Molecular characterization and field survey of Iranian potato virus X isolates

Hossain Massumi · Soodabe Poormohammadi · Shabnam Pishyar ·
Mohammad Maddahian · Jahangir Heydarnejad · Akbar Hosseini-Pour ·
Katherine van Bysterveldt · Arvind Varsani

Received: 5 January 2014 / Accepted: 8 May 2014 / Published online: 25 May 2014
© Indian Virological Society 2014

Abstract Six hundred and one symptomatic potato samples were collected from nine provinces in Iran. Screening by double-antibody sandwich enzyme linked immunosorbent assay using a potato virus X (PVX) together with a few potyviruses polyclonal antibodies, produced positive reactions in 4.3 % of samples against PVX. Based on symptoms on different test plant, the isolates were divided into two groups: the first groups causing blistering and malformation of leaves and the second showed mild mosaic and vein clearing in *Nicotiana glutinosa*. The almost complete nucleotide sequence of two isolates as a representative of severe and a mild isolates were determined. Genomes of two PVX Iranian isolates are identical to that of the most PVX isolates comprise 6435 nucleotides in length excluding 101 nucleotide in the 5' end of the genome and shares 94.8–96.7 % identities with European and Asian, and

77–96.1 % with American isolates. Furthermore, the 3'-terminal sequences, including the coat protein coding region of other 13 Iranian isolates were determined and compared with the GenBank sequences. Phylogenetic analysis of the *cp* gene of 13 Iranian isolates together all those available in public databases indicated that the 13 Iranian isolates all belong to low diversity clade I.

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

Introduction

Potato virus X (PVX) is the type species of the family *Alphaflexiviridae* (Genus *Potexvirus*) and its virions are flexuous filaments. The 5' end has a methylguanosine cap whereas the 3' end has a poly (A) tail [17]. PVX is primarily mechanically transmitted (e.g. plant to plant contact, seed graders and cutters etc.) [1]. PVX has a ssRNA genome of approximately 6,435 nt in length which encodes five open reading frames (ORFs). ORF 1 encodes the RNA-dependent RNA polymerase (RdRp; 166 kDa), which is required for replication and the production of three subgenomic RNAs. ORFs 2–4 are known as the triple gene block (TGB), encoding three polypeptides of 25, 12, and 8 kDa, respectively and are involved in viral movement [16, 24]. The TGB proteins are highly conserved in members of the genera *Potexvirus*, *Hordeivirus*, *Benyvirus* and *Carlavirus* [6, 15, 23]. ORF5 encodes the capsid protein (CP) with an estimated molecular mass of 25 kDa and plays an significant role in cell-to-cell movement of PVX particles and translational regulation of the PVX RNA genome [10, 14]. The CP gene is widely used for identification and classification of PVX isolates [3, 25, 26].

Electronic supplementary material The online version of this article (doi:10.1007/s13337-014-0222-z) contains supplementary material, which is available to authorized users.

H. Massumi (✉) · S. Poormohammadi · S. Pishyar ·
M. Maddahian · J. Heydarnejad · A. Hosseini-Pour
Department of Plant Protection, College of Agriculture, Shahid
Bahonar University of Kerman, Kerman, Iran
e-mail: masoomi@mail.uk.ac.ir

K. van Bysterveldt · A. Varsani
School of Biological Sciences, University of Canterbury,
Private Bag 4800, Christchurch 8140, New Zealand

A. Varsani
Biomolecular Interaction Centre, University of Canterbury,
Private Bag 4800, Christchurch 8140, New Zealand

A. Varsani
Electron Microscope Unit, Division of Medical Biochemistry,
Department of Clinical Laboratory Sciences, University of Cape
Town, Cape Town 7925, South Africa

PVX is one of the several viruses causing diseases in commercially important potato cultivars with yield reduction of between 10 and 50 % in certain potato cultivars and varieties [1, 9]. In general, potyviruses such as *Potato virus Y* (PVY), *Potato virus A* (PVA), *Tobacco vein mottling virus* (TVMV) and *Tobacco etch virus* (TEV) are found co-infecting with PVX in various solanaceous plants [8, 12, 25] and show synergistic disease.

Currently only 25 complete genomes of PVX and 157 sequences of the CP gene are available in public databases. Interestingly, despite the global distribution of PVX with significant crop losses, there is limited information on diversity of PVX in Iran and how these isolates relate to those found in other parts of the world. Esfandiari et al. [5] have identified and characterized the only genome of PVX from Iran (GenBak Acc. no. FJ461343) isolated from *Pisum sativum*. Given the limited knowledge of PVX in Iran, we undertook a large survey, characterized two near complete genomes and 13 CP sequences of PVX from various regions of Iran. We also determined the host range of some of these PVX isolates in green house experiments.

Materials and methods

Collection and maintenance of virus isolates

Six hundred and one potato plants showing symptoms were sampled between January 2009 and July 2012 from potato fields in Kerman, Fars, Northern Khorasan, Hamedan, Esfahan, East Azerbaijan, West Azerbaijan, Hormozgan and Ardebil provinces. Leaf samples were tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) [2] for the presence of PVX and other potyviruses i.e. (PVY, PVA and *Potato virus V*; PVV). Antisera and the positive controls for these assays were sourced from Bioreba (CH-4153 Reinach BL1, Switzerland). Positive and negative controls were included in all tests in each PVX and potyviruses identification assay.

Thirteen PVX positive samples (Table 1) were selected based on geographical location and the PVX isolates from these samples were biologically purified and cloned by three local lesion passages in *Gomphrena globosa* L. and one systemic infection on *Nicotiana glutinosa* L. All PVX isolates for biological and molecular studies were maintained on *N. glutinosa* L. in a temperature regulated insect-proof greenhouse. The isolates were named according to the abbreviated name of provinces and sampling regions, and assigned a specific number (see Table 1 for details).

Biological characterization of the PVX isolates

Thirteen isolates propagated on *N. glutinosa* were characterized biologically by symptomology based on inoculations

of a variety of range of test plants. These samples were ground in 1 % (w/v) solution of K_2HPO_4 at pH 7.5 containing 0.01 % Na_2SO_3 , 0.05 % ethylene diamine tetra acetic acid (EDTA) and 2 % poly vinyl pyrrolidone (PVP, MW = 40,000) and the extracts were then mechanically inoculated in triplicate onto the 11 plant species belonging in three families (Table 2). Inoculated test plants were maintained in a greenhouse at 25° to 28 °C. Two weeks later the presence of PVX was checked by ELISA and symptomology in inoculated plants.

RNA isolation and RT-PCR

Thirteen PVX isolates were mechanically inoculated to young tobacco plants (*N. glutinosa*). Total RNA was extracted from infected leaves of thirteen *Nicotiana glutinosa* plants mechanically inoculated with PVX isolates using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Germany). First-strand cDNA synthesis was performed using Moloney Murine leukaemia virus (MMLV) reverse transcriptase as described as previously by Sharifi et al. [22]. The resulting viral cDNAs were used for consecutive PCR reaction with appropriate primers (based on GenBank sequences; AB056718 and M95516) to amplify 9 overlapping fragments spanning the entire genome of two PVX isolates (Supplementary Fig. 1). The following thermal cycling conditions were used: an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 60 s, annealing at appropriate temperatures for 30 s, and extension at 70 °C for 60 s, and a final 10 min extension at 72 °C. The 3' terminus of the PVX sequence was determined using a 3' RACE kit (Roche, USA) according to the manufacturer's instructions. In all tests positive and negative control were used.

Additionally, a primer pair (PVX- 8F and PVX -7R, Supplementary Fig. 1) was designed to amplify a 714 bp amplicon for *cp* gene of thirteen PVX Iranian isolates (Table 1) collected from different regions in Iran and maintained by inoculations in *N. glutinosa*. Amplification of CP regions of Iranian PVX isolates was performed with the following thermal cycling conditions: initial denaturation for 3 min at 94 °C, followed by 30 cycles of denaturation for 60 s at 92 °C, annealing at primer optimized temperatures for 60 s at 50 °C, extension at 72 °C for 60 s and final extension at 72 °C for 5 min.

Molecular cloning and sequence analysis

The PCR amplicons including fragments of the genome encoding the CP gene of the 13 Iranian PVX isolate and nine overlapping fragment amplicons, spanning the entire genome of two Iranian isolates were cloned into a pTZ57R/

Table 1 GenBank accession numbers of 11 CP and two full length genome of Iranian PVX isolates characterized at a molecular level and their sampling locations

Isolate name	Province, region	GenBank accession no of coat protein	GenBank accession no of full length genome
ESF.CH.1	Esfahan, Chadegan	KF568902	–
ESF.AS.2	Esfahan, Ashgerd	KF568901	–
AZA.WE.UR.1	West, Azerbaijan, Yourghonabad	KF568900	–
AZA.WE.LA.2	West, Azerbaijan, Lak	KF568899	–
ARD.GI.1	Ardabile, Gilandeh	KF568897	–
ARD.GR.2	Ardabile, Garjan	KF568898	–
AZA.ES.TAB.1	East, Azerbaijan, Tabriz	KF537623	–
KH.SH.1	Khorasan, Shirvan	KF568904	–
FA.SI.1	Fars, Siakh	KF568903	–
KER.ZA.1	Kerman, Zarand	KF575174	KF575174
KER.ZA.2	Kerman, Zarand	KF568906	–
KER.LA.1	Kerman, Lalezar	KF568905	–
KER.LA.2	Kerman, Lalezar	KF575175	KF575175

Table 2 Symptoms induced by PVX isolates on selected plant species

Families, species and cultivars	Symptoms	Symptoms
<i>Solanaceae</i>	Group I ^a	Group II ^b
<i>Nicotiana glutinosa</i> L.	BL, MF, VB	MM, VC, VB
<i>N. tabacum</i> L. cv. Samsun.N	SM	VB, M
<i>N. clevelandii</i> Gray	M, BL, MF	VB, M
<i>N. debneyii</i> Domin	M, BL, MF	VB, M
<i>N. tabacum</i> L. cv. Turkish	–	–
<i>Datura stramonium</i> L.	M, VB	M, VB
<i>Datura.maxima</i> L.	–	–
<i>Chenopodiaceae</i>		
<i>Chenopodium amaranticolor</i> Coste et Reyn	–	–
<i>Ch. quinoa</i> Wild	–	–
<i>Cucurbitaceae</i>		
<i>Cucurbita pepo</i> L. cv. Khoy (local cultivar)	–	–
<i>Cucurbita pepo</i> L. cv. Maragheh (local cultivar)	–	–

MM mild mosaic, VC vein clearing, BL blistering, MF malformation, VB vein banding, SM sever mosaic, M mosaic, –, no symptoms and no virus recovered

^a Group I: Consisted of KER.LA.2 and AZA.WE.UR.1 isolates

^b Group II: Including ESF.CH.1, ESF.AS.2, AZA.WE.LA.2, ARD.GI.1, ARD.GR.2, AZA.ES.TAB.1, KH.SH.1, FA.SI.1, KER.ZA.1, KER.ZA.2 and KER.LA.1 isolates

T vector (Thermo Fisher, USA) and transformed into *Escherichia coli* strain DH5 α . Recombinant plasmids were extracted and Sanger sequenced at MacroGenInc. (Korea).

Sequence contigs were assembled using DNAMAN version 7 (Lynnon, Biosoft, Quebec, Canada).

PVX sequences (CP and full genome) were aligned with all sequences available in GenBank using MUSCLE [4]. The resulting alignments were used to infer maximum likelihood phylogenetic trees using PHYML [7] with GTR+I+G4 and TN93+I+G model of nucleotide substitution chosen as the best fit model by j Model Test [19] for *cp* dataset and full genome datasets respectively with 1,000 bootstrap replicates. Both the full genome and *cp* dataset trees were midpoint rooted and branches with less than 60 % bootstrap support were collapsed. Nucleotide pairwise sequence identity was calculated using SDT v1.0 [18].

Results

Survey for virus identification

Of the 601 symptomatic potato leaf samples tested by DAS-ELISA, 25 (4.2 %) tested positive for PVX. Additionally, 260 samples (43.3 % incidence) tested positive for PVY, 185 (30.8 %) for PVA and 16 (2.7 %) for PVV. The symptoms observed ranged from mild to severe mosaic, mottling, leaf malformation, and yellowing in the infected samples. Co-infections with two potyviruses were detected in 29.5 % (177/601) of the samples that tested positive by ELISA. Of these co-infected sampled, 130 (21.6 %) were infected with PVY and PVA. PVY + PVV and PVY + PVX co-infection combinations were found in 4.9 and 14.2 % of the collected samples, respectively. Triple infections involving combination of PVY + PVA + PVV and PVY + PVA + PVX were detected in 8 and 3 samples, respectively.

Fig. 1 Maximum likelihood phylogenetic tree of the full genomes of PVX isolates and a two dimensional pairwise sequence identity colour plot of the isolates. The PVX isolates form two distinct clades (I and II). Clade I has low diversity and isolates from Europe, Asia, North

America and the middle east whereas Clade II has more divergent isolates and all these are from South America. Branches with <59 % bootstrap support have been collapsed. The pairwise comparisons were performed using SDT v1.0 [19]. (Color figure online)

Host range analysis

Thirteen PVX isolates were mechanically inoculated to all the tested plants (Table 2). Based on the symptomology on test plants, the Iranian PVX isolates were classified into two groups. In *Nicotiana glutinosa*, group I isolates (AZA.WE.UR.1 and KER.LA.2) caused blistering, malformation and vein banding symptoms, whereas group II isolates (ESF.CH.1, ESF.AS.2, AZA.WE.LA.2, ARD.GI.1, ARD.GR.2, AZA.ES.TAB.1, KH.SH.1, FA.SI.1, KER.ZA.1 and KER.ZA.2) induced vein clearing, mild mosaic and vein banding symptoms.

PVX isolates AZA.WE.UR.1 and KER.LA.2 induced severe mosaic symptoms on *N. tabacum* cv. Samsun NN, whereas on *N. clevelandii* and *N. debneyii* they induced mosaic, blistering and malformation symptoms. AZA.WE.UR.1 and KER.LA.2 on *Datura stramonium* induced mosaic and vein banding symptoms. Group II isolate infection in *N. tabacum* cv. Samsun NN, *N. clevelandii* and *N. debneyii* caused vein banding and mosaic. Four (ESF.AS.2, ARD.GI.1, TAB.BO.1 and KER.ZA.1) of 10 group II isolates caused mosaic and vein banding on *D. stramonium*. None of the group I or II isolates showed any signs of infection on *Chenopodium amaranticolor*, *Ch. quinoa*, *N. tabacum* L. cv. Turkish, and *D. maxima*.

Molecular analysis of complete PVX genomes

The PVX genome sequence of two isolates (KER.LA.2 and KER.ZA.1) are of 6,435 nucleotides (nt) excluding 101 nucleotide in the 5' region of the genome. The sequence identity values of five ORFs RdRP, TGBp1, TGBp2, TGBp3 and CP between these two isolates were 95.5, 96.2, 99.7, 96.2 and 96.1 % on the nucleotide level and 98.3, 98.2, 100, 95.7 and 98.7 % on the amino acid level. The genome-wide pairwise identities and the maximum likelihood phylogenetic analysis are summarised in Fig. 1. The KER.LA.2 (KF575174) and KER.ZA.1 (KF575175) isolates share 96.2 % genome-wide pairwise identity and 96 and 98.1 % identity, respectively, with the Iranian PVX isolate from *P. sativum* [5]. Overall the three Iranian PVX isolates share >95 % pairwise identity with all other PVX genome sequences.

Molecular analysis of the capsid protein

Of the 25 PVX positive samples we amplified, cloned and sequenced, the *cp* (714 nt) genes of 13 isolates and analysed these with all CP sequences available in GenBank ($n = 149$). The 13 isolates represent five geographically

Fig. 2 Maximum likelihood phylogenetic tree of the *cp* gene sequences of PVX isolates from sampled around the world. The *cp* sequences cluster into two main clades. Clade I has *cp* sequences of isolates from Africa, Australia, Europe, Asia, North America and the

Middle East and show less sequence diversity than Clade II *cp* sequence of isolates from Europe, North America and South America. Branches with <59 % bootstrap support have been collapsed

distinct regions in Iran, the southwest (provinces of West Azerbaijan, East Azerbaijan and Ardabil), centre (province of Esfahan), northeast (Khorasan province) and southeast (provinces of Kerman and Fars) (Table 1).

Pairwise sequence analysis of the *cp* genes of the Iranian isolates indicate that they share >94.7 % nucleotide pairwise identity and >97 % amino acid pairwise identity. The 'AFDF' motif (161–164 aa) identified by Komatsu et al. [13] that is specific to filamentous plant virus CPs, is conserved in CP of all 13 Iranian isolates. Maximum likelihood phylogenetic analysis shows two clear clades of PVX (Fig. 2). The isolates in the clade with the largest

number of *cp* gene sequences share >91.7 % pairwise identity and all Iranian isolates fall within this clade. The second clade has *cp* genes sequences from isolates of only America and Europe and has greater diversity (sharing >80.1 % pairwise identity; data not shown).

Discussion

PVX in Iran has previously been detected using serological assays [11, 20], however, there is limited information on PVX variants/genotypes at a molecular level. The only

molecular characterisation of PVX in Iran is from an isolate from *P. sativum* [5]. As part of this study we decided to sequence two complete PVX genomes that showed severe (KER.LA.2) and mild (KER.ZA.1) symptoms in infected plants and analysis of the molecular diversity of the CP gene of 13 isolates from various locations in Iran.

Maximum likelihood phylogenetic analysis of the full PVX genomes shows that the PVX isolates form two distinct clades (I and II). The Iranian isolates are part of clade I with clade II representing isolates from South America. Interestingly, the two isolates (PVX 2.7 and PVX 2.7b) from U.S.A are part of clade I and hence clearly indicates that the isolates from North America are distinct from those in the southern America contradicting the previous suggestion of two distinct lineages of PVX, Eurasia and America [3, 13, 25, 26].

The five coding regions (RdRP, TGBp1, TGBp2, TGBp3 and CP) of complete genome sequences of the 27 PVX isolates were used in genetic diversity analysis to estimate genetic variability on different coding regions (data not shown). According to the results of this study and those of Yu et al. [25, 26] different selection pressure is operating on different genes of PVX, and the ORF encoding RdRp is under the highest selection pressure. Therefore, the RdRp ORF of 27 PVX complete genome were further divided into three parts as described by Yu et al. [26] and their dN/dS values separately were calculated. Unlike previous studies by Yu et al., 2010 our results indicated the dN/dS values for three parts of genome were <1, despite the fact that RdRp-B was higher than RdRp-A and RdRp-C. This indicted 5'-termini, central and 3'-termini of RdRp are under negative selection.

PVX strains based on amino acid sequence of CP, amino acid sequence length and their ability to overcome resistance conferred by three potato resistant gene (Nx, Nb and Rx1) have been previously classified in two type X and B [21]. All of the strains that were avirulent on potato cultivars carrying the Nx resistant gene and including 237 amino acids in length were found to have type X coat proteins. Whereas, PVX strains that are capable of overcoming the Nx resistant gene and were 236–248 amino acid in length were classified as type B CPs. In addition, K at position 20, A at 64, and M at 128 and T at 227 were conserved in the coat protein of all isolates in type X coat proteins. The alignment of amino acid sequence of CP of PVX isolates indicated that all 13 Iranian isolates can be classified as having type X CPs.

In this study we analysed the genetic diversity of PVX isolates from around the world and compare them to those determined in this study from Iran. We determine the full genome sequence of two PVX isolates and *cp* gene sequences of 13 isolates from Iran. Despite the fact that the two Iranian isolates caused severe (KER.LA.2) and mild

(KER.ZA.1) symptoms in infected test plants, the isolates shared 96.2 % genome-wide pairwise identity. We were not able to identify any relationship between the sequence variation and the symptom observed. Hence there is a clear need for further studies to identify the genomic region of the Iranian PVX responsible for this variation in symptoms. The Iranian isolates are most closely related to the isolates in clade I which show very low diversity (<6 %). It is interesting to note that the analysis of the *cp* gene sequences reveals that there is far greater diversity in the Clade II isolates which are primarily from America and Europe.

Acknowledgment This research work was supported by the Iran National Science Foundation (INSF; Grant No. 87041270).

References

1. Beemster ABR, Rozendaal A. Potato viruses: properties and symptoms. In: De Borkz JA, editor. Viruses of potatoes and seed-potato production. Wageningen: Pubdoc; 1972. p. 115–43.
2. Clark MF, Adams AN. Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J Gen Virol. 1977;34:475–83.
3. Cox BA, Jones RA. Genetic variability in the coat protein gene of Potato virus X and the current relationship between phylogenetic placement and resistance groupings. Arch Virol. 2010;155(8): 1349–56.
4. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5): 1792–7. doi:10.1093/nar/gkh340.
5. Esfandiari N, Kohi-Habibi M, Hohn T, Pooggin MM. Complete genome sequences of Potato virus X from the legume plant *Pisum Sativum*. Virus Genes. 2009;30:141–5.
6. Gilmer D, Bouzoubaa S, Hehn A, Guilley H, Richards K, Jonard G. Efficient cell-to-cell movement of beet necrotic yellow vein virus requires 3' proximal genes located on RNA 2. Virology. 1992;189:40–7.
7. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59(3):307–21. doi:10.1093/sysbio/syq010.
8. Hagiwara K, Ichiki TU, Ogawa Y, Omura T, Tsuda S. A single amino acid substitution in 126-kDa protein of Pepper mild mottle virus associates with symptom attenuation in pepper; the complete nucleotide sequence of an attenuated strain, C-1421. Arch Virol. 2002;147:833–40.
9. Hahn Y, Slack SA, Slattery RJ. Reinfection of potato seed stocks with Potato virus S and Potato virus X in Wisconsin. Am Potato J. 1981;58(2):117–25.
10. Karpova O, Zayakina O, Arkhipenko M, Sheval E, Kiselyova O, Poljakov V, et al. Potato virus X RNA-mediated assembly of single-tailed ternary 'coat protein–RNA–movement protein complexes. J Gen Virol. 2006;87(9):2731–40.
11. Khakvar R, Pourrahim R, Shamsbakhsh M. Study on six Potato viruses in Khuzestan Province. In: Proceeding of the 14th Iranian plant protection congress, Isfahan University of Technology, Iran 2000. p. 312.
12. Kobori T, Miyagawa M, Nishioka K, Ohki ST, Osaki T. Amino acid 129 of Cucumber mosaic virus coat protein determines local

- symptom expression and systemic movement in *Tetragonia expansa*, *Momordica charantia* and *Physalis floridana*. *J Gen Plant Pathol*. 2002;68:81–8.
13. Komatsu K, Kagiwada S, Takahashi S, Mori T, Yamaji Y, Hirata H, et al. Phylogenetic characteristics, genomic heterogeneity and symptomatic variation of five closely related Japanese strains of Potato virus X. *Virus Genes*. 2005;31:99–105.
 14. Leshchiner A, Minina E, Rakitina D, Vishnichenko V, Solovyev A, Morozo S, et al. Oligomerization of the Potato virus X 25-kD movement protein. *Biochem (Mosc)*. 2008;73:50–5.
 15. Memelink J, van der Vlugt CI, Linthorst HJ, Derk AFL, Asjes C. Homologies between the genomes of a carlavirus (lily symptomless virus) and a potexvirus (lily virus X) from lily plants. *J Gen Virol*. 1990;71:917–24.
 16. Morozov SY, Solovyev AG. Triple gene block: modular design of a multifunctional machine for plant virus movement. *J Gen Virol*. 2003;84:1351–66.
 17. Morozov SY, Zakchariev VM, Chernov BK, Prasolov VS, Kozlov YV, Atabekov JG, et al. The analysis of the primary structure and localization of the coat protein gene on the genomic RNA of Potato virus X. *Dokl Akad Nauk SSSR*. 1983;271:211–5.
 18. Muhire B, Martin DP, Brown JK, Navas-Castillo J, Moriones E, Zerbini FM, et al. A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus *Mastrevirus* (family *Geminiviridae*). *Arch Virol*. 2013;158(6):1411–24. doi:10.1007/s00705-012-1601-7.
 19. Posada D. Selection of models of DNA evolution with jModel-Test. *Methods Mol Biol*. 2009;537:93–112. doi:10.1007/978-1-59745-251-9_5.
 20. Pourrahim R, Farzadfar SH, Golnaraghi AR, Ahoonmanesh A. Incidence and distribution of important viral pathogens in some Iranian potato fields. *Plant Dis*. 2007;91:609–15.
 21. Santa Cruz S, Baulcombe D. Analysis of Potato virus X coat protein genes in relation to resistance conferred by the genes *Nx*, *Nb* and *Rx1* of potato. *J Gen Virol*. 1995;76:2057–61.
 22. Sharifi M, Massumi H, Heydarnejad J, Hosseini Pour A, Shaa-banian M, Rahimian H. Analysis of biological and molecular variability of Watermelon mosaic virus isolates from Iran. *Virus Genes*. 2008;37:304–13.
 23. Skryabin K, Morozov SY, Kraev AS, Rozanov MN, Chernov BK, Lukashcheva LI, et al. Conserved and variable elements in RNA genomes of potexviruses. *FEBS Lett*. 1988;240:33–40.
 24. Verchot-Lubicz J. A new cell-to-cell transport model for potexviruses. *Mol Plant Microbe Interact*. 2005;18:283–90.
 25. Yu XQ, Wang HY, Lan YF. Complete genome sequence of a Chinese isolate of Potato virus X and analysis of genetic diversity. *J Phytopathol*. 2008;156:346–51.
 26. Yu X-Q, Jia JL, Zhang C, Li XD, Wang YJ. Phylogenetic analyses of an isolate obtained from potato in 1985 revealed Potato virus X was introduced to China via multiple events. *Virus Genes*. 2010;40:447–51.