

\*

✉

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

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

N. SHOKROLLAHI<sup>1</sup>, R. POURRAHIM<sup>2</sup>✉, SH. FARZADFAR<sup>2</sup> and S. NAZARI<sup>2</sup>

1- Former MSc student of Islamic Azad University of Damghan, Iran

2- Iranian Research Institute of Plant Protection, P. O. Box. 1454, Tehran 19395, Iran

#### 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.

\*

✉ Corresponding author: pourrahim@yahoo.com

( )

*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)

( )

*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)

Archive of SID

( )  
 ( )  
 ( )

PVX  
*Nb Nx*  
*Nx , Nb*  
 (hypersensitivity-HR)

HR  
*Nx Nb*  
 (Pourrahim *et al.*, 2007)

HR  
*Nb 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

PVA PVY

PVX

( )

*Nicotiana tabacum* L. )

(*N. tabacum* L. cv. Samsun) (cv. White Burley

*Chenopodium* ) (*N. rustica*) (*N. glutinosa* L.)

*Lycopersicon esculentum* ) (*amaranticolor*

(Mill.

(Loebenstein *et al.*, 2001)

IRN-HK2 IRN-HB1

PVX

)

(

(CP)

Reverse Transcriptase-Polymerase )

CP

(Chain Reaction; RT-PCR

( ) Jones Cox

Tri-reagent

( )

PVX

(Pourrahim *et al.*, 2009)

MMuLV-10X

(down primer)

dNTP

DEPCE H2O

/

PVX

/

(*Datura stramonium*)

( ) PLRV PVS PVY PVX

( ) DAS-ELISA

(Clark and Adams, 1977)

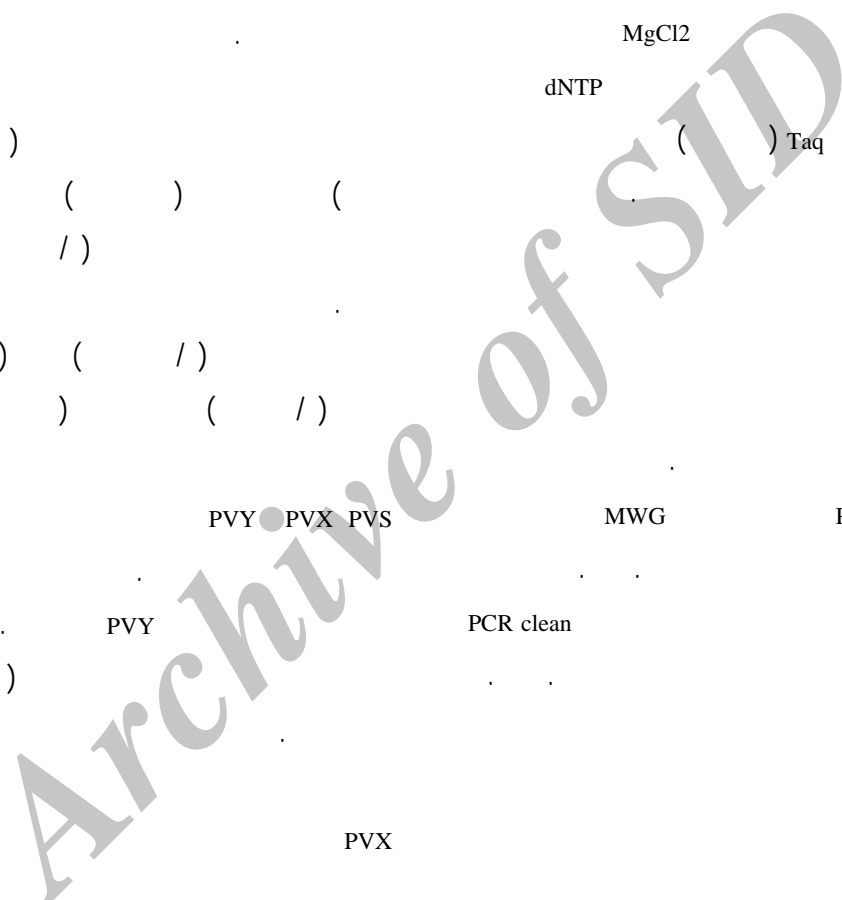


Fig. 1. Map of Hamadan province (the surveyed areas (Bahar, Razan and kabodar Ahang) were identified by star)

PVX

PVX

) RNase inhibitor  
 ( (Revert Aid MMuLV)  
 PVX (cDNA)  
 ( )  
 PVY PVX  
 MgCl<sub>2</sub>  
 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 )

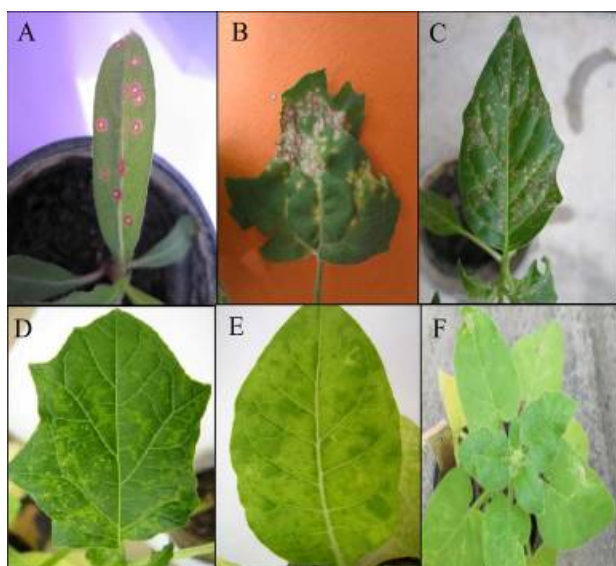


(IRN-HK2 IRN-HB1) PVX

( )

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										
			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	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	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										
			0.3	0.7	LR+S										Total (324 R/132 S)

PLRV PVS PVY PVX

PVX

PVX

( / )

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

.( )

.( )

PVX

Esfandiari *et al.*, ) (FJ461343)

PVX

(Group I-Eurasia)

(2006

PVX

)

(

.(Johnson, 2007)



PCR

PVX PVS

( / ) PVY  
PLRV

GeneRuler™ 100bp DNA Ladder Plus, )

(Farmentas, Lithuania

HR1 (HK2)

(HB7 HB1)

PVX

( )

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

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)

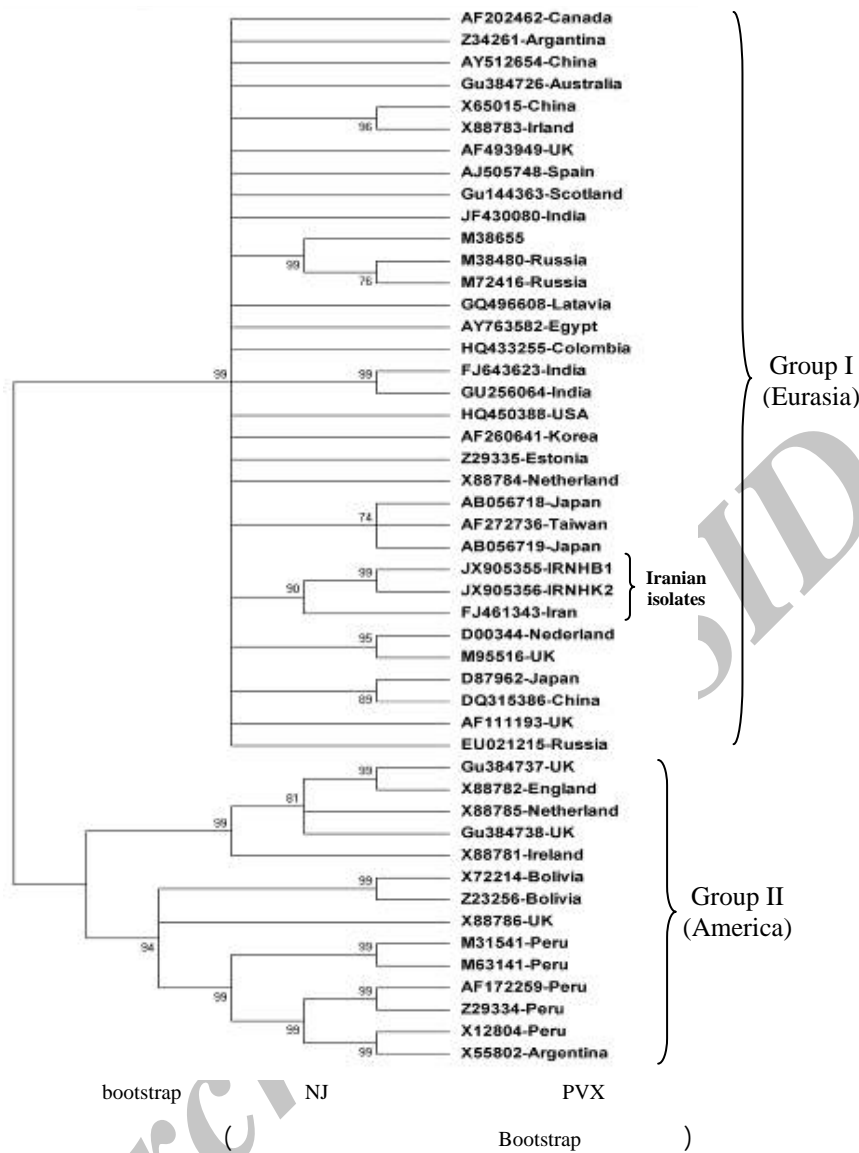


Fig 5. Phylogenetic tree of Iranian PVX isolates based on CP gene reconstructed using NJ method and 1000 bootstrap value (Values > 70% are shown)

PVX

Eurasia

(Cox and Jones, 2010)

( )

(Laufer, 1938)

## References

- ABEL, P. P., NELSON, R. S., De, B., HOFFMANN, N., ROGERS, S. G., FRALEY, R. T. and BEACHY R. N., 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* No. 232: 738–743.
- ADAMS, M. J., ACCOTTO, G. P., AGRANOVSKY, A. A., BAR-JOSEPH, M., BOSCIA, D., BRUNT, A. A., CANDRESSE, T., COUTTS, R. H. A., DOLJA, V. V., other authors, 2005. Genus *Potexvirus*. In *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1091–1095.
- ADAMS, M.J., CANDRESSE, T., HAMMOND, J., KREUZE, J.F., MARTELLI, G.P., NAMBA, S., PEARSON, M.N., RYU, K.H. VAIRA, A.M. 2012. Family *Alphaflexiviridae*. In: King A.M.Q., Adams M.J., Carestens E.B., Lefkowitz, E.J, *Virus Taxonomy Classification and Nomenclature of Viruses*. Ninth Report of The International Committee on Taxonomy of Viruses. ELSEVIER Academic Press San Diego, Calif. pp. 904-919.
- ANONYMOUS, 2008. Crop production year book 2008. Ministry of Jihad Agriculture, Deputy of Planning and Economy, Statistics and Information Technology Office, P 136.
- BARATOVA, L. A., GREBENSHCHIKOV, N. I., DOBROV, E. N., 1992. The organization of *Potato virus X* coat proteins in virus particles studied by tritium planigraphy and model building. *Virology*, No. 188: 175–180.
- BELINDA, A. C. and ROGER, A. C. J., 2010. Genetic Variability on the coat protein gene of *Potato Virus X* and the current relationship between phylogenetic placement and resistance groupings. *Archives of Virology*, No. 155: 1349-1356.
- BERCKS, R., 1970. *Potato virus X*. in *Descriptions of plant viruses*. Common W. mycol. Ins./Association of Applied Biologists England. No. 4: 4.
- BOSTAN, H. and HALILOGLU, K., 2004. Distribution of PLRV, PVS, PVX and PVY in the seed potato tubers in Turkey. *Pakistan Journal of Biological Science*. No.7:1140-1143.
- BURLAND, T. G. 2000. DNASTAR Lasergene sequence analysis software. *Meth. Mol. Biol.* No.132: 71-91.
- CLARK, M. F. and A. N. ADAMS, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* No.34: 475-83.
- COCKERHAM, G. 1955. Strains of *Potato virus X*. *Proceedings of the Second Conference on Potato Virus Diseases* (Lisse-Wageningen, 1954), pp. 89-92.
- COX, A. B. and R. A. C. JONES, 2010. Genetic variability in the coat protein gene of *Potato Virus X* and the current relationship between phylogenetic placement and resistance grouping. *Archives of Virology*, No. 155: 1346-1356.
- ESFANDIARI, N., KOHI-HABIBI, M., MOSAHEBI, G., 2006. Occurrence of viruses infecting pea in Iran. *Communication in Agriculture and Applied Biological Sciences*. No. 71: 1281-1287.
- FAO., 2009. FAO production year book. Available on line: [www. faostat.org](http://www.faostat.org).
- FRAILE, A., J. H., ALONSO-PRADOS, M. A., ARANDA, J. J., BERNAL, J. M., MALPICA AND, F., GARCIA-ARENAL., 1997. Genetic exchange by recombination or reassortment is infrequent in natural populations of a tripartite RNA plant virus. *Journal of Virology*, No. 71: 934–940.
- FRIBOURG, C. E., 1975. Studies on *Potato virus X* strains isolated from Peruvian potatoes. *Potato Research* No. 18: 216-226.
- GOULDEN, MG., BAULCOMBE, DC., 1993. Functionally Homologous Host Components Recognize *Potato Virus X* in *Gomphrena globosa* and Potato. *Plant Cell* No.5: 921–930.
- HUISMAN, M. J., LINTHORST, H. J. M., BOL, J. F. and CORNELISSEN, B. J. C., 1988. The complete nucleotide sequence of potato virus X and its homologies at the amino acid level with various plus stranded RNA viruses. *Journal of General Virology*. No. 69: 1789-1798.
- JOHNSON, D. A., 2007. *Potato Health Management*. American Phytopathology Society Press, 272.
- LAUFER, B., 1938. *The American Plant Migration*. Part 1: The Potato Anthropology Series. Field Museum of Natural History. Publication Chicago. 28, 14-18.
- LOEBENSTEIN, G., BERGER, P. H., BRUNT, A. A. and LAWSON, R. H., 2001. *Potato virus X* (PVX; Genus Potexvirus) Virus and virus-like diseases of potatoes and production of seed potatoes. Kluwer Academic

- Publishers. No. 25: 365-368.
- MALCUIT, I., MARANO, M. R., KAVANAGH, T. A., De JONG, W., FORSYTH, A., BAULCOMBE, D. C., 2000. The 25-kDa movement protein of PVX elicits Nb-mediated hypersensitive cell death in potato. *Molecular Plant Microb Interaction* 12, 536.
- PARKER, L., KENDALL, A. and STUBBS, G., 2002. Surface features of *Potato virus X* from fiber diffraction. *Virology*, No. 300: 291-295.
- POURRAHIM, R., FARZADFAR, Sh., GOLNARAGHI, A. R. and AHOONMANESH, A., 2007. Incidence and distribution of important viral pathogens in some Iranian potato fields. *Plant Disease*. No. 91: 609-615.
- POURRAHIM, R., S. FARZADFAR and A. R. GOLNARAGHI, 2009. Development and optimization of ELISA diagnostic Kit for detection of Iranian isolates of *Potato Virus X*. Seventh National Iranian Congress of Biotechnology, 11-13 September, Tehran, Energy Research.
- SANTA-CRUZ, S., ROBERTS, A. G., PRIOR, D. A., CHAPMAN, S. and OPARKA, K. J., 1998. Cell-to-cell and phloem-mediated transport of *Potato virus X*. The role of virions. *Plant Cell* No. 10: 495-510.
- SKRYABIN, K. G., MOROZOV, S. Y., KRAVE, A. S., ROZANOV, M. V., CHERNOV, B. K., LUKASHEVA, L. I. ATABEKOV, J. G., 1988. Conserved and variable elements in RNA genomes of potexviruses. *FEBS Letters*. No. 240: 33-40.
- SMITH, K. M., 1931. *Potato virus X*. *Proceeding of Royal Society*, No. 109: 251.
- STEVENSON, W. R., LORIA, R., FRANC, G. D. and WEINGARTNER, D. P. (eds.), 2004. *Compendium of potato diseases*, 2<sup>nd</sup> edn. APS Press, St. Paul., Minnesota The Netherlands. 300, 269-281
- SUTIC, D. D., Ford, R. E. Tosic, M. T., 1999. *HANDBOOK OF PLANT VIRUS DISEASE*. CRC Press LLC, 551pp.
- VARMA, A., GIBBS, A. J., WOODS, R. D. and FINCH, J. T., 1968. Some Observations on the Structure of the Filamentous Particles of Several Plant Viruses. *Journal of General Virology* No. 2: 107.
- YU, X-Q., JIA, J-L., ZHANG, C-L., LI, X-D. WANG, Y-J., 2010. Phylogenetic analyses of an isolate obtained from potato in 1985 revealed *Potato virus X* was introduced to China via multiple events. *Virus Genes*. No. 40: 447-451.
- YU, X-Q., WANG, H-Y., LAN, Y-F., ZHU, X-P., LI, X-D., FAN, Z-F., LI, H-F., WANG, Y-Y. 2008. Complete sequencing of a Chinese isolate of *Potato virus X* and analysis of genetic diversity. *Journal of Phytopathology* 156: 346-351.

Archive