216 52 83 08 95*

Article history:
Received December 2017; Received in revised form: January 2018.
Accepted: Jabuary 2018; Available online: April 2018.

Abstract

Potato is one of the most important crops growing in Tunisia. Potato virus Y (PVY) has been reported as one of the major viruses touching these crops. In this study, the nearly full-length nucleotide sequence of one Tunisian PVY isolate was determined using next generation sequencing (NGS). The genome of Tunisian PVY isolate was 9,657 nucleotides long encoding all potyviral cistrons. Phylogenetic analysis showed that the Tunisian isolate clustered with European recombinant NTN isolates. To the best of our knowledge, our analysis provides the first demonstration of genomic structure of Tunisian PVY strain and highlights the importance of recombination in the evolution of PVY. NGS Data screening of PVY infecting potato sample revealed a huge diversity of putative viral genomic sequences. More than 90% of the assembled contigs shared less than 70% identity with genomes from known mycoviruses species according to the BLASTx results, suggesting that they probably represent new viral species in these families. Interestingly, known viral infection like as PVY could curiously hide other unknown's agents which could be the subject of further study on mycoviruses.

Keywords: Potato virus Y, NGS, Genome, Recombination, Tunisia

1. Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop in Tunisia, planted on about 24.280 ha with a production of about 390.000 t (FAOSTAT Database Results, 2014). Although it is grown as three season crops, which the big part of production correspond to the main and late seasons (Khamassy, 1999). *Potato virus Y* (PVY; genus *Potyvirus*, family *Potyviridae*) firstly described by Smith (1931) is one of the most important viruses infecting potato, tobacco, and pepper (De Bokx and Huttinga, 1981). It is reported as one of the most detrimental potato viruses causing major yield losses for potato production worldwide (Gray et al., 2010) and also in Tunisia (Djilani-Khouadja et al., 2010).

There are more than thirty five recombinant PVY types reported by Green et al. (2017), which include nine relatively common recombinants found in many geographical locations, namely $PVY^{N:O}$, PVY^{N-Wi}, PVY^{NTNa}, PVY^{NTNb}, PVY-NE11, PVY^{E} , and PVY-SYR-I, -II, and -III, and ten rare recombinant types found and reported only once or twice, namely PVY^{N-Wi} -156var, $PVY^{N-Wi-261-4}$, PVY-SCRI-N, PVYFrN, PVY-Nicola, PVY-T13, PVY-nnp, NY110001, AL100001 and ND23 (Green et al., 2017 a). Later, the number of rare or novel recombinants was increased to 25 that were identified from a variety of hosts in 34 countries. Ten of which (1104, 1107, FZ10, SD1, GBVC_PVY_9, GBVC_PVY_23, 9703_3, E30, N_Nysa and FrKV15) had not been recognized previously. Genomic study of PVY isolates showed that several of them have recombinant genomes from only two "parental" genomes, PVY^{O} and PVY^{Eu-N}, with a small minority including segments from other parents, e.g. PVY^{C}, PVY^{NA-N}, and a segment of the recombinant strain PVY-NE11 (Green et al., 2017 b).

The disease induced by PVY is now well-known in Tunisia (Boukhris-Bouhachem et al., 2010) and in most countries cultivating potatoes (Kerlan et al., 2011). Research on PVY has interestingly shown the occurrence of the PVY^{NTN} variant and its high incidence in late season potato growing areas of the central and the Northern of Tunisia (Larbi et al., 2012; Djilani-Khouadja et al., 2010). Hereafter surveys conducted revealed the high prevalence of PVY with an average incidence of 80.2%, and the predominance of the necrotic PVY^{NTN} variants (~ 67%) in Tunisian potato crops (Tayahi et al., 2016). Analysis of the molecular evolutionary history of viruses is very composite as they require interpreting diversity caused by mutation, recombination, selection pressure and adaptation (Gibbs et al., 2017). These studies facilitate to know critical aspects of viral biology like switch in virulence and geographical area that result in 'emergence' of recent epidemics. Indeed, knowledge on this virus facet is essential to conduct control procedures in order to hinder their expansion. Previous researches have spotlighted on etiology, pathogenesis, ecology, molecular biology and control of the virus (Hosseini et al., 2011; Pourrahim et al., 2007); nevertheless, there are still lacuna in our information in terms of genetic diversity and population structure exclusively at whole genome level in Tunisia. The objectives of our study were to characterize and to determine whether Tunisian isolate represent novel types or share common origins with strain variants reported elsewhere. Here we report the sequencing and characterization of one such isolate.

2. Materiel and Methods

2.1 Identification of PVY infection

PVY isolate Tun_144 was from our laboratory collection. It was obtained from a potato Spunta sample showing typical symptoms of viral infection: dwarfing, yellowing and leaf deformation. It was collected in April 2013 during the main season from a farmer's field in Sahel province. It was identified as a PVY isolate based on DAS-ELISA using specific monoclonal antibodies (*Bioreba, Basel, Switzerland*) and RT-PCR profile. PCR amplification was carried out as described by Svanella-Dumas et al. (2013).

The PVY primers used (PVY-F 5'-CATCGATTATGGCAAATGATACAA-3' and PVY-R 5'-TGTCGACATTCACATGTTCTTGACTCC-3') were designed from an alignment of PVY sequences available in GenBank. The following thermal cycling conditions were used: an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 1 min and a final 10 min extension step at 72°C. Reaction products were analyzed by non-denaturing electrophoresis on 1% agarose gels, stained with ethidium bromide and visualized under UV light. The sequence of the uncloned amplicons was obtained by direct Sanger sequencing (GATC Biotech AG).

2.2 Sequencing

Viral double-stranded RNAs (dsRNAs) were extracted from dried potato leaves following the method of Valverde et al. 1986, modified by Gentit et al. 2001. cDNA synthesis and PCR tagging for NGS sequencing were performed as described by Candresse et al. 2013. The PCR products were sequenced, in multiplex using Illumina sequencing-by-synthesis with 2x150 bp paired-end protocols. The library was prepared using the TruSeq Rapid SBS Kit v2 (*Illumina Inc., San Diego, CA, USA*). DNA quantity and quality were validated and monitored by qPCR following the instructions of KAPA Library Quantification Kits (*KAPA Biosystems, Woburn, USA*). Sequencing was performed with an Illumina HiSeq Benchtop Sequencer. After demultiplexing the reads, CLC Genomics Workbench 8.0 (CLCGW) (http://www.clcbio.com) was used for *de novo* assembling. Scaffolds were annotated by BlastX and

BlastN comparison with GenBank database, using a 10^{-3} e-value cut-off and then used for further extension by mapping steps.
The nearly whole genome sequence was deposited in the GenBank database under the accession number MG696820.

2.3 Sequence, phylogenetic and recombinant analysis

In total, 43 representative PVY whole genome sequences, extracted from GenBank, were aligned with the nearly complete genome of Tunisian PVY-Tun_144. ClustalW was employed with default parameters for the multiple sequence alignment. The phylogenetic relationships of the aligned sequences were inferred by the maximum likelihood method (ML). The best-fit model of nucleotide substitutions was determined according to the best DNA models program. The bootstrap method based on 1000 pseudo replicates was used to evaluate the branch support. All these tools are implemented in MEGA 7 program (Tamura et al., 2013).
For recombinant analysis, 125 non-recombinant PVY^{O}, PVY^{O}-O5, PVY^{Eu-N}, PVY^{NA-N} and PVY^{C} isolates were used. It was performed using RDP4.92 in order to correctly identify the Tunisian isolate strain type by checking putative recombination using six recombination-detecting methods: RDP, GENECONV, BooTScan, MaxChi, Chimaera, 3Seq and SiScan (Martin et al., 2015). The analyses in the RDP 4.95 package were done using default settings and a Bonferroni-corrected *P-value* cut-off. Only recombination points detected as highly statistically significant ($p<0.0001$) by more than 4 methods in the RDP 4.95 program were taken into consideration.

3. Results

3.1 DAS-ELISA and RT-PCR detection assays

Tunisian isolate was detected by DAS-ELISA and confirmed by RT-PCR. This isolate was obtained in Monastir (Sahel) during the main season of 2013. A PCR product of the expected size (820 bp) was obtained in this case upon amplification of total RNAs extracted from infected leaves using a primer pair targeting a fragment of the PVY coat protein gene. The identity of the amplification products was validated by direct Sanger sequencing.

3.2 Sequence data

PVY-Tun_144 isolate was subjected to whole genome sequencing. The numbers of reads obtained from NGS after trimming were 15.516.678. Following de novo assembly of this sample using the CLCGW, the numbers of contigs produced were 3420 corresponding to 456.574 reads. Au total of 508 contigs were identified as viral in origin. Of those contigs, 3 were identified as belonging to PVY, the only infectious plant virus identified and characterized in this study. Contig of interest lengths were 1274, 1719 and 6726 nt in length with 25, 88 and 57-fold coverage respectively and the numbers of reads mapped to each contig were 247, 1177 and 2966. The consensus sequence resulted from merged contigs was 9615 nt and the numbers of reads mapped to the scaffold sequence were 4391 (0.93%). Final sequence length consisted of the consensus of the extended contigs from CLCGW mapping steps, and was 9648 nt in length excluding the 3' terminal poly (A) tail, with 53 nt missing sequence in the 5' untranslated region (UTR).

3.3 PVY Nearly complete genome analysis

PVY-Tun_144 isolate show the same typical genomic organization as other PVY isolates. The regions encoding the P1 (135-959 nucleotides), helper-component proteinase (HC-Pro) (960-2354 nucleotides), P3 (2355-3449 nucleotides), 6 kDa 1 protein (6K1) (3450-3605 nucleotides), cylindrical inclusion protein (CI) (3606-5507 nucleotides), 6 kDa 2 protein (6K2) (5508-5663 nucleotides), genome-linked viral protein (VPg) (5664-6227 nucleotides), nuclear inclusion a-proteinase (NIa-Pro) (6228-6959 nucleotides), nuclear inclusion b (NIb) (6960-8516 nucleotides) and coat protein (CP) (8517-9317 nucleotides) were 825, 1395, 1095, 156, 1902, 156, 564, 732, 1557 and 801 nucleotides long, respectively. Furthermore, the region from 2864 to 3094 (231 nucleotides) was determined for pipo ORF. Tunisian isolate coded for an open reading frame of 3,061 amino acids. All characteristic motifs of potyviral proteins fundamental for viral cycle life and vector transmission were conserved in the PVY-Tun_144 nearly complete genome as noted in Table 1.
Based on genome comparison, PVY-Tun_144 was found to be most closely related to the F17 (KX 184817/ Israel) and IUNG-4 (JF927752/ Poland) isolates with 99.13% and 99.12% identity at the nucleotide level (99.32 % and 99.26% at amino acid level) respectively.

3.4 Phylogenetic relationships and Recombination analysis

The grouping of PVY-Tun_144 was further confirmed by phylogenetic analysis of 43 representative PVY sequences available in NCBI. Tunisian PVY isolate was clustered with only PVY^{NTN} recombinant strains and in particular with those of PVY^{NTNa}. Its close evolutionary relationship with the PVY isolates from Israel and Poland (Fig.1).
Two major clades are observed. Clade I corresponded to all N-type sequences from 'parental', 'common' and 'rare' recombinants. All recombinants sequences of this clade are derived from non-recombinant PVY isolate segments like as PVY^{Eu-N}, PVY^{NA-N} and PVY^{O},

with the exception of six isolates (PVY^{E}, PVY-NE-11, -1104,-1107,-FZ10 and –DF) which present an undescribed parental sequence type with smaller segments from other PVY recombinants. In the second clade, there were two subgroups presented by PVY$^{O/Z}$ and PVY^{C} types. The NY110001 and PVY-nnp isolates were identified as recombinants between PVY^{C} and PVY^{O} genomes. In addition, most of the AL100001 isolate genome is derived from PVY^{C} parental isolate with a short region from NE-11 isolate.

When the genome sequences of 125 non-recombinant (PVY^{O}, PVY^{O}-O5, PVY^{Eu-N}, PVY^{NA-N} and PVY^{C} isolates), were aligned with the PVY-Tun_144 sequence, it had identical recombination structures with PVY^{NTNa} isolate. Remarkably, following the adopted criteria previously indicated for the RDP 4.95 program two significant recombination events were detected using at least six of the methods with > 98% nucleotide identity as detailed in Table 2. It was recombinant of parental isolates belonging to strain groups Eu-N (X97895) as major parent and O (KY848031) as minor parent. Three breakpoints were identified at the two junctions (HC-Pro/P3 and 6K2/NIa-VPg) and near the end of the CP gene. This corresponds to the typical structure of the PVY^{NTNa} isolate described by Karasev and Gray (2013).

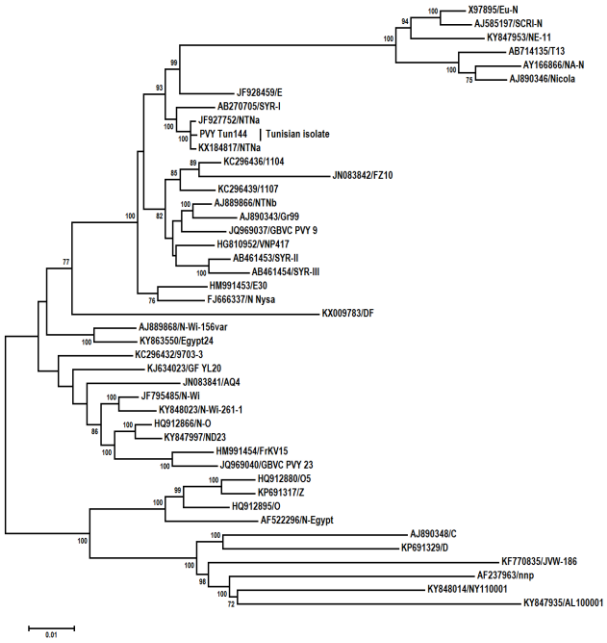

Fig. 1 Unrooted phylogenetic tree showing predicted relationship between the Tunisian PVY isolate and other PVY isolates based on complete genome sequence. Trees were bootstrapped with 1000 replications and only bootstrap values >70 % are indicated at each node.

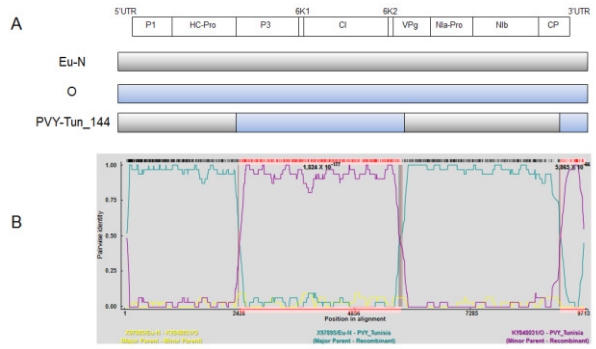

Fig. 2 Schematic representation of PVY TUN_144 recombinant structure. A- Schematic representation of the recombinant region, Parental sequence are colored differently vertical lines delineate recombinant genome segments. B- Graphical representation of recombinant region (Pink). Pairwise identities of each pair of sequences (y-axis) and their position (x-axis) are indicated.

Protein	Motif name / Sequence	Position	Associated role	Reference
HC-Pro	CCC	574-576	aphid transmission	Huet et al., 1994
HC-Pro	PTK	592-594		
HC-Pro (N-terminal domain)	KITC	334-337		-
-	Metal binding motif / FRNK	463-466	symptom expression	Gal-On and Raccah, 2000
CI	NTP binding motif / GAVGSGKST	1242-1250	helicase proteins	Kadare and Haenni, 1997
	VLLIEPTRPL	1262-1271		
	KVSAT	1358-1362		
	LVYV	1409-1412		
	VATNIIENGVTL	1460-1471		
	GERIQRLGRVGR	1504-1515		
NIb	QPSTVVDN	2587-2594	-	Domier et al,, 1987
	GDD	2626-2628	-	
CP (N-terminus)	DAG	2800-2802	aphid transmission	Atreya et al,. 1990

Table 1: Characteristic motifs of potyviral proteins identified in Tunisian PVY isolate. **(-):** Absence of information

Methods	Event 1	Event 2
RDP	1,824 .10^{-177}	5,865 10^{-66}
Gene Conv	1,337 10^{-174}	1,019 10^{-67}
BootScan	1,586 10^{-174}	3,899 10^{-67}
MaxiChi	7,820 10^{-58}	9,398 10^{-18}
Chimaera	3,279 10^{-59}	5,254 10^{-64}
3 Seq	3.330 10^{-16}	3.330 10^{-16}

Table 2: Methods implemented in the RDP 4.95 program used for Recombination breakpoint prediction

	Family	Reference sequence	No of reads	No of contigs	Blastx identity (%)							
					20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
dsRNA	***Amalgaviridae***	*Zygosaccharomyces bailii virus Z*	396	2	2							
	Chrysoviridae	*Aspergillus fumigatus chrysovirus*	1078	3		2		1				
		Grapevine associated chrysovirus-1	1261	4		1						3
		Grapevine chrysovirus	765	4		1			1	1	1	
		Helminthosporium victoriae 145S virus	16	2		1				1		
		Penicillium chrysogenum virus	351	2		1	1					
		Verticillium dahliae chrysovirus 1	26	2		1		1				
	Endornaviridae	*Chalara endornavirus CeEV1*	15	1			1					
		Grapevine endophyte endornavirus	2628	11			1	3	5	1	1	
		Phytophthora endornavirus 1	67	1			1					
	Hypoviridae	*Cryphonectria hypovirus 2*	15	1		1						
	Partitiviridae	*Botryosphaeria dothidea virus 1*	5763	14	4	5	2	3				
		Botryotinia fuckeliana partitivirus 1	9	1								1
		Cannabis cryptic virus	9	1		1						
		Rosellinia necatrix partitivirus 1-W8	9	1						1		
		Rosellinia necatrix partitivirus 2	103	2	1		1					
		Ustilaginoidea virens partitivirus 2	28	2				1		1		
		Vicia faba partitivirus 1	102	2		1		1				
	Totiviridae	*Black raspberry virus F*	793	38		4	21	9	1	3		
		Aspergillus mycovirus 1816	27	1		1						
		Ribes virus F	235	10			3	6	1			
		Saccharomyces cerevisiae virus L-A	112	5			2	3				
		Saccharomyces cerevisiae virus L-A-2	203	9			4	4		1		
		Saccharomyces cerevisiae virus L-A-lus	34	3			3					
		Saccharomyces cerevisiae virus L-BC (La)	4073	26	2	5	10	7	2			
		Scheffersomyces segobiensis virus L	1599	13		2	3	6	1	1		
		Tuber aestivum virus 1	1056	22		4	4	4	2	4		
		unidentified blackcurrent	32	1			1					
		Ustilaginoidea virens RNA virus 1	23	1			1					
		Xanthophyllomyces dendrorhous virus L1A	18693	105	5	14	28	39	14	5		
		Xanthophyllomyces dendrorhous virus L2	2872	56	2	5	11	11	10	6	1	
	unclassified	*Atkinsonella hypoxylon virus*	19	1		1						
		Circulifer tenellus virus 1	25	1				1				
		Curvularia thermal tolerance virus	11	1					1			
ssRNA	***Closteroviridae***	*Grapevine leafroll-associated virus 3*	8	1		1						
	Narnaviridae	*Gremmeniella abietina mitochondrial RNA virus S2*	53	1			1					
		Mitovirus AEF-2013	327	2			2					
		Ophiostoma mitovirus 6	30	1			1					
		Saccharomyces 23S RNA narnavirus	19	1		1						
		Sclerotinia sclerotiorum mitovirus 6	42	1			1					

		Sclerotinia sclerotiorum mitovirus 10	24	1			1					
	unclassified	*Blueberry necrotic ring blotch virus*	4706	4		2	2					
		Sclerotinia sclerotiorum ourmia-like virus 2 RNA 1	425	3			2	1				
		Mgnaporthe oryzae ourmia like virus	1308	6		2	3	1				
		Phytophthora infestans RNA virus 4	98	4			3		1			
		Sclerotinia sclerotiorum negative-stranded RNA virus 3	30	1		1						
		Alternaria tenuissima virus	591	2				1	1			
		Cladosporium cladosporioides virus 1	60282	39	1	11	15	9	3			
unclassified		*Grapevine associated mycovirus-1*	9460	91		7	16	43	21	2	2	
		Tomato matilda virus	29	1								1
Total			**119880**	**508**	17	76	145	155	64	27	5	5

Table 3: Category list of putative potato associated mycovirus

4. Discussion

Potato virus Y (PVY) is one of the most important viruses infecting potato which has a worldwide distribution. Particularly in Tunisia, its significant spread has been reported in a recent paper (Ben Hafsa et al., 2018). Since PVY was first described, the assignment of strains has been contentious. Thus, it occurs as a complex set of strains, including a growing number of recombinants, through the accumulation of mutation and recombination events. Currently, recombinant strains are prominent or dominant among PVY isolates circulating around the world. In this study, the nearly complete genome sequence of PVY-Tun_144 was determined from a single in-depth sequencing of potato's sample. This work endorses the reliability of NGS to decipher the genome structure and diversity of viruses. From this sequence, with only 53 nt missing sequence in the 5'UTR, phylogenetic relationships and recombination analysis were established. According to literature, it is not easy to study the origins of PVY recombinants based on phylogenetic reconstructions without taken the number of recombination events into consideration. Recently, a total of 285 PVY genomes were used in order to investigate the relation between the recombination and the origin of common PVY recombinants. These strains were shown more than once to be originated from different parental sequences (Green et al., 2017a). This can also explain the controversy between biological and phylogenetic classification of PVY strains/variants, as the example of PVY^{o} and PVY^{z} isolated, showing different biological proprieties after inoculation on potato cultivars carrying the *Nytbr* and *Nztbr* genes (Kehoe and Jones, 2015).

In addition to the phylogenetic analysis that showed the clustering of the Tunisian isolate in relation to the representative PVY isolates, recombination analysis showed a typical feature of the variant PVY^{NTNa}. Given that the most of potato's seeds are imported in Tunisia from Europe, this suggests the possibility that Tunisian NTN strain originated potentially from an ancestral European PVY strains. The polyprotein ORF presents 23 polymorphic nucleotides in comparison to F17 and IUNG-4 isolates. Only one site (C3226G) results in amino acid change in CI protein (L1076V) for F17 isolate and three sites (A712G; U5567C; G6019A) results in amino acid changes in P1 (I238V), 6K2 (V1856A) and VPg (G2007S) for IUNG-4 isolate. This is the first nearly complete PVY sequence from Tunisia, deposited to the GenBank.

Other than the PVY viral sequences, a great diversity of viral sequences has been revealed. A total of 503 contigs (118, 446 reads) were identified as belonging to numerous viral family. About 40% of these sequences corresponded to sequences with mycoviruses. More than 90% of the assembled contigs shared less than 70% identity with genomes from known mycoviruses species according to the BLASTx results, suggesting that they probably represent new viral species in these families. dsRNA extraction strategy promotes the detection of fungal viruses (Table 3). Many of them yield dsRNA molecules or dsRNA replicative intermediates in their hosts (Pearson et al., 2009). In our case, such sequence diversity revealed by this sequencing strategy suggests the hypothesis that mycoviruses infecting fungi may be present as epiphytes or as endophytes on the sequenced sample. These data can be exploited thereafter for the identification of these putative mycoviruses in fungi cultured from potato or directly from this sample after a direct viral detection of initial dsRNA.

References

Ben Hafsa, A., N. Nabi, B. M'rabet Saamali, M. S. Zellama, S. theil, C. Faure, A. Berard, D. Brunel, M.-C. Le Paslier, A. Marais, T. Candresse and M. Chaouachi, 2018. Status of potato viruses in Tunisia

and molecular characterization of Tunisian Potato Virus X (PVX) isolates. Eur J Plant Patho. DOI: 10.1007/s10658-017-1407-2.

Boukhris-Bouhachem, S., F. Djilani-Khouadja, H. Fakhfakh, L. Glais, M. Tribodet and C. Kerlan, 2010. Incidence and Characterization of Potato virus Y in Seed Potatoes in Tunisia. Potato Res. 53(3): 151-166.

Candresse, T., A. Marais and C. Faure, 2013. First report of Southern tomato virus on tomatoes in Southwest France. Plant Disease, 97:1124.

De Bokx, J. A. and H. Huttinga, 1981. Potato virus Y. CMI/AAB Descriptions of Plant Viruses, no. 242.

Djilani-Khouadja, F., L. Glais, M. Tribodet, C. Kerlan, H. Fakhfakh, 2010. Incidence of potato viruses and characterization of Potato virus Y variability in late season planted potato crops in Northern Tunisia. Eur. J. Plant Pthol. 126: 479-488.

FAO, 2014. FAO statistical databases FAOSTAT. http://faostat3.fao.org/ Accessed 5 Dec 2017.

Gentit, P., X. Foissac, L. Svanella-Dumas, M. Peypelut, G. Macquaire and T. Candresse, 2001. Biological properties and partial molecular characterization of two foveaviruses associated with similar disorders of cherry trees. Acta Horticulturae, 550: 161-168.

Gibbs A., K. Ohshima, R. Yasaka, M. Mohammadi, M.J. Gibbs and R.A.C. Jones, 2017. The phylogenetics

of the global population of Potato virus Y and its necrogenic recombinants. Virus Evol. 3(1): vex002.

Gray, S., S. De Boer, J. Lorenzen, A.Karasev, J. Whitworth , P. Nolte, R. Singh, A. Boucher and H. M. Xu, 2010. Potato virus Y: an evolving concern for potato crops in the United States and Canada. Plant Dis. 94: 1384-1397.

Green K.J., C.J. Brown, A.V. Karasev, 2017. Genetic diversity of potato virus Y (PVY): sequence analyses reveal ten novel PVY recombinant structures. Arch Virol. doi: 10.1007/s00705-017-3568-x.

Green, K.J., C.J. Brown, S.M. Gray, A.V. Karasev, 2017a. Phylogenetic study of recombinant strains of Potato virus Y. Virology. 507:40-52.

Hosseini, A., H. Massumi, J. Heydarnejad, A. P. Hosseini and A. Varsani, 2011. Characterization of Potato virus Y isolates from Iran. Virus Genes, 42:128-140.

Karasev, A. V. and S.M. Gray, 2013. Continuous and emerging challenges of Potato virus Y in potato. Annu. Rev. Phytopathol. 51: 571-86.

Kehoe M.A and R.A.C. Jones, 2015. Improving Potato virus Y strain nomenclature: lessons from comparing isolates obtained over a 73-year period. Plant Pathol. 65 : 322-333.

Kerlan, C., O.V. Nikolaeva, X. Hu, T. Meacham, S.M. Gray, A.V. Karasev, 2011. Identification of the molecular make-up of the Potato virus Y strains PVYZ: Genetic typing of PVYZ -NTN. Phytopathol.101: 1052-1060.

Khamassy, N., 1999. Contribution in the development of a system of potato seed production adapted to the Tunisian culture pressures. PhD dissertation, National Institute of Agronomic Research of Tunisia, North Africa.

Larbi, I., F. Djilani-Khouadja, N. Khamassy and H. Fakhfakh, 2012. Potato virus surveys and wide spread of recombinant PVYNTN variant in Central Tunisia. Afr. J. Microbiol. Res . 6(9):2109-2115.

Martin, D.P., B. Murrell, M. Golden, A. Khoosal and B. Muhire, 2015. RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evolution, 1: vev003.

Pearson, H.N., R.E. Beever, B. Boine and K. Arthur, 2009. Mycoviruses of filamentous fungi and their relevance to plant pathology. Mol. Plant Pathol. 10:115-128.

Pourrahim, R., S. Farzadfar, A.R. Golnaraghi and A. Ahoonmanesh, 2007. Incidence and distribution of important viral pathogens in some Iranian potato fields. Plant Disease, 91(5): 609-615.

Svanella-Dumas, L., T. Candresse, M. Hullé and A. Marais, 2013. Distribution of Barley yellow dwarf virus-PAV in the Sub-Antarctic Kerguelen Islands and Characterization of Two New Luteovirus Species. PLoSONE, 8:1-13.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30, 2725–2729.

Tayahi, M., C. Gharsallah, N. Khamassy, H. Fakhfakh and F. Djilani-Khouadja, 2016. Biological, serological and molecular typing of potato virus Y (PVY) isolates from Tunisia, 2016. Genet. Mol. Res. 15 (4): gmr15045165.

Valverde, R.A., A. Dodds and J.A. Heick, 1986. Double-stranded ribonucleic acid from plants infected with viruses having elongated particles and undivided genomes. Phytopathol. 76: 459-465.