The Journal of Animal & Plant Sciences, 25(4): 2015, Page: 1016-1021
ISSN: 1018-7081

POTATO VIRUS X FROM PAKISTAN: COAT PROTEIN SEQUENCE ANALYSIS

A. Khan, B. Tabassum, I. A. Nasir, M. Bilal, M. Tariq and T. Husnain

Affiliation of authors: Centre of Excellence in Molecular Biology, University of the Punjab, Lahore.
Corresponding author's email: anwarleo2003@gmail.com

ABSTRACT

Potato virus X (PVX) a most devastating pathogen of potato, tomato and tobacco all over the world was surveyed for its epidemics in Pakistan. A 891bp amplicon covering full capsid protein gene of potato virus X was amplified through reverse transcription-polymerase chain reaction by using gene specific primers from the samples collected all over Punjab, Pakistan. Deduced sequence of full capsid protein gene of PVX was reported as the first full length coat protein gene of potato virus X from Pakistan in NCBI database (Accession # KC757709). In phylogenetic analysis, Pakistani PVX isolate was found to be clustered closely with European sub linage while distinctly with Australian PVX and South American isolates. In contrast to the nucleotide sequence the amino acid sequence dendrogram of PVX, Pakistani isolate showed 100% homology with American isolates. Conclusively, a new isolate of PVX was identified from Pakistan which surprisingly exhibited close lineage with European isolate.

Key words: Potato Virus X, Capsid protein gene, Phylogenetic analysis, Reverse Transcription, amino acid sequence dendrogram.

Abbreviations: PVX (Potato virus X); CP (Capsid protein gene); PLRV (potato leafroll virus); PVY (potato virus Y); DAS-ELISA (double antibody sandwich Enzyme linked immunosorbent assay); cDNA (complementary DNA).

INTRODUCTION

Potato is one of the major food crops of countries like Pakistan on accounts of its high nutritional value and low cost of production. It is cultivated in Pakistan over an area of 184.9 thousand hectare with an annual production of 3392.5 thousand tones (GOP, 2012). Potato is used for human consumption, animal feed and as a source of starch and alcohol. It is one of the most important vegetable crops containing about 75 to 80% water, 16 to 20% carbohydrates, 2.5 to 3.2% crude protein, 1.2 to 2.2% true protein, 0.8 to 1.2% mineral matter, 0.1 to 0.2% crude fats, 0.6% crude fiber and some vitamins (Abbas *et al*., 2012). Viruses are considered to be major threat to potato yield which spread mechanically or through different vectors. About 40 viral species have been reported to infect potato worldwide (Valkonen, 2007). Among potato viruses PLRV, PVX and PVY have been recognized as major threat to potato crop (Zhang *et al*., 2010). Viral diseases are characterized by degeneration, decrease in vigor, productivity and disintegration of resistance against diseases after successive generations (Tabassum *et al*., 2011). Interestingly in Pakistan 80% losses of potato tubers are reported to occur through RNA viruses, (Mubin *et al*., 2009).

Potato virus X (PVX), the most wide spread potato virus worldwide also occupies all potato growing areas of Pakistan and causes mild mosaic, interveinal chlorosis and rugosity and sometimes top necrosis in several solanaceous crops including potato, tomato and tobacco along with some experimental species of the Chenopodiaceae, Amaranthaceae and Fabaceae. PVX affected plants produce fewer number of smaller and under sized tubers. PVX is a latent virus because it can remain latent in the infected plant and therefore sometimes unable to identify on symptom basis (Ahmad *et al*., 2011).

Genetically, PVX is a RNA virus and genome of PVX is a single-stranded positive sense RNA molecule of 6.4 kb encapsidated inflexuous filamentous particles, which contains five open reading frames (ORFs). The 5′ end has a methyl guanosine cap and the 3′ end has a poly (A) tail, (Huisman *et al*., 1988). PVX alone can cause up to 30% loss of yield but in mixed infection with PVY the yield loss increases up to 55% (Vance, 1991). It is transmitted mechanically by direct contact or by cutting tools before planting (Mandal *et al*., 2012).

To date, the most effective method of viral control is the use of RNAi technology. As coat protein gene of PVX interferes with viral uncoating, movement and translation (Fedorkin *et al*., 2001) thus a transgenic plant harboring resistance against CP gene, will be potentially more effective against invading PVX. The main objective of the present study was to get full sequence of coat protein gene of the local PVX isolate its correlation with phylogenetically distinct group on the basis of nucleotide and amino acid sequence so that this information can be utilized for designing potential siRNA to control virus.

MATERIALS AND METHODS

Virus survey for its epidemics in Pakistan: A total of 144 potato leaf samples showing potato virus X symptoms were collected from different regions of the Punjab, Pakistan during November to January 2012-13. The symptomatic samples were analyzed for potato virus X through DAS-ELISA (Clark and Adams, 1977) using BIOREBA AG, Reinacht, Switzerland.

Reverse Transcription and amplification of Potato virus X: Total RNA was extracted from PVX infected plants using TRIZOL reagent (Cat # TR118; Invitrogen) according to manufacturer's instructions. PVX- specific primers were designed by aligning the available complete genome from NCBI to MEGA 5.0, the conserved region upstream from CP gene along with few nucleotides downstream. Primers were got synthesized from gene link USA. Reverse transcription for cDNA synthesis from extracted RNA of PVX samples were done by using cDNA synthesis kit (cat # 1622; Thermo Scientific) as per manufacturer instruction. Amplification of 891nt fragment covering full length CP gene from synthesized cDNA was done by using primers PVXIF (GGACTGAACCTTGTGTCATCA) and PVFIR (ATGAAACTGGGGTAGGCGTC). The cycling conditions were optimized for amplification of CP gene by using gradient PCR. Amplified products were resolved on 1% agarose gel, stained with ethidium bromide.

Ligation of amplified product and sequence confirmation: PCR amplified product was gel purified by using gel extraction kit (cat no.K0691; Thermo Scientific) and ligated in pCR 2.1 vector (Invitrogen) in a ratio of 1:1 (vector to insert). Blue/white screening was done for initial screening. The white colonies were picked and inoculated in LB broth with ampicillin as screening marker (100mg/mL). After overnight growth plasmid DNA was isolated using GeneJet plasmid extraction kit (cat # K0503; Thermo Scientific). The plasmid was digested with EcoRI and resolved on 1% agarose gel. The confirmed plasmid was sent to Macrogen, Korea for sequencing.

Sequence alignment and phylogenetic analyses: The multiple sequence alignment of PVX deduced sequence with NCBI reported sequences was done using Clustal W multiple sequence alignment programs integrated in MEGA5.0. Neighbour-joining phylogenetic trees were drawn using MEGA 5.0 software package. Furthermore, nucleotide sequences were translated into amino acid sequences and percent amino acid similarities were calculated for CP gene that were used further for phylogenetic tree analysis.

RESULTS

Out of 144 symptomatic potato leaf samples, 46 samples were identified to be infected by PVX (29.48%) through DAS-ELISA. Symptomatic samples that were identified to be negative for PVX through ELISA were separated from the present study for the possibility to have any other virus or pathogen. PVX ELISA confirmed plantlets were maintained in MS basal media through single node cutting at 22± 2 °C with 16/8 hr photoperiod.

Reverse Trancription, cDNA synthesis, Amplification and Ligation in pCR 2.1: RT-PCR amplification of coat protein gene of PVX local isolate using PVXIF/PVXIR primer generated expected product of 891 bp as depicted in figure 1A. Annealing temperature was optimized to be 58°C via gradient PCR. The total base pair of complete CP gene were reported to be 714, but amplification was dome by primers designed from conserved region upstream and downstream from the gene, to get the complete gene, sequenced. Based on blue/white screening, selected white colonies were found putative transformants as clear from figure 1B where plasmid DNA was digested and resolved on 1% agarose gel, Two fragments appear on gel one of ~3.9kb vector pCR 2.1, while ~891 bp fragment represent CP gene of PVX.

The confirmed plasmid containing CP gene was sent to Macrogen, Korea for sequencing. The deduced sequence was submitted to NCBI Gene bank and accession number: KC757709 was obtained, as being the first full length coat protein gene of potato virus X reported from Pakistan.

Sequence alignment and phylogenetic analysis: The deduced sequence of the CP gene was used to study the homology of our isolate with already reported sequences of NCBI database. For this purpose, the coat protein gene sequence was downloaded using MEGA 5.0 and aligned by multiple sequence alignment tool of Clustal W. It was evident from the sequence alignment (Figure 2) that PVX isolates branched into three very distinct regions with respect to its nucleotide sequence; first branch is occupied by European PVX isolates, the second branch pointed out Australian isolate while the third region cover South American PVX isolates. The maximum similarity of Pakistani isolate was found to be 99% with GU144353.1, a Scottish isolate (UK), and the minimum homology of 81% was found with GU384738.1, an Australian isolate.

In CP-PVX sequence homology of 80-82% was obtained with South American isolate when subjected to comparison with phylogenetically distinct group of PVX isolates while South American isolates had 88-100% homology within themselves. However highest homology of a South American isolate, with isolates other than that region, (accession number: X55802.1) was 89% with UK isolate having accession number: X88786.1).

In the present study, a comparison of the deduced CP-PVX amino acid sequence with already reported sequence of NCBI was also done. Results depicted that Pakistani isolate shared maximum homology of 100% with an American isolate i.e. a reference sequence, (Figure 3) accession number, YP_002332933.1 and minimum homology of 85% with an Australian isolate, accession number ADL40976.1.

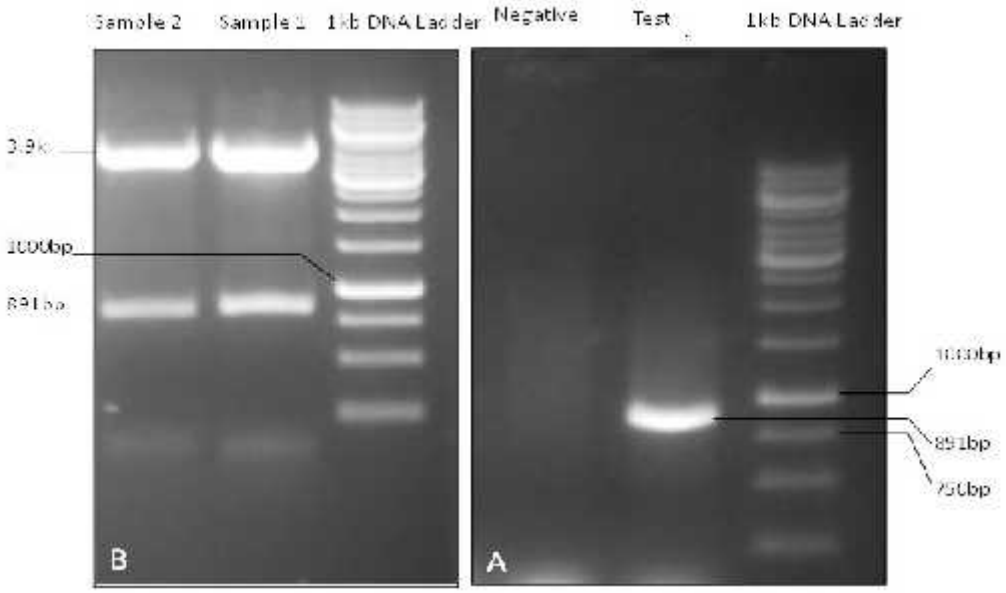

Figure 1 (A): RT-PCR amplification of capsid protein gene of PVX isolate collected from Punjab, Pakistan. Fragment of 891bp was amplified. (B): Restriction digestion of recombinant clone releasing ~3.9kb vector pCR2.1 and ~891bp CP gene fragment.

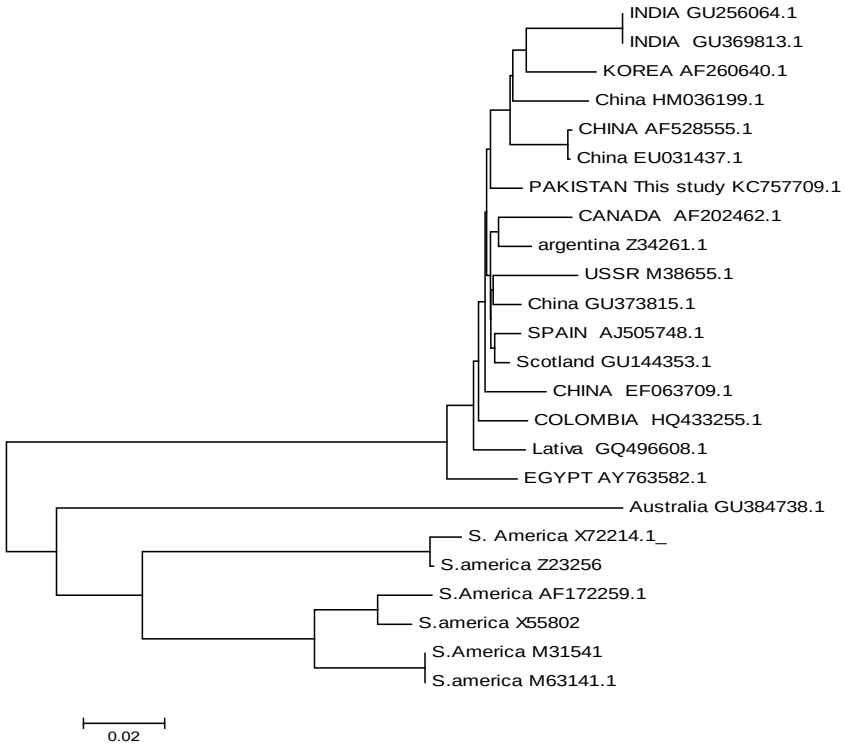

Fig.2. Dendrogram showing predicted relationship among Pakistani and other isolates of Potato virus X based on the nucleotide sequence. The dendrogram was constructed by the neighbour-joining method of Mega5.0 software.

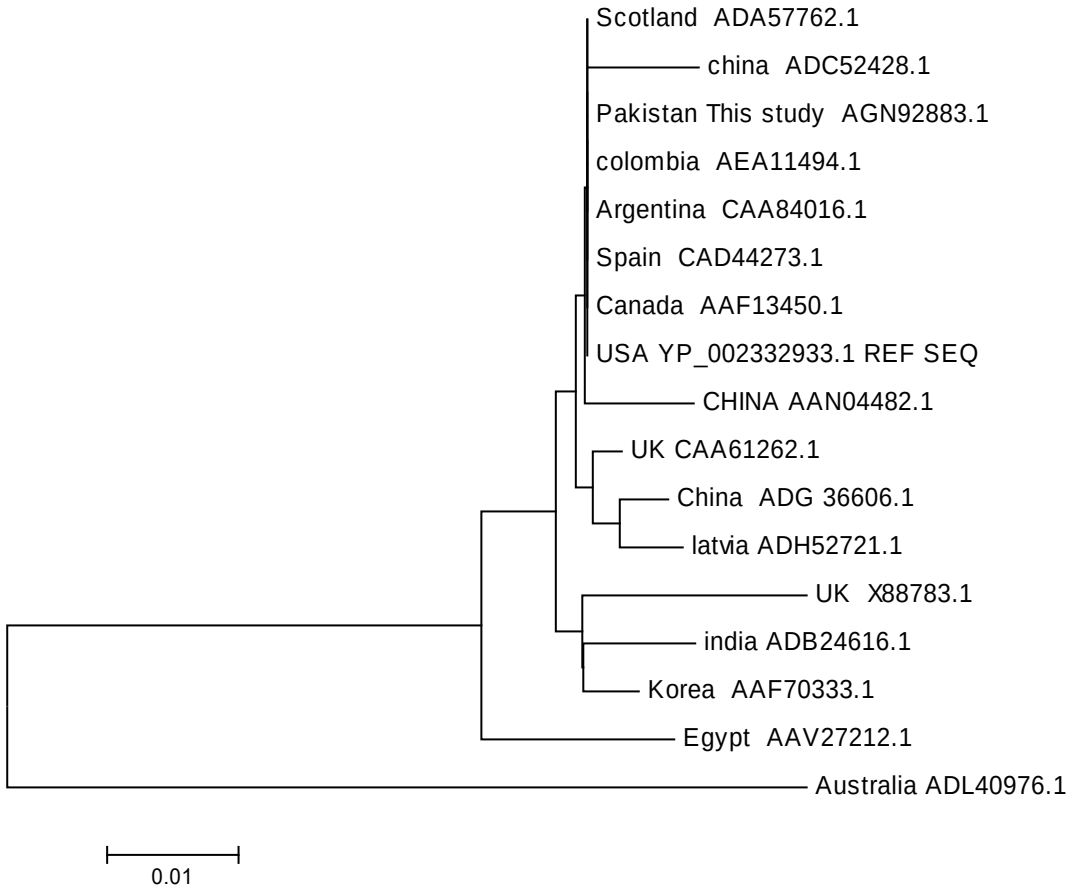

Fig. 3. Dendrogram showing predicted relationship among Pakistani and other isolates of Potato virus X based on the amino acid sequence. The dendrogram was constructed by the neighbour-joining method of Mega5.0 software.

DISCUSSION

Plant viruses are subjected to mutation at relatively high frequency because of their short generation time and high replication rate which lead to high evolution rate (Wu *et al.,* 2011). Phylogenetic trees showed the evolutionary relationships amongst various species by way of common ancestors. In present study sampling of potato leaf showing potato virus X symptoms was done from different regions of Pakistan as done by Park *et al*. (2011) from different regions of Korea. The amplification of full capsid protein gene of potato virus X was amplified through reverse transcription-polymerase chain reaction by using gene specific primers as done by Awan *et al*. (2010) for detection of Potato leaf roll virus. The sequencing of the amplified product was done from Macrogen Korea on request and Deduced sequence of full capsid protein gene of PVX was reported as the first full length coat protein gene of potato virus X from Pakistan in NCBI database (Accession # KC757709). Phylogenetic analysis of PVX Pakistani isolate was done for determining the homology and differences with other reported sequences on NCBI as done by Tian *et al*. (2011) and Cuevas *et al*. (2012). The phylogenetic analysis of this PVX Pakistani isolate deduced sequence was found to be clustered closely with European sub linage while distinctly with Australian PVX and South American isolates. These results conform to the findings of Cox and Jones (2010) who determined the genetic variability in the coat protein gene of Potato virus X isolates and established the phylogenetic placement of PVX isolates.

The interesting finding of current study is that China, Iran and India are geographically Pakistan's neighbors but with regard to CP-PVX sequence, homology was found to be 96%, 97% respectively. While USA and Canada are farthest from Pakistan but the homology between their isolate and Pakistani isolate is 97%, HQ450387.1 and AF202462.1 respectively. Significant homologies in sequences of PVX Pakistani isolate and that of a Scottish isolate suggested that transportation of virus infected potato and potato trade globalization are the key factors responsible for presence of geographically distinct isolate in Pakistan. The same

has been suggested by Ibaba and Gubba (2012) for existence of South American PVY isolates in KwaZulu-Natal.

Conclusion: Sequence of Potato Virus X of Pakistani isolate was reported on NCBI database. Rate of genetic variability between PVX isolates in the CP region between the Pakistani isolate and others already reported in NCBI database has been described through phylogenetic studies. Phylogenetic distribution of PVX isolates on the basis of amino acid sequences including Pakistani isolate in the deduced groups and subgroups has been described in the present study.

Acknowledgements: The authors wish to thank Higher Education commission (HEC) Pakistan for financial support and Potato Research Institute Sahiwal, Pakistan for their cooperation in sampling.

REFERENCES

Abbas, G., I. A. Hafiz, N. A. Abbasi and A. Hussain (2012). Determination of processing and nutritional quality atributes of potato genotypes in Pakistan. Pakistan J. Bot. 44(1): 201-208.

Ahmad, N., M. A. Khan, N. A Khan, R. Binyamin and A. Khan (2011). Identification of resistance source in potato germplasm against PVX and PVY. Pakistan J. Bot. 43(6): 2745-2749.

Bertolini, E., J. Garcia, A. Yuste and A. Olmos (2010). High prevalence of viruses in tablegrape from Spain detected by real-timeRT-PCR. Eur. J. Plant Pathol. 128: 283–287.

Clark M. F. and A. N. Adams (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475–483.

Cox, B. A. and R. A. C. Jones (2010). Genetic variability in the coat protein gene of Potato virus Xand the current relationship between phylogenetic placement and resistance groupings. Arch. Virol. 155: 1349–1356.

Cuevas, J. M., A. Delaunay, J. C. Visser, D. U. Bellstedt, E. Jacquot and S. F. Elena (2012). Phylogeography and molecular evolution of Potato virus Y. PLoS ONE. 7(5): 37853.

Digiaro, M., T. Elbeaino and G. P. Martelli (2007). Development of degenerate and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A, B and C. J. Virol. Methods. 141: 34-40.

Fedorkin, O. N., A. G. Solovyev, N. E. Yelina, A. A. Zamyatnin, R. A. Zinovkin, K. Makinen, J. Schiemann and S. Yu. Morozov (2001). Cell-to-cell movement of potato virus X involves distinct functions of the coat protein. J. Gen. Virol. 82: 449–458

Govt. of Pakistan (2007). Agricultural Statistics of Pakistan, p. 15. Govt. of Pakistan Ministry of Food, Agric. and Livestock Div. Economic Wing, Islamabad.

Huisman M. J., H. J. M. Linthorst, J. F. Bol and B. J. C. Cornelissen (1988). The complete nucleotide sequence of Potato virus X and its homologiesat the amino acid level with various plus-stranded RNA viruses. J. Gen. Virol. 69: 1789–1798.

Ibaba, J. D. and A. Gubba (2012). Phylogenetic studies of selected isolates of Potato virus Y (PVY) infecting vegetable crops in KwaZulu-Natal, Republic of South Africa. S. Afr. J. Plant & Soil. 29(2): 117-120.

Kagiwada, S., Y. Yamaji, H. Nakabayashi, M. Ugaki and S. Namba (2002). The complete nucleotide sequence of Potato virus X strain OS: The first complete sequence of a Japanese isolate. J. Gen. Plant Pathol. 68 (1): 94-98.

Li, F., D. Xu, J. Abad and R. Li (2012). Phylogenetic relationships of closely related potyviruses infecting sweet potato determined by genomic characterizaiton of Sweet potato virus G and Sweet potato virus 2. Virus Res. 45: 118-125.

Mandal. B., A. Kumar, P. Rani and R. K. Jain (2012). Complete Genome Sequence, Phylogenetic Relationships and Molecular Diagnosis of an Indian Isolate of Potato Virus X. J. Phyto Path. 160: 1–5.

Mubin, M., R. W. Briddon and S. Mansoor (2009). Complete nucleotide sequence of chili leaf curl virus and its associated satellites naturally infecting potato in Pakistan. Arch. Virol. 154(2): 365-368.

Park, J., S. Kim, E. Choi, H. R. Kwak, M. K. Kim, K. Y. Lee, H. S. Choi and S. Lee (2011). Molecular characterization of sweet potato leaf curl virus (SPLCV) isolates from Korea: phylogenetic relationship and recombination analysis. Acta. Virol. (Praha). 55(4): 327-35.

Saitou, N. and M. Nei (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Tabassum, B., I. A. Nasir and T. Husnain (2011). Potato virus Y mRNA expression knockdown mediated by siRNAs in cultured mammalian cell line. Virol. Sinica. 26 (2): 105-113

Tian, Y. P., J. L. Liu, C. L. Zhang, Y. Y. Liu, B. Wang, X.-D. Li, Z. K. Guo and J. P. T. Valkonen (2011). Genetic diversity of Potato virus Y infecting tobacco crops in China. Phytopath. 101: 377-387.

Valkonen, J. P. T. (2007). Viruses: economic losses and biotechnological potential. In Vreugdenhil, D.

(Ed.), Potato Biology and Biotechnology Advances: a Perspectives. Elsevier, Amsterdam, pp. 619-641

Vance, V. B. (1991). Replication of Potato virus X RNA is altered in co infections with Potato virus Y. Virol. 182: 486–494.

Walsh, K., J. North, I. Barker and N. Boonham (2001). Detection of different strains of Potato virus Y and their mixed infections using competitive fluorescent RT-PCR. J. Virol. Methods. 91: 167-173.

Wang, F., Z. L. Gao, A. N. Meng-nan, B. Zhou and W. U. Yuan-hua (2013). Sequencing and phylogenetic analysis of potato virus Y Liaoning isolate in China. J. I. A. 12 (7): 1195-1200.

Wu, B., A. Blanchard-Letort, Y. Liu, G. Zhou, X. Wang and S. F. Elena (2011). Dynamics of molecular evolution and phylogeography of Barley yellow dwarf virus-PAV. PloS One.6: 16896.

Zhang, W., Y. Q. Bai, Y. L. Gao, Y. Sheng, G. Q. Fan, H. W. Gen and X. X. Meng (2010). A survey on occurrence frequencies of potato viruses in major potato-producing provinces in China. Heilongjiang Agriculture Sciences. 4: 71-73.