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IDENTIFICATION OF POTATO VIRUS X IN UZABEKISTAN AND ITS BIOLOGICAL AND MOLECULAR GENETIC CHARACTERIZATION

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SUMMARY

Potato virus X (PXV) is one of the viruses infecting the potato plant, which requires the use of specific methods for its isolation and biological properties. In the research carried out in this direction, a "normal" isolate was isolated from Gala and Diyora potato varieties, and a "necrotic" isolate was isolated from Umid variety, and the characteristics of these isolates were studied and identified by molecular genetic method. To study the biological characteristics of the virus, both isolates were inoculated into several indicator plants, as a result, both isolates were *Chenopodium quinoa*, *Ch. amaranticolor* and *Ch.mural* in plant species yellow chloritic spots, in *Gomphrena globosa* plant red ring necrosis, in *D. stramonium* plant, the normal isolate of the virus causes dark systematic mosaic, and the necrotic isolate in this plant causes shrinking of the tips of the leaves and necrotic symptoms in the leaf plate was distinguished by its output. The method of immunoblotting on nitrocellulose membranes was used to determine the natural reservoir plants of the virus, and as a result, a number of plants that were unclear at the initial examination were re-examined, and the virus was identified as *Atriplex micrantha* C.A. May, *Solanum nigrum*, *Chenopodium murale*,. New natural reservoir plants of the virus, such as *Ch.quonea*, *Amaranthus retroflexus, Artemisia annua, A. vulgaris, Sinapis arvensis L., Brassica juncea (L) Czern,* which were excluded from IFA refreshment, were identified. In the molecular-genetic identification of the virus, ORFs5 (CP) gene was used as a basis, and as a result, it was confirmed that both isolates were specific for PVC as a result of PCR analysis, and a phylogenetic tree was built based on the sequence of the nucleotide sequence, and the necrotic isolate was placed in the NCBI database under the number MN702769. From the phylogenetic tree compiled on the basis of Blastn bioinformatics programs, it was found that the necrotic isolate of PVC isolated in Uzbekistan was 97.48% similar to the Dutch isolate D00344.1, Australian isolate GU384732.1, and AY297843.1, AY297842.1 and M95516.1 isolated from Great Britain. and isolates were found to share 97.06% homology, suggesting their descent from a common ancestor.

Key words: Potato virus X, necrotic isolate, reservoir plant, PCR, molecular identification, phylogenetic tree, electrophoresis, CP gene.

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Key findings: In this article, the results of the research conducted on the isolation, study of characteristics and molecular-genetic identification of PVC isolates distributed in the ecological conditions of Uzbekistan are reflected.

INTRODUCTION

Today, all over the world, the type and number of viruses infecting plants is increasing day by day. More than 50 viruses have been found to infect the potato plant, and each or several of them infect the potato plant and reduce the yield by 10-80%, causing great damage to agriculture. PVC is one of the viruses that causes disease symptoms such as spotty mosaic and growth point necrosis in plants [3; 11]. To date, a number of strains and isolates of this virus have been identified, which differ from each other in biological and molecular genetic characteristics [3; 5; 16; 18; 19]. In Uzbekistan, a number of isolates of this virus were isolated by the authors, and its effect on plant physiological characteristics, including the amount of plant leaf pigment, was studied. It was found that it decreased by four to five times compared to the plant [11]. To date, a number of scientific researches have been carried out in Uzbekistan on the isolation of phytopathogenic viruses and the study of their molecular and genetic characteristics, including the viruses of oat, potato, plum and leguminous plants, which were studied by the authors [11; 12; 13], in recent years the molecular-genetic characteristics of viruses of wheat, peas, tomatoes, alfalfa research is also being conducted on learning and identification. A number of studies are being carried out on the isolation of potato plant viruses, including M, X and L-viruses of potatoes, the study of the effect of the virus on some physiological properties of the plant, and its molecular identification [7; 22; 24].

The degree of spread of phytopathogenic viruses depends on environmental factors, virus type, plant type, variety, and the soil type, composition, and genesis of each region are different from each other, which has a serious effect on the spread of the virus [9; 10]. The biological characteristics of the virus include its storage, spread, ways of transmission, carriers, etc [11; 12; 13; 15].

A number of strains and isolates of PVC have been studied all over the world, and their molecular-genetic characteristics have been studied on the basis of some genes or the complete genome [14; 20; 21; 23], which serves as a basis for virus identification. To date, indicator plants, immunological and molecular-genetic methods are used to identify phytopathogenic viruses, each of them differs from each other in its sensitivity [15; 23]. Along with the identification of the virus using molecular genetic methods, it provides an opportunity to study its molecular-biological characteristics [13; 14].

Identification of resistant varieties for the development of measures to fight against viruses, obtaining lines and varieties resistant to new stress factors by crossing wild species [1; 6; 17], in order to physiologically increase the plant's resistance to the virus, it is possible to carry out genetic selection work on the varieties with such properties and to obtain physiologically resistant varieties by crossbreeding them [8]. In addition, in addition to the genetic aspects of resistance to the virus, there are also aspects related to the characteristics of the virus, strains and isolates. Therefore, it is very important to isolate the virus, to know its biological, physico-chemical and molecular-genetic characteristics in order to develop measures to fight against the virus. Based on this situation, the main goal of this study was to distinguish PVC, to study its biological and molecular-genetic properties, and to identify it.

MATERIALS AND METHODS

These studies were conducted in 2014-2020 in the "virology" scientific laboratories of the National University of Uzbekistan and "molecular biology and bioinformatics" of the Chirchik State Pedagogical University and were based on the collected materials. In this work, indicator plants are used for the separation, biological purification and identification of the virus [11; 15], the method of immunoblotting analysis on nitrocellulose membrane for the identification of natural reservoir plants of the virus [4; 13], while the RT-PCR method was used to determine and identify the molecular genetic characteristics of the virus, Sequence was used to determine the viral nucleotide sequence, and programs such as BLASTn were used for bioinformatic analysis [12]. Invitrogen™ PureLink™ RNA Mini Kit from Thermo Fisher USA was used to extract total RNA (mRNA) from the examined plant leaf. Spectral classification and quantification of RNA was determined using a spectrophotometer NanoDrop Eight (USA). The following reagents and materials for obtaining cDNA based on reverse transcription (TT) of viral matrix genomic RNA or total RNA: (Lu1+Eco Integrated DNA Technologies Belgium), олиго-dT_{21} primer (specific to the poly-A sequence to the 3'-end of the viral RNA) or the reverse (Lu4+Eco) oligo-dT20 primer was used. µl (20 pmol) of oligonucleotide primer and 5 µl of triton eluate or total RNA were placed in a thin-walled microcentrifuge tube (SnapSeal Graduated Microtubes, SSIbio, SShA). The mixture was kept in a thermostat "HB120-S DLAB" at 65°C for 5 minutes to bind the primer. The following reagents were used to prepare the TT mixture (1 reaction): 3 µl water (Water, nuclease-free, Thermo Scientific), 1 µl Lu4-R, 4 µl deoxynucleotide triphosphates (dNTPs), (Invitrogen™), 5 µl RNA, 4 µl 5x RT buffer RT, 1 µl 0.1 M DTT, 1 µl RNA ase out, 1 µl Loewe revertease. In most cases, reverse transcriptase enzymes from other companies were also used: SuperScript II (Invitrogen, SShA). Reverse transcription thermocycling program: 40°C - 115 minutes, 70°C - 1 cycle per 10 minutes. PCR-mix (for 1 reaction) 6.6 ddH_2 O, 12.5 µl 2X Master Mix, 0.9 µl MgCl2, 0.5 µl Lu1-F , 0.5 m1Lu4-R was inserted. PCR was performed on the amplifier "T960" (China). PCR sample denaturation (94°C, 1 min), 40-45 cycles of amplification, polynucleotide chain recovery 5-10 min at 72°C and storage at 4°C. The PCR product required for sequencing was obtained in an analogous manner, but the reaction was carried out in 9 µl, and the necessary components were calculated and mixed in the same manner as above. The annealing temperature of the primers and the duration of the elongation process are determined by the characteristics of the primers and the length of the PCR product to be synthesized. Analysis of PCR products was determined by electrophoresis on an agarose gel prepared in 10x tris-borate-EDTA buffer (TBE, Thermo Scientific) with ethidine bromide. 2 µl of DNA Gel Loading Dye (6X) and 1.3 µl of DNA marker M100BP are added to the well of agarose together with the sample for 10 µl of sample. A mixture of 100 bp DNA ladder molecules of known size of DNA was used as markers. Electrophoresis was performed using horizontal electrophoresis SE-1 (Helikon, Russia) at 80V for 60-100 minutes. The gel was analyzed and photographed on a transilluminator BK AG-100 (Gel Imaging Ayalysis System Biobase China).

The PCR product for sequencing was separated on a 2% agarose gel. After electrophoresis, the gel was illuminated using a transilluminator BK AG-100, and the desired zone was cut out using a scalpel and transferred to a 1.5 ml centrifuge tube. DNA was extracted from agarose on a spin column using the PureLink™ Quick Gel Extraction Kit (Invitrogen, USA) according to the instructions provided by the company. Purified DNA was sequenced on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific) using forward and reverse primers.

The primers were synthesized by Eurogen (No. pr-63530) and their sequences are listed in the table (Table 1). In this method (RT-PCR) Ahmed et all. (2013) used primers [3]:

Table 1

Structure of PVX ORFs5 (CP) gene primers

Primers	5´-3´- sequence	The number of nucleotides
Forward (F)	AGTGAGGACTGAACCTTGTGTC	22
Reverse (R)	ATGAAACTGGGGTAGGCGTC	20

The sequence of PCR products was determined using EditSeq and MegAlign (DNASTAR Lasergene, SShA) software based on the sequence result.

Nucleotide and their underlying amino acid sequences were compared using ClustalW v.2.1 software. The obtained comparative information was used to determine sequence divergence and identity and phylogenetic analysis. Phylogenetic analysis was performed using MEGA11 software using neighbor joining and Kimura-2 evolutionary models.

OBJECT OF RESEARCH

In this research work, Diyora, Gala and Umid varieties of potato plant and simple and necrotic isolates of PVC isolated from them, and in the biological identification of the virus, *Chenopodium quinoa, Ch. amaranticolor, Ch. murale, Gomphrena globosa* and *D. stramonium* plant species were used.

RESULTS OF RESEARCH

In many cases, the viruses infecting the potato plant come together in the state of mixed infection (X+Y+A; X+S; X+Y) which makes it difficult to separate them and obtain a homogeneous drug. Therefore, specific indicator-test plants and a scheme were selected for virus isolation and biological purification, which is presented below (Fig. 1).

sample => homogenization => *D. stramonium* => *G. glabosa* => *D. stramonium*
0,02M ФБ, pH 7-8 (differentiator) (mononecrosis) (collector)

Isolation of PVX- necrotic isolate from Umid variety

=> homogenization => *D. stramonium* => *70°C да 10 дақ.* => *N. tabacum*
0,02 M ФБ, pH 7-8 (systematic mosaic) (in a water bath) (systematic mosaic)

Isolation of PVX-simple isolate from Diyora variety

sample => homogenization => *D. stramonium* => *G. glabosa* => *D. tatula*
0,02M ФБ, pH 7-8 (differentiator) (mononecrosis) (collector)

Isolation of PVX-simple isolate from Gala variety

Figure 1. Scheme of separation and biological treatment of PVX isolates

Through this scheme, virus isolates were isolated from the host plant S. tuberosum and biologically purified. During the isolation process, attention was paid to the uniqueness of each isolate, including the mononecrosis method and temperature resistance of the virus were used to isolate the normal isolate of the virus from Diyora and Gala varieties, and if it was biologically purified, *N. tabacum* and plants such as *D.stramonium* have been used. *D. stramonium* var *tatula* species was used as the last collecting plant when isolating the necrotic isolate from the Umida variety, unlike other isolates (Fig. 1). At each stage, the purity of the virus was checked using the IFA method and the degree of purity was determined. For the biological identification of virus isolates, it is important to study their symptoms on indicator plants. For this purpose, abraded and biologically purified isolates were mechanically inoculated into indicator plants belonging to five families and 16 species, and the appearance of disease symptoms was observed in them. was monitored, the results obtained are presented in the table (Table 2).

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Table 2

Test-indicator symptoms of PVX isolates in plants

Name of indicator plant family and species	Strains of PVX			
	* PVX_o		** PVX_n	
	A symptom of illness	Emergence period	A symptom of illness	Emergence period
Chenopodiaceae				
Chenopodium quinoa	ClS	13-14	ClS	10-12
Ch. amaranticolor	ClS	10-12	ClS	8-10
Ch. murale L.	ClS	6-7	CH	5-6
Ch. album L.	-	-	-	-
Solanaceae				
Datura stramonium	SM	10-12	N	8-10
D. metel L.	GM	18-20	-	-
Phisalis floridana L.	-	-	-	-
Nicotiana barley	SM	20-22	GM	18-20
N. rustica	-	-	N	14-16
Petunia hybrida	-	-	-	-
L. esculentum Mill.	GM	10-15	LT, GM	6-8
S. melon-gena L.	-	-	-	-
C. annum)	M	10-12	SM	8-10
Leguminosae				
Vigna sinensis	-	-	-	-
Labiatae				
Ocimum basilicum L.	-	-	-	-
Amaranthus				
G. globosa	RRN	5-6	MN	5-6

Note: in the table disease symptoms column: CHS– chlorite stain, SM- spotted mosaic, GM – green mosaic, SM - systematic mosaic, M – mosaic, RRN - red ring necrosis, YN – yellow necrosis, N – necrosis, MN – major necrosis; the ” * “ – symbol is a” simple “ isolated from the gala and Diyora varieties of the virus, while ” * * “is a” necrotic " isolate isolated from the Umid variety [11]. (I did not know that the names of the disease change because it is a scientific abbreviation)

The "normal" isolate of the virus isolated from Gala and Diyora varieties is a number of species belonging to the *Chenopodiaceae* family, namely *Chenopodium quinoa, Ch. amaranticolor* and *Ch. Murale* caused yellow chloritic spots of different sizes, scattered *mosaic* in *Datura stramonium* belonging to *Solanaceae* family, green mosaic symptoms in metel and *Lycopersicum esculentum Mill* plants, and red in *Gomphrena globosa* belonging to *Amaranthus* family. was found to cause ring necrosis, the disease symptoms in the rest of the plants are listed in the table (Table 2).

In general, as a result of this study, the "normal" isolate of the virus isolated from the Gala and Diyora varieties was compared with the "necrotic" isolate isolated from the Umid variety, and the differences between them were determined.

In the implementation of virus circulation, its natural storage, i.e. reservoirs, is very important, especially if such reservoirs are from plants with a perennial life form, it is very important. For this purpose, in the following studies, potato fields and 37 species of cultivated and wild plants belonging to 16 families with disease symptoms or without any disease symptoms growing in the climatic conditions of the Tashkent region were examined using IFA

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and the natural reservoir plants of the virus spread in the Tashkent region were studied, and the reaction indicators were unknown in these studies. A number of plants with (±) were identified, and in subsequent studies, these plants, along with the plants initially examined, were examined by immunoblotting in NTsM. According to the authors who used the immunoblotting method in virological studies, the sensitivity of the method is high, and it is noted that it is close to the PCR method [4; 11]. The obtained results are presented in the table (Table 2).

Table 2

Detection of PXV in wild and cultivated plants using immunological methods

Plant family and species designation	**Specific serum of PVX**	
	Reaction indicators	
	ELISA	**NCR IB***
Solanaceae		
Solanum tuberosum, variety Diyora	+	++++
S. tuberosum, variety Umid	+++	++
S. tuberosum, variety Tuyimli	±	+++
S. tuberosum, variety Sante	++++	+
Solanum melon-gana	++++	++++
Solanum nigrum	±	++
Datura stramonium	+	++++
Datura metel	±	-
Petunia hybrida	+++	++++
Lucopersicum esculentum Mill	+++	++++
Chenopodiaceae		
Atriplex micrantha C.A.Mey	±	++
Chenopodium murale	±	+++
Ch. Quonea	±	+
Amaranthus retrofflexus	±	++
Ch. Amaranticolor	±	+++
Gramineae		
Cynodon daktulon (L) Pers	-	-
Zea mays	±	-
Alopecurus geniculatus	±	-
Sorghum helepense	-	-
Cyperaceae		
Cyperus rotundus	±	-
Compositae		
Xanthium strumarium	-	-
Artemisia annua L.	±	+
Artemisia vulgaris L.	-	++
***Leguminoseae*)**		
Medicago sativa L.	-	-
Alhagi Adans	-	-
Portulacaceae		
Portulica oleraceae L.	-	-
Cucurbitaceae		
Cucumis sativus L.	++++	++++
Cruciferae		
Sinapis arvensis L.	±	+++
Brassica juncea (L) Czern	±	++
Plantaginaceae		

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Plantago lanceolata L.	±	-
Onagraceae		
Onagra biennus Scop	±	-
Convolvulaceae		
Convolvulus arvensis L.	+++	++++
Cuscutaceae		
Cuscuta approximata Babing	±	-
Labiatae		
Mentha asiatica Boriss	+	++
Ocumus basilicum	-	-
Malvaceae		
Althaea officinalis L.	++++	++++
Malva neglesta Wall	+++	+++
Abutilon theophrasti Medic)	±	-
Poiygonaceae		
Rumex crispus L.	+++	+++
R. syriacus Meisn	-	-

Изох: "-"- no reaction at all; "±"- the presence or absence of a reaction is abstract;"+" - the course of the reaction is very pale yellow; "++" - reaction progress is yellow; "+++"- reaction progress is orange; "++++"- that the transition of the reaction is extremely orange; **NCR IB***nitrocellulose refers to the immunobloting method in the membrane.

As can be seen from the table, except for potatoes, the X-virus is *Cucumis sativus L.*, *Solanum nigrum L.*, *Rumex crispus L.*, *Datura stramonium*, *Solanum melon - gana L.*, *Petunia hybrida*, *Brassica juncea (L) Czern*, *Convolvulus arvensis L.*, *Althaea officinalis L.*, *Lycopersicum esculentum Mill*, *Malva neglesta Wall*. Absence of the virus in plants such as *Cynodon dactulon (L) Pers*, *Xanthium strumarium*, *Sorghum helepense*, *Capsicum annum L.*, *Ocumus basilicum L.* and subsequent immunoblotting in **NCR IB*** was also confirmed as a result (Table 2).

As a result of the tests carried out using the ILISA method, there is a virus in plants such as *Atriplex micrantha C.A. Mey*, *Chenopodium quenoa*, *Cyperus rodundus*, *Datura metel*, *Amaranthus retroffexus L.*, *Sinapsis arvensis L.* absence is unknown, that is, the reaction indicator showed "+-" [2; 7; 11; 12; 19]. In further studies, these plants were examined using the immunoblotting method in NTsM, and the virus was detected in *Atriplex micrantha C.A. May*, *Solanum nigrum*, *Datura metel*, *Ch. murale*, *Ch. quonea*, *Amaranthus retroflexus*, *Artemisia annua*, *Artemisia vulgaris*, *Sinapis arvensis L.*, *Brassica juncea (L) Czern* and new natural reservoir plants of the virus, which are not sensitive to ELISA, were identified (Table 2).

Based on the above information, it should be noted that PVX infects annual and perennial plants belonging to *Solanaceae, Malvaceae, Cruciferae, Amaranthaceae,* and *Compositae* families, and in different amounts paid (3+,4+). Therefore, these plants undoubtedly serve as reservoir plants of PVX.

Molecular biological methods are based on PCR and are more sensitive than immunological methods. To date, several variants of the PCR method have been developed, and QT-PCR, which is performed by obtaining cDNA using the enzyme reverse transcriptase, is used for the detection of RNA-capturing plant viruses.

For molecular identification of PVX isolates, isolated from *Diyora* variety and propagated on *Datura stramonium* plant (Fig. 1,a) and Gala variety, which causes sporadic mosaic symptoms (Fig. 1, b), tomato with mosaic symptoms (Fig. 1,c), and samples of the *Umid* variety

of potatoes with dark mosaic symptoms were collected and molecularly identified using the RT-PCR method.

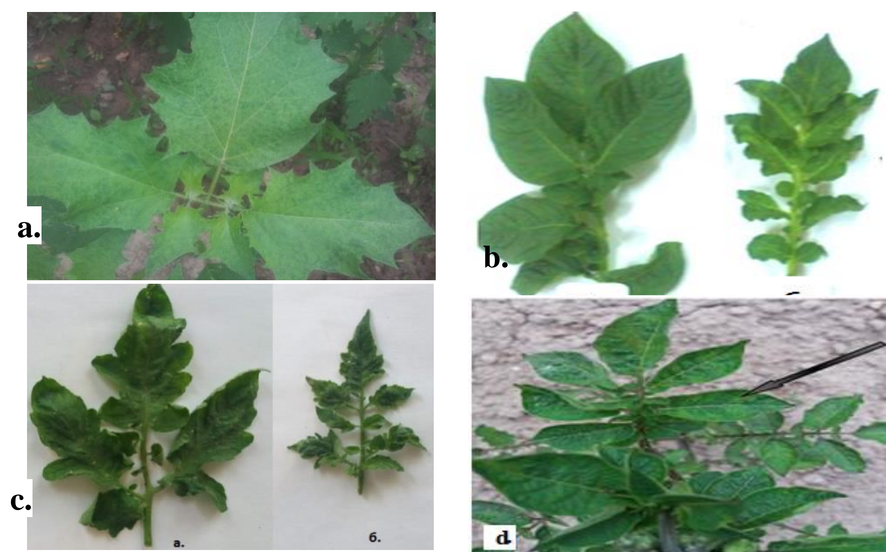
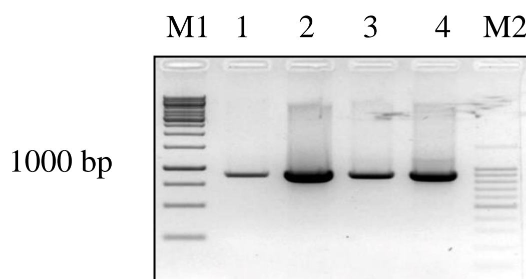


Figure 1. Plants tested for PXV by RT-PCR method: a – Systemic mosaic symptoms in *D. stramonium* plant isolated from Diyora cultivar; b - A leaf of a potato plant with mosaic symptoms (*S. tuberosum*), Gala variety; c - tomato plant with mosaic symptoms (*L. esculentum* Mill); d-A leaf of a potato plant with mottled (mosaic) symptoms, variety Umid.

The results of QT-PCR were visualized on a 2% agarose gel in an electrophoresis device and presented in an electrophorogram (Figure 2).



Electrophoresis was performed in 2% - agarose gel. M1-O'generuler 1 kb DNA ladder (Fermentas); M2 - 100 bp DNA ladder Plus. 1 is a simple isolate isolated from a Diyora variety of potatoes, propagated in a Hawthorn (Datura stramonium) plant (Figure 1, a); 2 is a PVX isolated from a sample from a Gala variety of potatoes (Figure 1, B); 3 is a kxv isolated from a sample from an existing tomato plant (Lycopersicum esculentum Mill) (Figure 1, C); 4 is a PVX isolated from a sample from a Umid variety of potatoes (Figure 1, d). Primers are PVXF/ PVXR. Pzr Ahmed et al. da (2013) is done in the same way as quoted [3].

Figure 2. PVX in plants using the RT-PCR method make a diagnosis

As it can be seen from the picture, as a result of the investigation carried out using the RT-PCR method, all four sampled plants were infected with PVX based on the lines of the electrophorogram.

Therefore, as a result of the investigations, PVX was diagnosed using the RT-PCR method from plants with disease symptoms, and this serves as a basis for conducting further research, that is, studying the molecular-genetic characteristics of the virus.

For study and phylogenetic analysis of the isolates isolated from the climatic conditions of Uzbekistan based on the ORFs5 gene, the PCR product generated as a result of electrophoresis

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was cut and purified using a scalpel and submitted for sequencing (Evrogene, Russia) and as a result, it was isolated from the Umid variety and caused necrotic symptoms on the leaves of the *Datura stramonium* plant. A new isolate with the following nucleotide sequence was isolated from the sequence of the PCR product isolated from the source sample:

ATGTCAGCACCAGCTAGCACAACACAGGCCACAGGGTCAACTACCTCAACTACCACGAAA
ACTGCAGGCGCAACTCCTGCCACAGCTTCAGGCCTGTTCACCATCCCTGATGGGGATTTCTTCA
ATACAGCCCGTGCCATAGTAGCCAGCAATGCTGTCGCAACAAATGAAGACCTCAGCAAGATTGA
GGCTATTTGGAAAGACATGAAGGTGCCCACAGACACTATGGCACAGGCTGCTTGGGACTTAGTC
AGACACTGTGCTGATGTGGGATCGTCTGCTCAAACAGAAATGATAGATACAGGTCCCTATTCCA
ACGGCATCAGCAGAGCTAGACTGGCAGCAGCAGTCAAAGAGGTGTGCACACTTAGGCAATTTTG
CATGAAGTATGCTCCAGTGGTGTGGAACTGGATGTTAGCTAACAACAGTCCACCTGCTAACTGG
CAAGCACAGGGGTTCAAGCCTGAGCACAAATTCGCTGCATTCGACTTCTTCAATGGAGTCACTA
ACCCAGCTGCCATCATGCCCAAAGAGGGGCTCATCCGGCCACCGTCTGAAGCTGAAATGAATGC
CGCCCAAACTGCTGCCTTTGTGAAGATTACAAAGGCCAGGGCACAATCCAACGACTTTGCTAGC
CTAGATGCAGCTGTAACTCGAGGTCGTATCACTGGAACAACAACCGCTGAGGCTGTTGTCACTC
TACCACCACCATAA

According to the sequence results, the nucleotide sequence of the ORFs5 gene of the PVX isolate isolated in the climatic conditions of Uzbekistan was determined and it was deposited in the International Gene Bank - NCBI under the name PVX-UZ under the number MN702769 (Fig. 3).

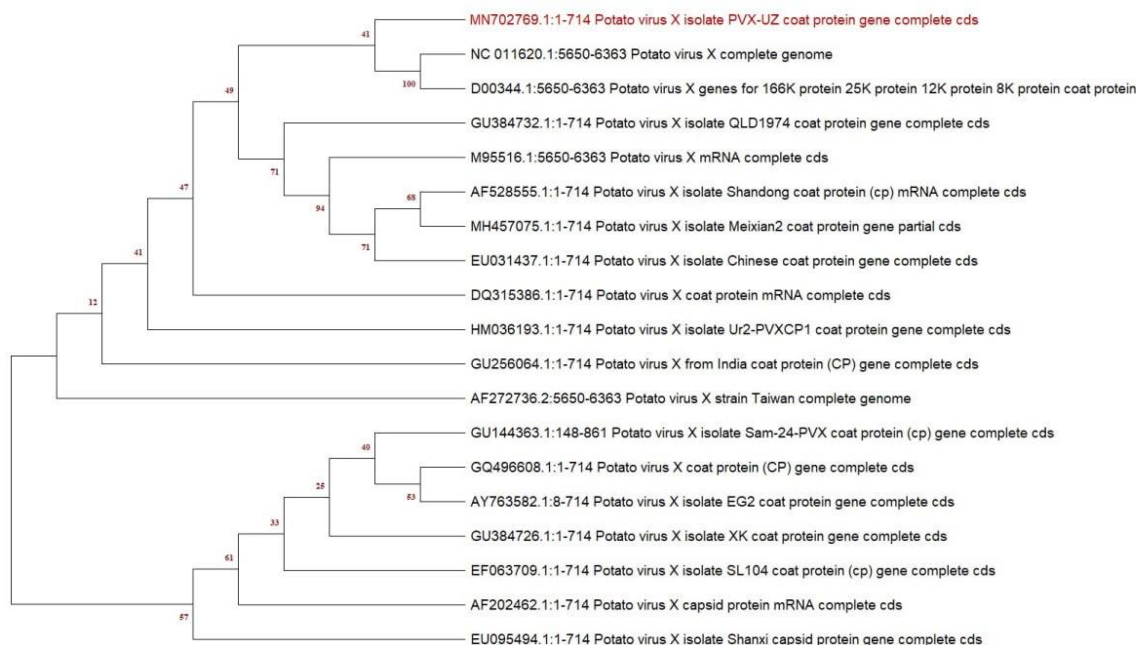

Figure 3. Phylogenetic tree of PXVn isolate isolated in Uzbekistan

In general, based on these obtained results, it can be concluded as follows, that is, the isolate MN702769 identified in Uzbekistan with the nucleotide sequence of ORFs5 gene Д00344.1 97.48%, AY297843.1, AY297842.1, M95516.1 97.06% with European isolates % affinity can be noted that this isolate originates from this European isolate and as a new isolate that underwent a certain change after the climatic conditions of our country.

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CONCLUSION

The PVXn isolate of the virus is different from the PVXo isolate *Ch. murale* causes yellow necrosis, *D. stramonium* causes symptoms such as dark flesh necrosis and plant growth retardation, *N. barley* causes green mosaic, N. rustica causes necrotic spots, and *Capsicum annum* causes scattered mosaic symptoms. was found to cause, the symptoms of the disease in the rest of the plants are listed in the table (Table 2).

The PVXn isolate of the virus differs from the PVXo isolate of tobacco. Both isolates belong to the *Chenopodiaceae* family album plant, *Phisalis floridana*, *Retunia hybrida*, *Solanum melon-gena, Capsicum annum* belonging to the *Solanaceae* family, Vigna sinensis belonging to the *Leguminosae* family, *O. basilicum* belonging to the *Labiatae* family, etc., did not cause disease symptoms (Table 2).

So, as a result of the conducted research, a "necrotic" isolate of PVXn was isolated in the climatic conditions of our country, causing systemic black mosaic symptoms in the Umid variety of potatoes, causing large necrosis in plants such as *Datura stramonium*, *Ch. murale*, *Gomphrena globosa*, and in Diyora and Gala varieties. Sporadic mosaic, and in *Datura stramonium* plants, compared with the "normal" PXVo isolate of the virus causing simple systemic mosaic symptoms, it was identified with the help of test-indicator plants and the disease symptoms were studied.

Potato virus diseases have been studied since 1916 by Kvainer, Botes, Shultsem, Folsom, Cassanis, Martin, Yora, Morel, and Ambrosov in countries such as England, Holland, USA, Germany, Russia, Estonia [13]. However, in the conditions of Uzbekistan, the sensitivity of the work was carried out only at the sensitivity level of indicator plants, drop method or ABV-test (0.2 μg/ml). A small number of viruses in many host plants, potato cultivars, remained outside the style sensitivity level. In this work, we used highly specific, high-titer antisera with a sensitivity level of 0.01ng [11; 12].

Many plants are asymptomatic and are being analyzed for the first time. For example, the plants that were studied for the first time are *Cucumis sativus*, *Rumex crispus*, *Brassica juncea (L) Czern*, *Althaea officinalis*, *Malva neglesta* Wall *Atriplex micrantha* C.A.Mey, which hides the virus, *Solanum nigrum*, *Datura metel*, *Ch. murale*, *Ch. quonea*, *Amaranthus retroflexus*, *Artemisia annua*, *Artemisia vulgaris* have been identified. So, there is no doubt that these plants are being included as reservoirs for the first time in phytovirology.

Nucleotide sequence of the isolate isolated from climatic conditions of Uzbekistan and studied molecular-genetic characteristics was used for further bioinformatic analysis. As a result of the analysis, PVXn isolate distributed in our country (MN702769) was 97.48% with isolate D00344.1 (Netherlands), 97.48% with isolates GU384732.1 (from Australia), AY297843.1, AY297842.1 and M95516.1 (Great Britain). 06% homology was found and its phylogenetic tree was constructed (Figure 3). The phylogenetic tree shows that this isolate is very closely related to the Dutch isolate D00344.1, and to the GU384732.1 isolate on a long branch in the phylogenetic tree, as a result of their dispersal from a common ancestor and subsequent divergence due to their geographic distribution indicates its origin.

REFERENCES

1. Amanov, B., Muminov, K., Samanov, S., ...Arslanov, D., Tursunova, N. (2022). Cotton introgressive lines assessment through seed cotton yield and fiber quality characteristics. Sabrao Journal of Breeding and Genetics, 54(2), pp. 321–330.
2. Arshad, J., Ahmad, N., Bushra, T., Muhammad, T., Abdul, M. (2012). Molecular characterization of capsid protein gene of potato virus X from Pakistan. *African Journal of Biotechnology*, Vol. 11(74), pp. 13854-13857.
3. Ahmed, Sh., Ahmed, I., Yaqub, T., Waseem, M., Tabassum, B., Masood, F., Khan, A. (2013). Molecular detection, phylogenetic analysis and designing of siRNA against Potato Virus X. *Advance ments in Life Sciences,* Vol . 1, Issue 1. pp. 37-44.
4. Eshboev, F., Yusupova, E., Piyakina, G., Mejlumyan, L. (2019). Isolation and study of the amino acid composition of proteins from soybean for producing conjugates for elisa//*Universum: Химия и биология: электрон. научн. журнал,* №11(65), –с. 62-65.
5. Belinda, A., Roger, A., Jones, C. (2010). Genetic variability in the coat protein gene of Potato virus X and the current relationship between phylogenetic placement and resistance groupings. *Arch Virol*, 155:1349–1356.
6. Baboev, S.K., Buranov, A.K., Bozorov, T.A., Adylov, B. S., Morgunov, A. I., Muminzhonov, K. H. (2017). Biological and agronomical assessment of wheat landraces cultivated in mountain areas of Uzbekistan. Sel'skokhozyaistvennaya Biologiya,*52*(3), 553-560.
7. Jovliyeva, D.T., Sadriyeva, Z.N., Muhamadaliyev, O.D. & Fayziyev V.B. (2022). Determination of the effect of Alstromeria X virus on chlorophyll amount in plant leaves. Modern biology and Genetics, 1-2 (1), 8-13.
8. Qulmamatova, D.E., Baboev, S.K., Buronov, A.K. (2022). Genetic variability and inheritance pattern of yield components through diallel analysis in spring wheat. Sabrao Journal of Breeding and Genetics, 54(1), pp. 21–29.
9. Ramazonov, B.R., Mutalov, K.A., Fayziev, V.B., Koraev, S.B. (2020). Morphogenetic characteristics and biological activity of Takyr and meadow soils of the Republic of Karakalpakstan (On the example of soils of chimbay district)morphogenetic characteristics and biological activity of Takyr and Meadow soils of the Republic of Karakalpakstan (on the example of soils of Chimbay District). Journal of Critical Reviews, 7(5), pp. 243–24.
10. Ramazonov, B.R. (2020). Soil cover of the aral sea region and change of soil properties under the influence of desertification (On the example of soils of the karakalpakstan region). Journal of Advanced Research in Dynamical and Control Systems, 12(6 Special Issue), pp. 620–631.
11. Fayziev, V., Jovlieva, D., Juraeva, U., Shavkiev, J., Eshboev, F. (2020). Effects of PVXN-UZ 915 necrotic isolate of Potato virus X on amount of pigments of Datura stramonium leaves. Journal of Critical Reviews, 7(9), pp. 400–403.
12. Sattorov, M., Sheveleva, A., Fayziev, V., Chirkov, S.(2020). First Report of Plum Pox Virus on Plum in Uzbekistan. Plant Disease , 104(9), pp. 2533

13. Sobirova, Z.S., Fayziev, V.B., Abduraimova, K.I. (2020) Effect of the virus of the yellow dwarf corn mosaic growth and development of varieties of corn in various phases. Journal of Advanced Research in Dynamical and Control Systems, 12(6 Special Issue), pp. 602–606.
14. Ceroska, N., Moravec, T., Plchovec, H., Hoffmeisterova, H., Folwarezna, J., Dedic, P. (2010). Production of polyclonal antibodies to Potato Virus X using recombinant coat protein. *J. Phytopathology,* Vol.158, pp. 66-68.
15. Chirkov, S., *et al.* 2016. *Arch. Virol*., 161: 425. https://doi.org/10.1007/s00705-015-2658-x.
16. Mohammad, H., Nemat, S. (2017). Population genetic analysis of potato virus X based on the CP gene sequence. *Virus Dis.,* (January–March), 28(1): 93–101.
17. Muminov, K., Amanov, B., Buronov, A., Tursunova, N., Umirova, L. (2023). Analysis of yield and fiber quality traits in intraspecific and interspecific hybrids of cotton. Sabrao Journal of Breeding and Genetics, 55(2), pp. 453–462.
18. Hossain, M., Soodabe, P., Shabnam, P., Mohammad, M., Jahangir, H., Akbar, H., Katherine, B., Arvind, V. (2014). Molecular characterization and field survey of Iranian potato virus X isolates. *Virus Dis*, (July–September 2014) 25(3): 338–344.
19. Neda, E., Mina, K., Thomas, H., Mikhail, M. (2012). Complete genome sequence of an Iranian isolate of Potato virus X from the legume plant Pisum sativum. *J. Virus Genes*, Vol. 39, pp. 141–145.
20. Khan, A., Tabassum, B., Nasir, I., Bilal, M., Tariq, M. and Husnain, T. (2015). Potato virus X from Pakistan: coat protein sequence analysis. *The Journal of Animal & Plant Sciences*, 25(4), Page: 1016-1021. ISSN: 1018-7081.
21. Kagiwada, S., Yamaji, Y., Komatsu, K., Takahashi, S., Mori, T., Hirata, H., Suzuki, M., Ugaki, M., Namba, S. (2005). A single amino acid residue of RNA-dependent RNA polymerase in the Potato virus X genome determines the symptoms in Nicotiana plants. *Virus Res*, 110:177–182.
22. Kholmatova, M.M. & Fayziyev V.B. (2023). Detection and identification of some biological characteristics of potato L-virus using real-time PCR method. Modern biology and Genetics, 1 (3), 19-26.
23. Karpova, O., Zayakina, O., Arkhipenko, M., Sheval, E., Kiselyova, O., Poljakov, V., Yaminsky, I., Rodionova, N. & Atabekov, J. Potato virus X RNA-mediated assembly of single-tailed ternary 'coat protein–RNA–movement protein' complexes. *J. Gen. Virol*, 87, 2006. – P. 2731–2740.
24. Yusubakhmedov, A.A. & Fayziev V.B. (2022). Determination of some biological properties and identification of potato virus using the PCR method. Modern Biology and Genetics, 1-2(1), 14-20.