Occurrence of Diverse Recombinant Strains of *Potato virus Y* Circulating in Potato Fields in Egypt

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Abstract

Potato is one of the staple crops in Egypt, grown under irrigation almost continuously year-round. *Potato virus Y* (PVY) has been reported as one of the main viruses affecting potatoes in Egypt, but limited information is available on PVY strains circulating in potato fields in the country. From 2014 to 2016, virus surveys were conducted in several potato-growing governorates of Egypt, and PVY-positive samples were found to represent at least five distinct recombinant PVY strains, including PVY^{NTN} and PVY^{N-Wi}. Whole genome sequences were determined for four

isolates representing strains PVY-SYR-III (Egypt7), PVY-261-4 (Egypt11), PVY^{NTNa} (Egypt35), and a novel recombinant named Egypt24 that combined molecular properties of strains PVY-261-4 and PVY-Wilga156var. At least three recombinants found in Egypt in potato were previously found associated with potato tuber necrotic ringspot disease (PTNRD). The identification of multiple recombinant types of PVY in potato in Egypt, including the novel recombinant Egypt24, suggests a wide presence of PTNRD-inducing virus strains in the country.

Viruses affect potato production in two ways: either by affecting tuber yield (Nolte et al. 2004), or by affecting tuber quality. The tuber damage can be internal, like net necrosis caused by *Potato leafroll virus* (PLRV) (Jayasinghe and Salazar 1998; Peters and Jones 1981) and extensive internal necrosis caused by *Alfalfa mosaic virus* (AIMV) (Nie et al. 2015; Slack 1981), or external damage like potato tuber necrotic ringspot disease (PTNRD) caused by *Potato virus Y* (PVY) in susceptible potato cultivars (Beczner et al. 1984; Le Romancer et al. 1994). Tuber quality problems affect the marketable value of the crop and may lead to significant losses in addition to reduction in yields. Hence, the three viruses known to affect tuber quality, PVY, AIMV, and PLRV, are a major concern for potato producers all over the world.

In Egypt, potato is an important staple crop produced under irrigation almost year-round, and virus infections represent a significant burden on potato production (Elawady and Abdulkheir 2015; International Potato Center 2007; Shalaby et al. 2002). Epidemiology of potato viruses in the Middle East is complicated, since the exchange of seed potato between neighboring states in the area is largely absent. A substantial proportion of potato production is targeting European markets, where most seed potato is coming from. The rest is destined for internal consumption in most of the countries of the eastern Mediterranean and the Middle East. Egypt is an exception in the region, since it also exports a significant share of its potato crop to other Arab countries and to Russia (Elawady and Abdulkheir 2015; International Potato Center 2007). Three viruses that can affect potato tuber quality were found in Egypt (Abdalla et al. 2016a, b; El-Absawy et al. 2012; El-Borollosy 2015; El-Helaly et al. 2012).

PVY has long been recognized as a threat to potato cultivation in Egypt (Abdalla et al. 2016b; El-Absawy et al. 2012; El-Borollosy 2015; Shalaby et al. 2002), but only limited studies were conducted to reveal the genomic diversity of PVY population in the country. An isolate of possibly a PVY^{NTN} strain from the Al Daqahliya

governorate was reported based merely on capsid protein (CP) cistron sequence comparisons (El-Absawy et al. 2012), and another isolate representing the PVY^O strain was reported from the Al Giza governorate (El-Borollosy 2015). A recent report suggested presence of the PVY^{NTN} isolates in potato in Asyut, Upper Egypt, based again only on sequence analysis of a small fragment of the genome in the CP region (Abdalla et al. 2016b).

PVY exists as a complex of strains that are classified genetically based on reactions in indicator plants and differential potato cultivars (Cockerham 1970; Jones 1990; Karasev and Gray 2013; Kehoe and Jones 2016; Kerlan 2006; Singh et al. 2008), and also molecularly based on genome sequences and recombinant structures (Karasev and Gray 2013; Singh et al. 2008). At least nine strains have been defined so far, plus a growing number of recombinant variants (Glais et al. 2002; Karasev and Gray 2013; Kerlan 2006; Singh et al. 2008). In recent years, PVY became a major problem for potato production worldwide (Blanchard et al. 2008; Gray et al. 2010; Karasev and Gray 2013) due to the spread of new recombinant strains often associated with induction of PTNRD in susceptible cultivars (Gray et al. 2010). Due to the wide spread of recombinant strains of PVY, partial sequences from the CP region of the virus genome were found insufficient to correctly determine the strain assignment of a virus isolate (Karasev and Gray 2013; Singh et al. 2008). Consequently, either whole genome sequencing or special typing techniques utilizing multiple primers probing for the presence or absence of main recombinant junctions would be necessary to type the PVY recombinants (Chikh-Ali et al. 2013; Lorenzen et al. 2006). Multiple recombinants of PVY have been found in potato in the eastern Mediterranean and the Middle East outside of Egypt, including PVY^{NTN} and PVY^{N-Wi} in Jordan (Anfoka et al. 2014, 2016), PVY-SYR-I, -SYR-II, and -SYR-III in Syria, Jordan, and Saudi Arabia (Anfoka et al. 2014, 2016; Chikh-Ali et al. 2010b, 2016a), and PVY-NE11 in Saudi Arabia (Chikh-Ali et al. 2016a).

To fill in the knowledge gap about the viruses potentially affecting tuber quality in the Nile Delta area of Egypt, we conducted surveys for the three potato viruses in 10 governorates of Egypt during three seasons, 2014 to 2016. Special emphasis was placed on PVY, to identify virus strains present in potato crops grown in the Nile Delta of Egypt. The main objective of this study was to determine the virus genetic diversity present in potato fields, including nearly whole genome sequencing for four recombinant Egyptian isolates of PVY. A novel PVY recombinant type was found in Egypt, not reported previously from any potato-producing area in the world.

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

Accepted for publication 10 April 2017.

Sample collection. A total of 260 potato leaf samples were collected over the period from 2014 to 2016 from 10 different governorates of the Nile Delta in Egypt as shown in Figure 1. During the survey, leaf samples were collected from plants showing various types of virus-related symptoms such as chlorosis, yellowing, mosaic, necrosis, leaf rolling, or stunted growth. Symptoms were evaluated in the fall season in each of the three years, during the month of November. Most of the potato fields surveyed were small plots of approximately 0.42 hectares (0.42 ha = 1.038 acres = 1 Feddan, a local unit of area) or less for each, growing commercial potato for local consumption. Potato cultivar names were provided by the plot owners. In all cases they were planted with potato seed produced locally, and not with imported seed. Samples were collected from plants exhibiting virus-like symptoms, and one sample constituted a single symptomatic leaf. All samples were placed in plastic bags kept in a cooler, labeled, and once delivered to the laboratory, kept frozen at -20°C until tested.

RNA extraction, RT-PCR, and sequencing of PVY positive samples. RNA was isolated from all 12 symptomatic samples using

Sequence analysis. MUSCLE (Edgar 2004) implemented in the program MEGA version 7 (Tamura et al. 2007) was used with the

default parameters for the multiple sequence alignment. Alignments were generated based on the whole genomes of representative isolates of all nine characterized PVY strains (PVY^O, PVY^O-O5, PVY^N, PVY^C, PVY^{NA-N}, PVY-NE11, PVY^{N:O}, PVY^{N-Wi}, and PVY^{NTN}), as well as PVY^E, the three Syrian types, and the rare PVY-261-4 and PVY-Wilga156var recombinant variants. The aligned sequences were checked for recombination using the RDP, GENECONV, BOOTSCAN, MAXCHI, CHIMAERA, 3Seq, and SiScan methods in the RDP v4.61 software (Martin et al. 2010). The analyses in the RDP 4.61 package were done using default settings and a Bonferroni-corrected *P*-value cut-off of 0.05. Only recombination points detected as highly statistically significant (*P* < 0.0001) by more than four methods in the RDP 4.61 program were taken into consideration. The phylogeny inference was conducted based on the whole genomes using the neighbor-joining method (NJ) implemented in MEGA version 7 (Tamura et al. 2007) with 1,000 bootstrap replicates. The condensed tree was then generated in MEGA version 7 with a cutoff value of <70%, so that all nodes with less than 70% support were collapsed.

Results

In order to characterize local virus isolates affecting potato in Egypt, a survey was conducted in the main potato production area, in Nile Delta governorates, targeting the three most common viruses, PVY, AIMV, and PLRV. A total of 260 potato leaf samples were

collected from local fields in 10 governorates in the Nile Delta area of Egypt (Fig. 1), over a three year period of 2014 through 2016. Collected samples exhibited various virus-like symptoms, such as different types of mosaic (yellow, bright yellow, pale or dark green), leaf necrosis, chlorosis, stunted growth, leaf distortions, or leaf blade rolling. A summary of the initial virus testing is shown in Table 1: 107 samples or 41.2% were found infected with one or more of the three viruses tested for, i.e., PVY, AIMV, or PLRV. PVY was the most common in the symptomatic samples (13.5%), followed by AIMV (11.5%) and PLRV (4.6%). In addition to single infections for each of the three viruses, double and triple infections in different combinations were detected at a relatively low incidence (Table 1). Given the dominance of the PVY infection in symptomatic potato leaf samples, and due to the limited information on PVY strains circulating in potato in Egypt, 12 samples were randomly selected for a more thorough study of genetic diversity and identification of possible recombinants in representative local PVY isolates.

PVY strain typing using RT-PCR assays. Of the 12 randomly selected potato leaf samples subjected to the RT-PCR testing for nine different potato viruses, nine were found positive for PVY (Table 2). Five of the nine PVY-positive samples also contained another potato virus, most often PLRV, while one sample contained both AIMV and PLRV in addition to PVY. Hence, mixed infections were easily found in field potato samples (Table 2). Aside from the three viruses targeted by the survey, namely PVY, AIMV, and PLRV (Table 1), no

Table 1. Summary of the surveys conducted in 10 Nile Delta (Egypt) governorates for *Potato virus Y* (PVY), *Alfalfa mosaic virus* (AIMV), and *Potato leafroll virus* (PLRV). PVY positive samples were detected using DAS-ELISA, and AIMV and PLRV positive samples were detected using RT-PCR, as described in Materials and Methods.

Governorate	Potato cultivar	No. of samples	Total infected samples		PVY	PLRV	AIMV	PVY+AIMV	PLRV+AIMV	PVY+AIMV	PVY+AMV+PLRV
			No.	%							
Alexandria	Barum	34	9	26.5	4	1	4	-	-	-	-
Kafr El-Shaykh	Cara	11	5	45.5	5	-	-	-	-	-	-
Damietta	Spunta/Cara	38	20	52.6	6	1	5	1	1	2	4
Al-Gharbiya	Spunta/Diamant	41	14	34.1	3	4	5	2	-	-	-
Al-Sharqiya	Spunta/Diamant	36	19	52.7	5	1	3	1	7	2	2
Al-Beheira	Spunta/Barum	34	14	41.2	6	1	4	3	-	-	-
Al-Qualioubiya	Spunta/Barum	25	11	44	1	1	6	3	-	-	-
Al-Giza	Cara	3	2	66.7	1	-	-	-	-	1	-
Al-Dakahlia	Spunta	35	12	34.3	4	2	3	-	1	-	2
Al-Menoufia	Spunta	3	1	33.3	-	1	-	-	-	-	-
TOTAL		260	107	41.2	35	12	30	10	9	5	8

Table 2. Detailed molecular characterization of a select set of 12 potato samples collected from Nile Delta governorates in Egypt. *Potato virus Y* (PVY) strain typing was conducted using RT-PCR, with two different primer sets according to Lorenzen et al. (2006) and Chikh Ali et al. (2010a). *Potato mop-top virus* (PMTV), *Tobacco rattle virus* (TRV), *Alfalfa mosaic virus* (AIMV), *Potato virus M* (PVM), *Potato virus A* (PVA), *Potato virus X* (PVX), *Potato virus S* (PVS), and *Potato leafroll virus* (PLRV) using RT-PCR.

Sample ID	Governorate	Potato cultivar	RT-PCR assay ^a		Strain typing call			Potato viruses							
			Lorenzen ^b	Chikh-Ali ^c	RT-PCR	Sequence ^d	GenBank accession	PMTV	TRV	AIMV	PVS	PVX	PVA	PVM	PLRV
Egypt2	Al Giza	Spunta	-	-	-	-	-	-	-	POS	-	-	-	-	POS
Egypt3	Damietta	Spunta	181	441	A recombinant	NTNb?	KY863552	-	-	-	-	-	-	-	-
Egypt6	Al Beheira	Spunta	181, 452	441, 633	NTNa or PVY-E	-	-	-	-	POS	-	-	-	-	-
Egypt7	Damietta	Spunta	181, 452	278, 441, 1,076	SYR-III	SYR-III	KY863549	-	-	-	-	-	-	-	-
Egypt8	Al Beheira	Spunta	181, 452	441, 633	NTNa or PVY-E	-	-	-	-	-	-	-	-	-	-
Egypt11	Al Sharqia	Spunta	181, 689	278	261-4	261-4	KY863548	-	-	-	-	-	-	-	POS
Egypt13	Al Sharqia	Spunta	181	-	A recombinant	-	-	-	-	-	-	-	-	-	POS
Egypt14	Alexandria	Barum	181	441, 853	N-Wi	N-Wi	KY863553	-	-	-	-	-	-	-	-
Egypt23	Al Daqahlia	Spunta	-	-	-	-	-	-	-	-	-	-	-	-	-
Egypt24	Al Daqahlia	Spunta	181	278	261-4 (?)	New	KY863550	-	-	-	-	-	-	-	POS
Egypt35	Al Gharbiya	Spunta	181, 452	441, 633	NTNa or PVY-E	NTNa	KY863551	-	-	POS	-	-	-	-	POS
Egypt108	Al Shraqia	Spunta	-	-	-	-	-	-	-	-	-	-	-	-	POS

^a Numbers represent PCR product sizes in base pairs (bp).

^b RT-PCR typing using methodology of Lorenzen et al. (2006).

^c RT-PCR typing according to Chikh Ali et al. (2010b).

^d Typing based on whole or partial genome sequence analysis.

other major potato viruses were found in these selected samples (Table 2).

All nine PVY-positive samples were identified as representing recombinant strains of PVY, with three typed as PVY^{NTNa} or PVY^E, one as PVY-SYR-III, one as PVY^{N-Wi}, two as PVY-261-4, and two as undefined recombinants (Table 2). No nonrecombinant PVY sequences were found, and no evidence was found for mixed infections between different PVY strains. In most cases, both typing primer sets, Lorenzen et al. (2006) and Chikh-Ali et al. (2010b), identified the presence of the recombinant junction RJ2 at position nt 2,415, characteristic of the main recombinants of PVY (Karasev and Gray 2013).

Sequencing and sequence analysis. Four PVY isolates were subjected to whole genome sequencing: those tentatively assigned by RT-PCR to strains PVY-SYR-III (Egypt7), PVY-261-4 (Egypt11), possibly PVY-261-4 (Egypt24), and PVY^{NTNa} or PVY^E (Egypt35). These four sequences represented nearly whole genomes of the respective PVY isolates, missing only 30-70 nt at the extreme 5'-end due to the amplification strategy used (Green et al. 2017). Two

isolates, Egypt3 and Egypt14, were subjected to partial sequencing that covered about 15% and 34% of their genomes, including the 3'-terminal area. This partial sequencing, together with RT-PCR typing, suggested Egypt3 to be either a PVY^{NTNa} or PVY^{NTNb} isolate, and confirmed the RT-PCR assignment of Egypt14 as a PVY^{N-Wi} isolate (Table 2). Nearly complete genome sequences of Egypt7, Egypt11, and Egypt35 were subjected to the recombinant analysis using the programs from the RDP package, and found to represent strains PVY-SYR-III (Egypt7), PVY-261-4 (Egypt11), and PVY^{NTNa} (Egypt35), matching the assignments based on the RT-PCR typing (Fig. 2; Table 2). The nearly complete sequence of the Egypt24 isolate was found unique: it partly resembled the PVY-261-4 strain by having an N-sequence fragment between positions 680 and 2,390, and partly resembled another recombinant named PVY-Wilga156var by having another N-sequence fragment between positions 5,850 and 6,855 (see Fig. 2).

In phylogenetic reconstructions, whole genomes of the four Egyptian isolates were placed in respective clades with PVY^{NTNa}

Fig. 2. Summary diagrams of the *Potato virus Y* (PVY) recombinants identified by sequence analysis in Egyptian potato. (A) A schematic diagram presents the main features of the PVY genome, with individual cistrons marked as unshaded rectangles. Below, two parental genomes, O (PVY^O) and Eu-N (PVY^N), are presented as unshaded and shaded in black, respectively. Underneath the parental sequences, four recombinant structures found in PVY isolates collected in Egypt are presented: (B) PVY^{NTNa} (Egypt35); (C) PVY-SYR-III (Egypt7); (D) PVY-261-4 (Egypt11); (E) a new recombinant, distinct from the closest PVY-Wilga156var (Egypt24); positions of the two recombinant junctions are indicated as nt numbers in the alignment.

(Egypt35), PVY-SYR-III (Egypt7), PVY-261-4 (Egypt11), and PVY-Wilga156var (Egypt24) (Fig. 3). Isolate Egypt7 grouped in the same clade with two isolates from Syria and Saudi Arabia (Fig. 3) belonging to strain PVY-SYR-III (Chikh-Ali et al. 2010a). Interestingly, three other isolates—Egypt11, Egypt24, and Egypt35—each grouped closely with respective isolates from strains PVY-261-4 (accession number AM113988), PVY-Wilga156var (AJ889868), and PVY^{NTNa} (AJ890345), all reported from Europe (Schubert et al. 2007).

Discussion

Potato has become a staple crop in Egypt, with over 172,000 ha planted in 2014 (FAOSTAT 2014). In 2014, more than 4.6 million tons of potato were produced in Egypt, with 516,000 tons estimated to be seed potato (FAOSTAT 2014). As a vegetatively propagated

crop, potato is prone to virus infections, and viruses affecting tuber quality are of particular concern for potato producers. Here, we conducted a survey in the Nile Delta, one of the major potato producing areas of Egypt, and collected data on the prevalence of three potato viruses affecting tuber quality, PVY, PLRV, and AIMV, during three seasons of observation, 2014 to 2016 (Table 1). Because not all strains of PVY affect tuber quality, we conducted a more detailed study of the strain composition on a subset of the samples collected in 2015.

The diversity of the PVY strains circulating in potato in Egypt was found remarkable – at least five different recombinant types were identified among PVY-positive samples subjected to a detailed study (Table 2; Figs. 2 and 3). No nonrecombinant isolates were found among the tested samples, which was consistent with the similar dominance of recombinant strains of PVY in neighboring Saudi

Fig. 3. Phylogenetic analysis of nucleotide sequences of the whole genomes for the four Egyptian and 48 additional, representative PVY isolates conducted with the neighbor joining (NJ) algorithm. Only nodes with bootstrap values 70% or higher were retained. Brackets designate individual strains and variants of PVY as described in Karasev and Gray (2013); the clade with the new recombinant Egypt24 is designated with an underlined font. 'Non-P' designates a sequence from a non-potato isolate SON41 (PVY^C). Bold font designates Egypt7, Egypt11, Egypt24, and Egypt35 sequences. Both an isolate name and a corresponding accession number in the GenBank database are given for each reference sequence.

Arabia (Chikh-Ali et al. 2016a), Jordan (Anfoka et al. 2014, 2016), and Syria (Chikh Ali et al. 2010b). The suggested presence of a possible PVY^{NTNb} isolate based on RT-PCR and partial sequencing that did not span the 5'-proximal segment of the genome should be viewed with caution, as similar indications for Indonesian PVY isolates were not confirmed after whole genome sequencing (Chikh-Ali et al. 2016b). Three of the PVY strains found in Egypt, PVY^{NTNa}, PVY^{N-Wi}, and PVY-SYR-III, were found in Syria and Jordan (Anfoka et al. 2014, 2016; Chikh Ali et al. 2010b), while strain PVY-SYR-III was also found in Saudi Arabia (Chikh-Ali et al. 2016a). On the other hand, the PVY-261-4 strain found in Egypt (Table 2; Fig. 2) was previously reported only once from Germany (Schubert et al. 2007). The newly described strain Egypt24 (Fig. 2) resembled the previously reported strain PVY-Wilga156var from Germany (Schubert et al. 2007). However, the position of the first recombinant junction RJ1 for Egypt24 was located at nt 680 of the whole genome alignment (Fig. 2). It was shifted by approximately 180 nt to the 3'-terminus relative to the RJ1 of Wilga156var, and matched the RJ1 of another European strain described by Schubert et al. (2007), PVY-261-4.

Recent study of the prevalence of PVY strains circulating in the U.S. revealed that recombinant strains dominated in the U.S., and two of them, PVY^{NTNa} and PVY^{N-Wi}, made up almost 80% of the PVY isolates circulating in potato (Funke et al. 2017), with the rest represented by PVY^O, PVY^{N-O}, and PVY-NE11 strains (Funke et al. 2017). Dominance of recombinant strains of PVY was also demonstrated in Europe (Glais et al. 2002; Schubert et al. 2007; Sihelska et al. 2016), in South America (Galvino-Costa et al. 2012a, b), and in Asia (Anfoka et al. 2014, 2016; Chikh-Ali et al. 2010a, 2016a, b; Schubert et al. 2015). PVY strains PVY-SYR-III, PVY-261-4, and Egypt24 were never found in the U.S. or Canada (Funke et al. 2017; Karasev and Gray 2013; Nie et al. 2013). The remarkable resemblance of PVY recombinants found in potato crops in several countries in the eastern Mediterranean and the Middle East may suggest a common source of the infection, most likely seed potato acquired through international trade. The closest relatives for three of the four Egyptian PVY genomes sequenced were found among the European PVY sequences (Fig. 3), which may suggest that the original source of these recombinants resided somewhere in Europe.

Four of the five recombinant types identified in Egypt, PVY^{N-Wi}, PVY-SYR-III, PVY-261-4, and Egypt24, are expected to have an O serotype based on the CP gene sequence (Chikh Ali et al. 2010b; Green et al. 2017; Nikolaeva et al. 2012; Schubert et al. 2007). At the same time, at least three of the identified recombinant strains, PVY^{NTNa}, PVY-SYR-III, and PVY-261-4, were reported to induce PTNRD in susceptible potato cultivars (Chikh Ali et al. 2010b; Le Romancer et al. 1994; Schubert et al. 2007). This unusual combination of the O serotype and PTNRD-inducing capabilities for the PVY strains circulating in Egypt poses special challenges for the diagnosis of the most damaging PVY strains in the country.

Acknowledgments

This work was supported by the Science and Technology Development Fund (STDF) Project 5564, in Egypt for the survey, identification of viruses, and for travel of Egyptian collaborators to the U.S. It was also funded in part through grants from the Northwestern Potato Research Consortium, the Idaho State Department of Agriculture, the USDA National Institute of Food and Agriculture (NIFA) Hatch project IDA01560, and by the Idaho Agricultural Experiment Station.

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