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(% / ) (% / ) (% / )  
IRN-HK2 IRN-HB1  
(Eurasia)  
PVX  
) /  
(FJ461343 ) / (X88785  
mid-Eurasia PVX

### Biological and molecular characterization of two *Potato virus X*- isolates from Hamedan province

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#### Abstract

The main potato growing areas in Hamadan province including Bahar, Razan and Kabodar-Ahang were surveyed during 2010 and 2011 to find distribution of *Potato virus X*. A total of 426 leaf samples (132 and 324 symptomatic and random, respectively) were collected from 9 potato fields. Totally 42 random samples were showed positive reaction with PVX specific antibodies in ELISA assay. The highest virus incidence was recorded in Bahar (18.3 %), followed by Razan (12.5 %) and Kabodar Ahang (9.2 %). Coat protein gene (CP) of two Iran-HB1 and Iran-HK2 PVX isolates from Bahar and Kabodar-Ahang districts, respectively, were amplified (size about 750 bp) by reverse transcriptase polymerase chain reaction (RT-PCR) using specific primers. Evolutionary relationship demonstrated that Iranian isolates fell into Eurasia group. These two isolates with other Iranian PVX isolate from *Pisum sativum* are closely related together with high bootstrap support. The complete CP nucleotide sequences of Iranian potato isolates were 714 nucleotides long, encoding an ORF with 237 amino acids as previously reported for isolates belong to Eurasia group. The lowest (% 78.7) and highest (% 99.2) identities were found with Netherland (group II) and Iranian (group I) isolates with accession numbers X88785 and FJ461343, respectively, whereas for two Iranian isolates the identities were 100%. Although Iranian isolates were found in the Eurasia population we do not know yet whether these are dominant isolates in this region or not. However, evolutionary comparisons of a large number of isolates from Asia Minor and mid-Eurasia with representative worldwide isolates are necessary to determine this. The present study to our knowledge shows for the first time, the evolutionary relationships of PVX from potato collected in the mid-Eurasian region of Iran.

**Key words:** *Potato virus X*, Phylogeny, Iran.

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( )  
*Alphaflexiviridae* *Potexvirus* (*Solanum tuberosum* L.)  
 (Adams *et al.*, 2005; Adams *et al.*, 2012) *Tymovirales*  
 (*S. tuberosum*) (FAO, 2009)  
 Smith (1931)  
 PVX ( )  
 PVX (Loebenstein *et al.*, 2001)  
 (FAO, 2009)  
 (Bostan and Halioglu, 2004)  
 (ssRNA) RNA / / /  
 Baratova *et al.*, 1992; Parker *et al.*, 2002; ) (Anonymous, 2008)  
 (Varma *et al.*, 1968)  
 (Open reading frame-ORF)  
 (Skryabin *et al.*, 1988)  
 (coat protein-CP)  
 (Adams *et al.*, 2012)  
 (Huisman *et al.*, 1988; Santa-Cruz *et al.*, 1998) (Stevenson *et al.*, 2004)  
 (Abel *et al.*, 1986) (Loebenstein *et al.*, 2001)  
 ( ) *Potato virus S-* (*Potato virus Y-PVY*)  
 (*Potato virus A-PVA*) (*Potato virus M-PVM*) (PVS  
*Potato leaf roll*) (PVX)  
*Rx1 Nx Nb* PVX (virus- PLRV)

PVX  
 $Nb \quad Nx$   
 ( )  
 ( )  
 ( ) (hypersensitivity-HR)  
 HR  
 $Nx \quad Nb$   
 (Pourrahim *et al.*, 2007)  
 HR  
 $Nb \quad Nx$   
 $Nb \quad Nx$   
 Cockerham, 1955; Belinda and ) HR  
 (Roger, 2010)  
 PVX  
 (Loebenstein *et al.*, 2001)  
 (Belinda and Roger, 2010) X B  
 X  
 B  
 PVX Bii Bi  
 X B  
 $Nx$   
 (  $Nx$  )  
 X CP  
 $Nx$  )  
 (Goulden *et al.*, 1993) B CP (HR  
 PVX  
 ( )



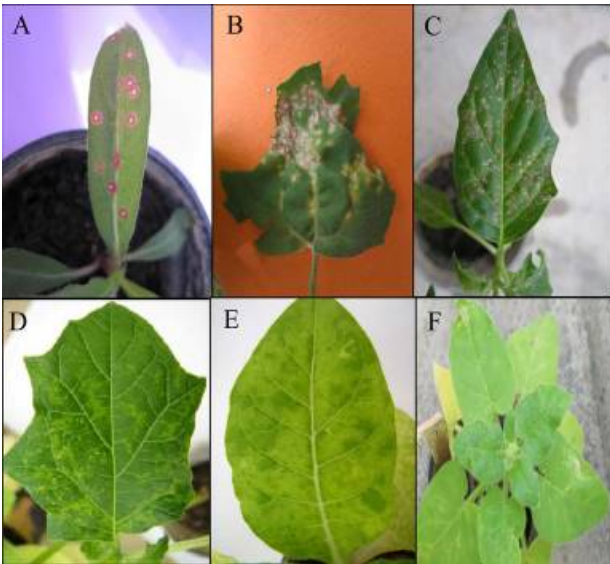
) RNase inhibitor  
 ( (Revert Aid MMuLV)  
 PVX (cDNA)  
 ( PVY PVX  
 MgCl2  
 dNTP  
 ) ( ) Taq  
 ( ) ( )  
 PVX ( / )  
 ( / ) ( / )  
 ( ) ( / )  
 PLRV  
 PVY PVX PVS MWG Primus  
 PVX  
 PVY PCR clean ( MN) up  
 PVX ( / )  
 PVX  
 Wisconsin Madison DNASTAR ( ) Megalign  
 ( A ) (*G. globosa*) (bootstrap) Neighbor Joining-NJ  
 ( B ) (*Ch. amaranticolor*)  
 ) (*D. stramonium*)  
 ( C ) (Burland, 2000)  
 ( D )

(IRN-HK2 IRN-HB1) PVX

( )

IRN-HK2 IRN-HB1

JX905356 JX905355



IRN-HK2

**Fig. 3.** Hosts reaction after mechanical inoculation by IRN-HK2

- A
- B
- C
- D
- E
- F

- A. Necrotic local lesions in *Gomphrena globosa*
- B. Necrotic local lesions in *Chenopodium amaranticolor*
- C. Necrotic local lesions in *Datura stramonium*
- D. Mosaic symptom in *Datura stramonium*
- E. Mosaic symptom in *Nicotiana tabacum* cv. White Burly
- F. Mosaic symptom in *Nicotiana glutinosa*

*N. tabacum* )

(*N. glutinosa*)

(cv. White Burly

( F E )

(*N. tabacum* cv. Samsun)

*Capsicum Solanum lycopersicum*

*S. Solanum tuberosum* cv. Agria *N. debneyi annuum*

*tuberosum* cv. Marphona

*Cucumis*

*Citrullus lanatus C. melo* cv. Gold Seed *sativus* cv. Green

*Vigna unguiculata* cv. Mashhad *Phaseolus vulgaris* cv. Sefid

IRN-HK2 IRN-HB1



**Fig. 2.** Mosaic and Necrotic symptoms in potato plant collected from Hamadan province

**Table 1.** Total infection rates (percent) of the studied viruses in random and symptomatic samples

PVY		PVX		PVS		PLRV		Region/No of samples	
R	S	R	S	R	S	R	S <sup>1</sup>		
20.4	51.9	18.3	21.2	16.1	28.8	5.4	15.4		(S ) (R ) Bahar (93 R/52 S)
15.2	51.2	12.5	12.2	13.4	43.9	3.6	9.8	(S ) (R ) Razan (112 R/41 S)	
12.6	55.1	9.2	14.3	14.3	36.7	4.2	14.3	(S ) (R ) Kabodar-ahang (119 R/49 S)	
15.7	56.8	12.9	17.4	14.5	38.6	4.3	14.4	(S ) (R ) Total (324 R/132 S)	
(random samples)				:R (symptomatic samples)				.S	

**Table 2.** Single and mixed infection rates (percent) of the studied viruses in random and symptomatic samples

Mixed infection rate of 3 and 4 viruses			Mixed infection rate of 2 viruses			Single infection rate								Region/No of samples	
%infection		Virus	%infection		Virus	PVY		PVX		PVS		PLRV			
R	S		R	S		R	S	R	S	R	S	R	S		
0	0	X+Y+LR	3.2	11.5	X+Y										
1.1	0	Y+LR+S	0	0	X+LR										(R )
1.1	0	X+Y+S	1.1	0	X+S										(S )
0	0	X+LR+S	0	9.6	Y+LR	15.1	17.3	12.9	9.6	10.8	13.5	3.2	3.8		
0	0	X+Y+LR+S	1.1	13.5	Y+S										
0	0		1.1	1.9	LR+S										Bahar (93 R/52 S)
0.9	0	X+Y+LR	1.8	2.4	X+Y										
0	2.4	Y+LR+S	0	0	X+LR										(R )
0	2.4	X+Y+S	1.8	2.4	X+S										(S )
0	0	X+LR+S	0.9	2.4	Y+LR	9.8	22.0	8.0	4.9	9.8	17.1	1.8	4.9		
0	0	X+Y+LR+S	1.8	19.5	Y+S										
0	0		0	0	LR+S										Razan (112 R/41 S)
0	2.0	X+Y+LR	1.7	4.1	X+Y										
0	0	Y+LR+S	0	0	X+LR										(R )
0	0	X+Y+S	0.8	0	X+S										(S )
0.8	0	X+LR+S	0	6.1	Y+LR	7.6	26.5	5.0	8.2	10.1	20.4	2.5	6.1		
0	0	X+Y+LR+S	2.5	16.3	Y+S										
0	0		0	0	LR+S										Kabodar-ahang (119 R/49 S)
0.6	0.7	X+Y+LR	2.2	6.8	X+Y										
0.3	0.7	Y+LR+S	0	0	X+LR										(R )
0.3	0.7	X+Y+S	1.2	0.7	X+S										(S )
0.3	0	X+LR+S	0.3	6.8	Y+LR	10.5	23.5	8.3	8.3	10.2	18.2	2.5	5.3		
0	0	X+Y+LR+S	1.9	17.4	Y+S										
0	0		0.3	0.7	LR+S										Total (324 R/132 S)

PLRV PVS PVY PVX

PVX

( / )

.( )

PVX

)

.(Johnson, 2007)

(

PVX PVS

( / ) PVY

/ / / PLRV

PVX

) / /

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PVX

PVX

(Group II-America) (Group I-Eurasia)

.( )

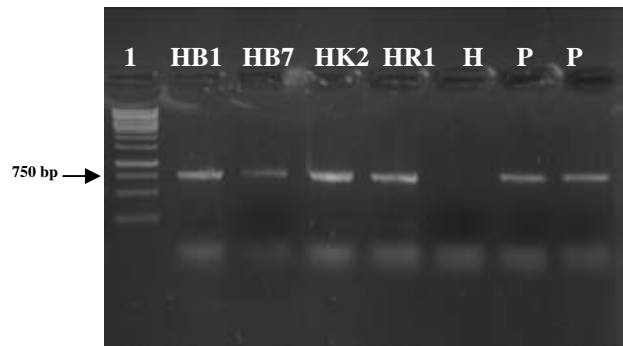
PVX

Esfandiari *et al.*, ) (FJ461343)

PVX

(Group I-Eurasia)

(2006



PCR

GeneRuler™ 100bp DNA Ladder Plus, )

(Farmentas, Lithuania

HR1 (HK2)

(HB7 HB1)

PVX

( )

**Fig 4.** Electrophoresis of PCR products corresponding to PVX coat protein gene amplified using specific primers. Columns 1: Molecular weight marker (GeneRuler™ 100bp DNA Ladder Plus, Farmentas, Lithuania), 2-5: Infected samples, HB1 and HB7 from Bahar, HK2 from Kabodarahang and HR1 from Razan areas which resulted to an amplicon of about 750 bp, H: Negative (healthy) control and P: Positive (infected) controls.

PVX



Eurasia

PVX

(Cox and Jones, 2010)

PVX

PVX

(Fribourg, 1975; Bercks, 1970)

(Cox and Jones, 2010; Yu *et al.*, 2008; 2010)

PVX

America)

(America)

(Bercks, 1970)

(Subgroup II-1)

(group

(*Solanum lycopersicum*)

Bercks, 1970; )

PVX

(Subgroup II-2)

*D. stramonium*

(Sutic *et al.*, 1999

Loebenstein )

PVX

PVX

(*et al.*, 2001

(II-2)

(Cox and Jones, 2010)

IRN-HB1)

PVX

PVX

(IRN-HK2

(Malcuit *et al.*, 2000)

*Tobacco mild green mosaic virus*

PVX

(Esfandiari *et al.*, 2006)

(TMGMV)

(Fraile *et al.*, 1997)

PVX

( )

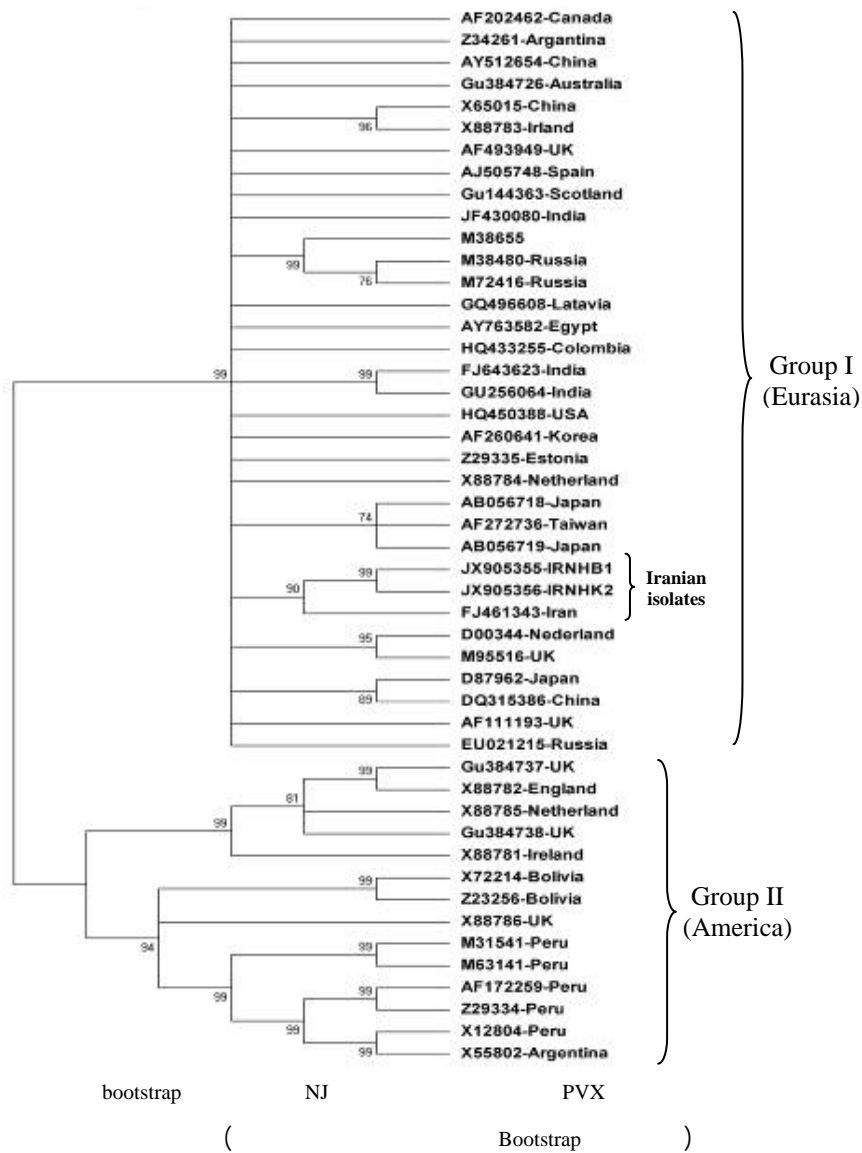
( / )

(Yu *et al.*, 2010)

(X

) (Eurasia)

(Belinda and Roger, 2010)



**Fig 5.** Phylogenetic tree of Iranian PVX isolates based on CP gene reconstructed using NJ method and 1000 bootstrap value (Values > 70% are shown)

PVX

Eurasia

.(Cox and Jones, 2010)

( )

.(Laufer, 1938)

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