Incidence and characterization of *Potato virus V* infections in Iran

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Abstract A survey was carried out to determine the extent of *Potato virus V* (PVV) infection, together with other potyviruses, in Iran in both commercial and local potato varieties. We found a low incidence of PVV in commercial varieties compared to a local potato cultivar Zardi, in which we noted a PVV infection up to ~32.9 %. We determined the genomic sequence 9,812 nucleotides of one isolate (KER.LAL.P) from cultivar Agria and the 3'-terminal sequence including coat protein (CP) gene of four additional isolates from cultivar Zardi. The Iranian isolate PVV KER.LAL.P was found to share 91 % sequence identity with the Scottish isolate DV-42 (AJ243766) of PVV. The CP gene sequences of the PVV isolates from Iran shared 96.5 to 99.3 % pairwise nucleotide identity and they shared <97.5 % pairwise identity with the CP sequences of all other PVV isolates available in public databases. Our host studies indicated that the Iranian PVV isolate had a narrower host range and infected *Nicotiana debneyi* and *N. glutinosa* test plants. Within the commercial varieties of potato in Iran, we noted a significant amount of mixed potyvirus infection. This study is the first report of occurrence and complete genome of PVV in Iran.

Keywords *Potato virus V* · Complete genome · Phylogenetic analysis · Iran

Introduction

Potato virus V (PVV) is a member of the economically significant plant virus genus Potyvirus, family Potyviridae [1]. The virus has flexuous filamentous particle which are approximately 760 nm in length [2, 3] and have a positive-sense RNA genome of ~9851-nts that has a covalently linked 5'-terminal viral protein (VPg) and a 3'-terminal poly (A). The genome has a single open reading frame (ORF) coding for a polyprotein of 3,067 amino acids [1] and through nine putative cleavage sites yield 10 mature proteins [1].

PVV was first reported by Rozendaal et al. [4] as a deviating strain of *Potato virus Y* (PVY), namely strain C (Y^C-G1) in the Netherlands and has subsequently been reported in potato cultivars in the Andean region of South America and parts of Europe including Netherlands, Finland, Scotland, Norway and Sweden [1, 3, 5, 7]. Other natural hosts are unknown, and no strains for PVV have been described. Within Europe isolates of PVY and *Potato virus A* (PVA) are commonly found infecting potato cultivars, whereas PVV is found at a low incidence [1]. The latter being attributed to the presence of genes for hypersensitive resistance (HR) and a broad-spectrum potyvirus resistance against PVV in many potato cultivars [8, 9].

At a genomic level, only a single isolate (DV 42) of PVV from Scotland has been sequences completely [1]. Most other studies have mainly focused on determining either the coat protein (CP) gene and/or P1 protein genes [1, 3, 5, 6, 7, 10].

Despite the large agricultural industry in the Middle East, almost no information of the occurrence of PVV

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incidences within the local potato cultivars is available. In this study, we report occurrence of PVV in Iran, in both commercial and local potato cultivars. We further characterize the virus isolates at biological and molecular level.

Materials and methods

Collection and maintenance of virus isolates

Two hundred and forty potato leaf samples (from 8 commercial cultivars; Table 1) were sampled regardless of symptoms from 20 locations from the six main potato producing provinces (Kerman, Fars, Khorasan, Hamedan, Ardabil and Esfahan provinces) between 2007 and 2010. All samples were screened for PVV, PVY, PVA, *Potato virus X* (PVX) and *Potato virus S* (PVS) infections by double antibody sandwich enzyme-linked immunosorbent (DAS-ELISA; Clark and Adams, 1977) using PVV, PVY, PVA, PVX and PVS specific polyclonal antibodies (Bioreba, CH-4153 Reinach BL1, Switzerland). Additionally 210 leaf samples from a local potato cultivar (Zardi) in the Zarand region of Kerman province were screened with PVV and PVY specific polyclonal antibodies (Bioreba, CH-4153 Reinach BL1, Switzerland). In all ELISA assays a sample was considered virus-positive only if its OD exceeded the mean plus three standard deviations of the OD of the healthy controls.

Of the PVV positive samples from two locations (Zarand and Bardsir regions in Kerman province), five specimens were selected for further studies and maintained in *Nicotiana glutinosa* L. in a temperature regulated insect-proof greenhouse. These isolates were named and numbered according to the abbreviated name of provinces and regions of collection, KER.LAL.P (*S. tuberosum* cv. Agaria), KER.ZAR.1, KER.ZAR.2, KER.ZAR.3, KER.ZAR.4 (*S. tuberosum* cv. Zardi).

Host range determination

The five PVV isolates were biologically characterized by inoculating a range of test plants, namely *N. debneyi* L., *N. tabacum* cv. Whiteburly L., *N. glutinosa* L., *N. clevelandii* L. Gray., *N. tabacum* L. cv. Samsun.NN, *N. bentamiana* L., *N. tabacum* cv. Turkish, *Datura metel* L., *D. straminium* L., *Chenopodium amaranticolor* L. and *Gompharina globosa*. Extracts were prepared by grinding the inoculum in 1 % (w/v) solution of K₂HPO₄ at pH 7.5 containing 0.01 % Na₂SO₃, 2 % polyvinylpyrrolidone (PVP), and 0.05 % ethylenediaminetetraacetic acid (EDTA). Inoculations were carried out in triplicates and the plants were maintained in a greenhouse (25–28 °C). Inoculated plants were

examined regularly and the resulting symptoms were recorded.

DNA extraction and RT-PCR

Total RNA was extracted from the *N. glutinosa* plants inoculated with the five PVV isolates using the High Pure Viral nucleic acid kit (Roche Biochemical, Germany). The first strand cDNA was synthesized with Moloney murine leukemia virus (MMLV) reverse transcriptase as described as by Sharifi et al. [11]. The resulting cDNA was used as template to assemble a full genome by PCR using pairs of primers with the Fast PCR program (<http://www.Biocenter.helsinki.fi/bi/programs/fastPCR.html>). The PCR parameters used were as follows: an initial denaturation at 98 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 10 s, annealing at appropriate temperatures for 30 s, and extension at 70 °C for 2 min, and a final 10 min extension at 70 °C. A primer specific (F13 and R13, Supplementary Fig. 1) were used to amplify CP gene of four PVV Iranian isolates from Kerman province isolated from Zardi and Agria cultivars. Amplification of CP regions of Iranian PVV isolates was performed with the following programs: initial denaturation for 3 min at 94 °C, followed by 35 cycles of denaturation for 60 s at 92 °C, annealing for 60 s at 56 °C, extension at 72 °C for 90 s and final extension at 72 °C for 10 min. The 3' terminus of the PVV sequence was determined using a 3' RACE kit (Roche) according to the manufacturer's instructions.

Cloning, sequencing and sequence analysis

Products obtained from RT-PCR amplifications were cloned into a pTZ57R/T vector using InsT/Aclone PCR Product Cloning Kit (Fermentas, USA) and transformed into *Escherichia coli* strain DH5 α . Recombinant plasmids were sequenced at Macrogen Inc. (South Korea) by dideoxy nucleotide chain termination method using universal primers for the plasmid. Both nucleotide and deduced amino acid sequences of Iranian isolates were compared with the counterpart sequences of other PVV isolates in the GenBank database using DNAMAN software package (Lynnon, Biosoft, Quebec, Canada).

CP and P1 gene sequences were aligned using MUSCLE (Edgar, 2004) implemented in MEGA 5 [12]. Pairwise identities were calculated with pairwise deletion of gaps using SDT 1.0 [13]. Maximum likelihood phylogenetic analysis was carried out using PHYML 3.0 [14] with GTR+I+G4 (CP gene dataset) and GTR+G4 (P1 gene dataset) as the best substitution models selected by RDP4 [15], with 1,000 bootstrap replicates. Branches with less than 50 % branch support were collapsed. The trees were

Table 1 Occurrence of potato viruses on potato cultivars collected from different regions of Iran

Province	Region	Cultivars	Number of samples	DAS-ELISA ^a				Single		Double		Triple			
				PVV	PVY	PVA	PVS	PVX	PVA	PVY+	PVA+	PVY+	PVY+	PVA+	PVA+PVY+PVX
Esfahan	Fereydunshahr	<i>S. tuberosum</i> cv. Picasso	18	0	6	2	3	1	6	2	0	3	1	0	0
Esfahan	Faridan	<i>S. tuberosum</i> cv. Maradonna	10	0	2	5	1	1	2	2	1	0	0	1	1
Esfahan	Chadegan	<i>S. tuberosum</i> cv. Agria	12	0	4	1	3	2	6	1	0	3	0	0	0
Kerman	Jiroft	<i>S. tuberosum</i> cv. Maradonna	11	0	5	3	1	6	9	3	0	0	2	1	0
Kerman	Jiroft	<i>S. tuberosum</i> cv. Sante	9	0	5	1	2	4	8	0	0	0	4	0	0
Kerman	Kerman	<i>S. tuberosum</i> cv. Agria	12	0	6	2	1	5	7	1	0	0	5	1	0
Kerman	Zarand	<i>S. tuberosum</i> cv. Agria	10	0	5	4	1	2	5	4	0	0	0	2	1
Kerman	Bardsir(lalehzaar)	<i>S. tuberosum</i> cv. Agria	18	3	9	2	9	5	17	2	0	2	5	2	0
Fars	Shiraz	<i>S. tuberosum</i> cv. Agria	9	0	1	3	1	1	2	1	1	0	0	0	1
Fars	Eqlid	<i>S. tuberosum</i> cv. Marfona	8	0	2	4	2	1	5	2	2	0	0	0	0
Fars	Qeshlaq	<i>S. tuberosum</i> cv. Agria	9	0	1	1	3	0	3	1	0	0	0	0	0
Fars	Eqlid	<i>S. tuberosum</i> cv. Agria	8	0	0	2	3	1	3	0	2	0	0	0	1
Hamedan	Kabodarahang	<i>S. tuberosum</i> cv. Marfona	14	0	4	1	2	1	4	1	0	2	1	0	0
Hamedan	Asadabad	<i>S. tuberosum</i> cv. Mondial	14	0	3	2	1	0	2	2	0	1	0	1	0
Khorasan	Tabadkan	<i>S. tuberosum</i> cv. Agria	9	0	4	1	2	2	3	0	0	2	2	1	1
Khorasan	Chenaran	<i>S. tuberosum</i> cv. Agria	10	0	2	3	1	1	4	2	1	0	0	0	0
Khorasan	Torbat-jam	<i>S. tuberosum</i> cv. Marfona	9	0	6	1	2	3	5	1	0	2	3	1	0
Khorasan	Torbat-heydariyeh	<i>S. tuberosum</i> cv. Marfona	10	0	6	2	1	1	3	2	1	1	1	1	1
Ardabil	Ardabil-Garehlar	<i>S. tuberosum</i> cv. Agria	16	0	5	3	2	2	7	3	0	2	0	0	0
Ardabil	Ardabil-Garjan	<i>S. tuberosum</i> cv. Draga	13	0	3	2	1	0	2	2	0	1	0	1	0
Ardabil	Ardabil-Gilandehe	<i>S. tuberosum</i> cv. Kayzer	11	0	1	3	2	3	6	1	2	0	0	0	0
Commercial Cultivars			240	3	80	48	44	42	108	33	10	19	24	12	6
Kerman	Zarand	<i>S. tuberosum</i> cv. Zardi	210	69	75										

^a Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for Potato virus V (PVV), Potato virus Y (PVY), Potato virus A (PVA), Potato virus S (PVS) and Potato virus X (PVX)

rooted with *Wild potato mosaic virus* (AJ437279) and *Peru tomato mosaic virus* (AJ437280).

Results

Potyvirus detection in potato samples

A local cultivar (Zardi) and eight commercial cultivars of potato were surveyed and tested in this study. PVV, PVA, PVS, PVY and PVX were detected in various cultivated *Solanum tuberosum* cultivars using specific monoclonal antibodies (Table 1). *Solanum tuberosum* cv. Agria was the highest sampled cultivar in our study (~47 % of the 240 samples) and within these we found an incidence of potyvirus infection of 32.7 % for PVY, 19.5 % for PVA, 23 % for PVS, 18.6 % for PVX and 2.7 % for PVV. In the remaining seven cultivars we noted infections with PVY, PVA, PVS and PVX. PVX was detected in all the cultivars except *S. tuberosum* cv. Draga and cv. Mondial. It was interesting to note that PVV was only detected in one of the commercial potato varieties, *S. tuberosum* cv. Agria with only three of the 113 samples testing positive. We also detected multiple infections in the eight commercial varieties (Table 1). In summary, of the 240 potato samples collected from six provinces, based on DAS-ELISA results, 33.3 % (80), 20 % (48), 18.3 % (44), 17.5 % (42) and 1.3 % (3) were infected with PVY, PVA, PVS, PVX and PVV, respectively. Double and triple infections involving different combinations of PVY, PVA, PVX and PVS were noted in 35.8 % (86) and 7.5 % (18) samples, respectively. In all commercial cultivars, non-symptomatic to severe mosaic was observed to be induced by PVY and against PVS we observed severe mosaic, mottling and leaf malformation against PVS. PVA infection in potato cultivars was

identified as mild mosaic and yellowing symptoms, whereas PVX showed blistering and malformation symptoms.

A local potato cultivar *S. tuberosum* cv. Zardi growing in Zarand region (Kerman province) was found to be severely infected by PVV. On the basis of ELISA assays, of the 210 potato leaves sampled, we found an incidence of 32.9 % PVV infection and 35.7 % PVY infection. No infection against PVA, PVS and PVX was detected. Additionally, we noted that in general the concentration of PVV (based on DAS-ELISA) in samples was far higher in *S. tuberosum* cv. Zardi than in cv. Agria. The symptoms of PVV on infected leaves of this local potato cultivar were generally mosaic, blistering and vein clearing (Fig 1).

Biological characteristics of the PVV isolates

Five PVV isolates including four from *S. tuberosum* cv. Zardi and one from *S. tuberosum* cv. Agria were chosen as representative for further infectivity studies and characterisations. All five PVV isolates were successfully inoculated on *N. glutinosa* plants, causing systemic vein clearing and mosaic. *N. debneyi* showed vein banding in the inoculated leaves after inoculation with the five PVV isolates. Whereas the leaves of *N. tabacum* cv. Samsun NN, *N. tabacum* cv. Turkish, *N. Clevelandi*, *tabacum* T. cv. whiteburley, *N. benthamiana*, *Datura metel*, *D. stramonium*, *Chenopodium amaranticolor* and *Gomphrena globosa* did not show any symptoms to PVV inoculations.

Sequence analysis of full PVV genome

The nearly complete sequence of the Iranian isolate comprised of 9,812 nucleotides (nt) excluding 72 nucleotide from the 5'-terminal sequence was determined and deposited in the GenBank with the accession number (KC433411). This

Fig. 1 Leaf (a) and plant (b) of potato Iranian local cultivar showing mosaic, blistering and vein clearing symptoms infected by *potato virus V*

isolate (KER.LAL.P) shares 91 % pairwise identity with the only reported complete genome of PVV isolate DV-42 (AJ243766) from Scotland (complete genome). Like isolate DV-42, the Iranian isolate has a large open reading frame (ORF) of 9,198 nt with an AUG (153–157 nt) start codon and a UAG stop codon (9,351–9,354 nt). The 5' untranslated region (UTR) is 152 nt and the 3' UTR 462 nt, followed by a poly-A tail. The base composition of the viral genomic RNA is adenine 30.9 %, cytosine 19.1 %, guanine 23.7 % and uracil 26.2 %. The 5' UTR has a high AU content (63.2 %) and a low GC content (36.8 %) similar to other potyviruses. The ORF encodes a polyprotein of 3,066 amino acids (aa) and has nine

putative proteinase cleavage sites yielding ten proteins: P1 (289 aa), HC-Pro (456 aa), P3 (358 aa), 6K1 (52 aa), CI (634 aa), 6K2 (52 aa), VPg (188 aa), NIa (246 aa), NIb (519 aa) and CP (272 aa). The pairwise identities to other related potyviruses at genomic and at individual gene/region level are summarized in Fig. 2.

Sequence analysis of the CP and P1 genes

RT-PCR amplicons, 808 bp in length were obtained using the primer set PVV-F13 and PVV-R13 (Supplementary Fig. 1) which flank most of CP gene of PVV with

Fig. 2 Schematic representation of the genome of the PVV isolates and pairwise comparisons of genomes, regions and genes of the Iranian PVV isolate to other potyviruses

PVV -KER.LAL.P [KC433411]			P1		HC-Pro		P3	6K1	CI		6K2	VPg	NIa-Pro		NIb		CP	
Genome wide																		
PVV -DV42 [NC_004010]	91.0	94.1	79.3	81.0	92.4	94.2	94.4	97.4	93.1	93.2	93.3	96.5	97.1					
PVY [HM367075]	61.0	51.9	50.2	61.6	50.4	64.7	65.5	60.0	67.4	65.0	66.1	67.0	47.6					
PVA [NC_004039]	53.8	43.8	43.5	54.7	42.7	55.5	55.9	47.7	53.0	56.7	59.5	63.0	40.4					
ZYMV [AF014811]	53.2	46.1	37.6	50.9	43.6	55.0	54.8	54.7	52.8	54.1	59.3	60.8	35.6					
WMV [EU660584]	52.6	46.0	37.6	50.2	43.7	52.1	56.4	48.3	54.2	53.7	59.0	60.2	46.3					
BCMV [EU761198]	52.3	33.9	35.9	48.7	45.3	48.4	57.4	45.8	53.0	51.9	58.5	61.3	43.5					
TuMV [DQ648591]	51.3	59.4	43.3	52.2	44.9	57.7	58.5	51.6	56.0	53.0	60.8	61.5	43.6					
BYMV [FJ492961]	50.9	50.9	43.8	52.8	45.6	53.0	56.2	56.3	56.7	52.2	59.9	62.8	40.2					
PRSV [HQ424465]	50.5	40.3	42.4	51.6	43.4	59.0	57.7	51.0	51.8	51.0	59.8	59.4	37.9

Fig. 3 Maximum likelihood phylogenetic analysis of the Coat protein genes of *potato virus V* isolates from around the world with their pairwise nucleotide comparisons (calculated using Muhire et al. [13])

Fig. 4 Maximum likelihood phylogenetic analysis of the P1 genes of PVV isolates from around the world their pairwise nucleotide comparisons (calculated using Muhire et al. [13])

exception of the 5'-terminal sequences of 8 nucleotides. The coat protein-coding sequences from the five PVV Iranian isolates (Fig. 3) were aligned with other PVV coat protein-coding sequences ($n = 29$) available in the GenBank. The five Iranian isolates share >96.5 % pairwise nucleotide identity. In contrast, these five isolates share 93.8–97.1 % pairwise identity with all other PVV isolates other than the three divergent isolates from Peru and USA (AJ516021, AJ516022 and AY521595). The maximum likelihood phylogenetic analysis of the CP gene clearly reveals the distinction between the European isolates, the Middle-East and American isolates. The CP of the USA isolate PA-4 is the most divergent sharing ~86 % pairwise identity with all the other PVV isolates whereas the two Peruvian isolates PA1.1 and PA1.0 share ~90 % pairwise identity with all other PVV isolates (Fig. 3).

Discussion

In previous studies, based on the CP sequence no distinct groups between PVV isolates collected from European countries were observed [1, 3]. However in this study since isolates from other countries including Iranian and Peruvian isolates were added and therefore existence of at least three more distinct groups of PVV isolates can be demonstrated (Fig. 3). In contrast to the CP gene, the P1 gene of the Iranian isolates is the most divergent based on current sequence information (Fig. 4) sharing ~77.2–80 % pairwise identity with all other P1 gene sequences. All the P1 genes of the European isolates share >97 % identity. The highly divergent P1 gene of the Iranian isolate may suggest that it is recombinant, however, at this stage we are

unable to analyse for recombination since only two full genomes of PVV are now available. Host range and symptoms have been used in the identification and differentiation of strains and pathotypes of viruses [16, 17]. Sap inoculation of five isolates of PVV onto *N. debneyii* and *N. glutinosa* showed similar symptoms, suggesting that they are closely related.

In summary, in this study we have attempted to survey various potato cultivars in Iran for potyviruses, in particular PVV. We note that the local variety *S. tuberosum* cv. Zardi is more susceptible to PVV infection than commercial varieties. The genomic sequence of an Iranian isolate KER.LAL.P shares 91 % pairwise identity with the Scottish PVV isolate DV-42 (AJ243766). The CP gene analysis of the five isolates of PVV characterized in this study share >95 % pairwise identity amongst themselves and phylogenetic analysis of the CP indicates that the Iranian isolates group in a distinct clade compared to the European and American isolates. Overall we note that throughout our sampling regions there are multiple infections of potyviruses in the commercial potato varieties.

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