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Genetic and biological signature of Potato Virus X circulating in Kazakhstan

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ABSTRACT

This research studied ten isolates of potato virus X (PVX) isolated from potato cultivars growing in Kazakhstan (KZ), Belarus (BY) and China (CN). Additionally, the potato samples were screened for Potato virus Y (PVY), Potato virus S (PVS), and Potato leafroll virus (PLRV) using multiplex reverse transcription-polymerase chain reaction (RT-PCR). PVX was isolated from the potato varieties 'Tamasha', 'Red Scarlet', 'Gala variety', 'Krone', 'Juwel', 'Gala', and 'b2-7'. The identified isolates of PVX were sequenced and used to construct a phylogenetic tree, which demonstrated that KZ and BY isolates form clusters closely related to isolates from Asia and Europe, however, CN isolate formed a separate clade. KZ5 and KZ7 PVX isolates showed local symptoms of mosaic and light green speckled spots on the upper leaves of *Datura stramonium* in pathogenicity test.

Keywords: Potato, Potato Virus X, isolates, ELISA, PCR, Targeted sequencing, Indicator plants.
Article type: Research Article.

INTRODUCTION

Potato, *Solanum tuberosum* is the third most important crop grown in 150 countries around the world (Birch *et al.* 2012). It is one of the main food products of the population characterized by great plasticity, adaptability, and potential productivity (Haq *et al* 2021; Suraganova *et al.* 2022). In the distribution of production volumes, the most important agricultural potato crops are within the following limits for the EAEU countries: Armenia 0.4 million tons, Kyrgyzstan 1.4 million tons, Kazakhstan 3.9 million tons, Belarus 6.1 million tons, Russia 22.1 million tons. The average potato yield in Kazakhstan in 2022 was 205.4 kg ha^{-1}, which is 2-3 times less than in potato-growing countries (Netherlands, France, Israel, USA, etc.) (Devaux *et al.* 2021). The yield and quality of potatoes depend on the degree of their infection with several pathogens, including bacteria, fungi, viruses, and viroids (Al Zaidi *et al.* 2023). According to the United Nations Food and Agricultural Organization (FAO), global potato crop looses from diseases amount to about 90 million tons annually. The viral diseases of potatoes are the most harmful. Losses due to viruses and related pathogens are not limited to the year of initial infection (Kuldybayev *et al.* 2023). They continue to increase gradually until the crop is saturated with one or more viruses, i.e., every time seed material from an infected population is used (Chirkov 2009). Even though more than 40 potato viruses active under natural conditions have been described, only nine of them achieved economic significance globally. These are potato leafroll virus (PLRV), potato viruses A, M, S, V, X, and Y (PVA, PVM, PVS, PVV, PVX, and PVY), potato mop-top virus (PMTV), and tobacco rattle virus (TRV; Anisimov 2010)
According to the data on the distribution of viruses, potato varieties in North Kazakhstan are infected with various combinations of viruses: PVX, PVS, and PVM: 95-99%, PLRV: 30%, gothic: 27%, and rugose mosaic: 16%.

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The Kokshetau region is characterized by a low spread of viral diseases: 0.2-12%, mainly mottled mosaic. The incidence of viral diseases in the mountainous regions of the Almaty region is also low: 5-27%. According to studies by Ospanova *et al.* (2014) the distribution of viral diseases in South Kazakhstan in 2014 were as follows: PLRV: 5%, PVM: 8.5%, PVS: 31.7%, PVX: 41.9%, and PVY: 14.5%. Researchers in Southeast Kazakhstan have found instances of mono-infection by each of the three viruses: PVM: 36.13%, PVS: 0.84%, and PVY: 5.88%. PVM was the only example of mono-infection (28.74%) in the Northern region, while PVS and PVY were detected only as a part of multiple infection. In general, the prevalence of single infections was 42.85% in Almaty region and 28.74% in Kostanay region (Aleksandrova *et al.* 2018; Aleksandrova *et al.* 2018). According to the virus screening conducted in various regions of Kazakhstan (Beisembina 2021), the Republic enjoys a uniform distribution of almost all main potato viruses: PLRV (39.6%), PVS (38.8%), PVY (36.8%), and PVX (35.8%) with a slight predominance of PVM (55.2%). Of all the listed common potato viruses, PVX has been studied the least in Kazakhstan. In addition, PVX was included in the list of 10 major plant viruses in plant molecular pathology in 2011 due to its scientific development as a model for studying interactions between plants and viruses (Scholthof *et al.* 2011; King *et al.* 2012). This virus does not cause visible symptoms in many varieties of potatoes, so it remains unnoticed. Depending on the pathogenicity of the strain, the virus can damage crops in various ways: weak pathogenic strains can reduce yield by 12%, and the strong pathogenic ones by 45%. The decrease in yield, even in the absence of symptoms of the disease, can reach 10% and 45% with mosaic (Jari 2015). PVX affects almost all potato varieties, in all areas of its cultivation. The latent form of infection is the most common, since they are difficult to distinguish from healthy plants. Currently, PVX can be identified using the classic (serological and biological testing) and modern diagnostic methods such as enzyme-linked immunosorbent assay (ELISA), Indirect hemagglutination assay, RT-PCR, and DNA sequencing. In the present research, the ten isolates of PVX were genetically characterized and two of KZ isolates induced virus specific symptoms in indicator plant (Blotskaya 1989).

MATERIALS AND METHODS

Sampling and detection of viruses

The potato plants showing the symptoms of viral infection were collected in the fields of Kazakhstan, Belarus, and China. The young leaves and tubers of each plant were tested for viruses' presence. Around 300 samples were analyzed (Shvidchenko *et al.* 2011). ELISA was the first step in the routine detection of viruses and was performed using commercial PVX detection kit from Loewe (Germany), according to the manufacturer's instructions. PVX positive samples were additionally tested by RT-PCR. Besides PVX, other viruses such as PLRV, PVY, and PVS were also analyzed to reveal the samples with multiple infection. To test the samples by RT-PCR, the total RNA of each sample was isolated using Trizol reagent according to the protocol supplied by the manufacturer (Thermo Fisher Scientific, USA). Complementary DNA (cDNA) was synthesized using RevertAid Reverse Transcriptase (Thermo Scientific, USA). A mixture of 200 ng of RNA, 2 μM Oligo-dT, and 2 μM random hexamer primers to the final volume of 15 μl, was incubated for 10 min at 72 ℃ and then cooled on ice. The next step included the addition of 5 × RT reaction buffer, 0.5 mM dNTPs, and 100 U reverse transcriptase. Then cDNA was synthesized at 45 ℃ during 1 h. The PCR was conducted in the reaction mix containing 2 μL cDNA from RT step, 1U DNA polymerase (Thermo Scientific, USA), 0.2 mM of each dNTP, 1× PCR buffer, 2.5 mM $MgCl_2$, and 0.2 μM of each primer (Gritsenko *et al.* 2022). The PCR program consists of denaturation at 96 ℃ during 3 min followed by 30 cycles of denaturation at 96 ℃ for 20 sec, primer annealing at 46 ℃ for 20 sec, and elongation at 72 ℃ for 1 min. The final elongation was performed at 72 ℃ during 5 min.

Targeted sequencing

The detected PVX isolates were sequenced using the primers for full capsid protein gene (forward – 5'-atgactacaccagccaacac-3' and reverse - 5'- gagttatggtggtgggagag-3'). The sequencing PCR was performed using BigDye terminator V.3.1 kit according to manufacturer protocol followed by capillary electrophoresis on a 3500 genetic analyzer (Applied Biosystems, California, USA) using the StdSeq50_POP7 mode.

Phylogenetic analysis

All CP gene sequences obtained in this research were aligned with available CP sequences of PVX isolates retrieved from NCBI using MUSCLE algorithm (link). The phylogenetic tree was constructed in MEGA XI (link)

program by the maximum likelihood method. The visualization of the phylogenetic tree was performed in FigTree v.1.4.4. software.

Pathogenicity test

The PVX inoculum was prepared in 0.1 M phosphate buffer (pH 7.2) by grinding leaves of infected potato plants [20]. The crude sap was mechanically inoculated onto the two leaves of 3-week old *Datura stramonium* L. plant. Four plants for each virus isolate were inoculated. The mock inoculation was performed by buffer alone as a control. The infection symptoms were evaluated on the 14th to 16th day post inoculation (DPI).

RESULTS

Detection of viruses

Out of 300 potato samples, 35 were positive for PVX infection identified by ELISA (Table 1). The virus was detected in both leaves and tubers. Each PVX isolate was assigned a name based on the country of origin and its serial number. The PVX positive samples were additionally tested by multiplex RT-PCR to confirm the ELISA results. Moreover, PVY and PVS were identified in six tested samples (Fig. 1).

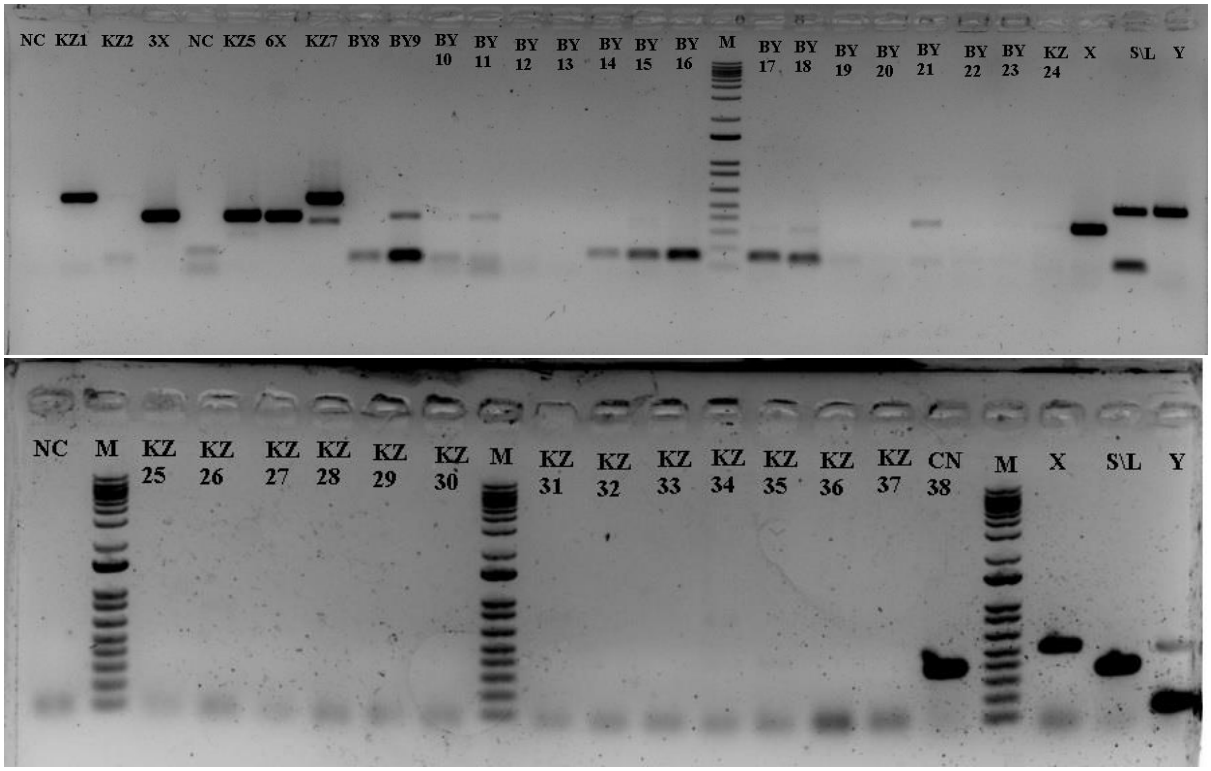

Fig. 1. The results of multiplex PCR for the viruses PVX (376 bp), PVS (149 bp), PVY (535 bp), and PLRV (249 bp). NC – negative control, 3X, 6X, X positive controls for PVX; S – positive control for PVS; L – positive control for PLRV; Y – positive control for PVY, M – molecular marker 1 kb plus (Invitrogen).

The results of multiplex PCR did not confirm the presence of PVX isolates BY 12, BY 13, BY 19, BY 20, BY 22, BY 23, and BY 24 in the plants. These plants were also negative for other viruses. The only PVX infection was confirmed in the plants KZ 3, KZ 5, KZ 6, BY 21, and CN 38. The 'Alliance' (KZ 1) was infected only by PVY. The 'Red Scarlet' (KZ 7) was positive for both PVY and PVX. The infection induced by both PVS and PVX was confirmed in the samples BY10, BY11, BY17, and BY18. PLRV was not found in the tested samples. The samples KZ 2, BY 8, BY 14, BY 15, and BY 16 were infected only by PVS. Out of 35, only 10 samples were confirmed as PVX positive by RT-PCR.

Phylogenetic analysis

The full CP gene of each PVX isolate obtained in this research was sequenced and aligned to 58 available PVX isolates from NCBI. The results of the phylogenetic analysis showed that KZ and BY isolates formed a clade with

PVX from India, Argentina, Ukraine and the UK. The genetic diversity according to host variety or place of isolation was not confirmed. Notably, CN38, isolated from a Chinese field, formed cluster with other Chinese isolates which were the most represented inside the GenBank. China isolates were represented in each large clade.

Table 1. Results of ELISA analysis of potato samples for PVX infection.

No.	Variety name	Country, region/city	Sample/ isolate	Extinction A450, p.u.	Ao/Ok	Result of reaction, +/-
1	Alliance	Kazakhstan, Akmola region	KZ 1	0.880	3.2	+
2	Colomba	Kazakhstan, East Kazakhstan Region	KZ 2	1.201	4.3	+
3	Tamasha	Kazakhstan, Akmola region	KZ 5	1.461	5.3	+
4	Red Scarlet	Kazakhstan, East Kazakhstan Region	KZ 7	0.808	2.9	+
5	Nixe.	Belarus	BY 8	1.534	5.5	+
6	Nixe.	Belarus	BY 9	1.628	5.9	+
7	Paroli	Belarus	BY 10	1.317	4.7	+
8	Paroli	Belarus	BY 11	1.534	5.5	+
9	Gala	Belarus	BY 12	2.308	8.3	+
10	Gala	Belarus	BY 13	1.390	5.0	+
11	Krone	Belarus	BY 14	0.840	3.0	+
12	Krone	Belarus	BY 15	3.009	10.8	+
13	Krone	Belarus	BY 16	0.915	3.3	+
14	Krone	Belarus	BY 17	1.637	5.9	+
15	Krone	Belarus	BY 18	2.308	8.3	+
16	Krone	Belarus	BY 19	0.996	3.6	+
17	Juwel	Belarus	BY 20	2.420	8.7	+
18	Juwel	Belarus	BY 21	3.009	10.8	+
19	Juwel	Belarus	BY 22	1.058	3.8	+
20	Juwel	Belarus	BY 23	1.412	5.1	+
21	Gala	Kazakhstan, Karaganda region	KZ 24	0.877	3.2	+
22	Gala	Kazakhstan, Karaganda region	KZ 25	0.850	3.1	+
23	Gala	Kazakhstan, Pavlodar region	KZ 26	0.899	3.2	+
24	Gala	Kazakhstan, Pavlodar region	KZ 27	0.920	3.3	+
25	Gala	Kazakhstan, Pavlodar region	KZ 28	0.845	3.0	+
26	Gala	Kazakhstan, Pavlodar region	KZ 29	0.840	3.0	+
27	Gala	Kazakhstan, Pavlodar region	KZ 30	0.895	3.2	+
28	Gala	Kazakhstan, Pavlodar region	KZ 31	1.020	3.7	+
29	Vega	Kazakhstan, Kostanay region	KZ 32	0.980	3.5	+
30	Vega	Kazakhstan, Kostanay region	KZ 33	0.903	3.2	+
31	Gala	Kazakhstan, Pavlodar region	KZ 34	0.908	3.3	+
32	Gala	Kazakhstan, Pavlodar region	KZ 35	0.870	3.1	+
33	Gala	Kazakhstan, Pavlodar region	KZ 36	0.996	3.6	+
34	Gala	Kazakhstan, Pavlodar region	KZ 37	0.975	3.5	+
35	b 2-7	China, Chongqing	CN 38	1.588	5.77	+
	Blank			0.102	0.4	
	Positive			0.984	3.5	+
	Negative			0.278	-	-
	Note: "+" is a positive result; Ao/Ok is the ratio of the average optical density of the test sample to the average optical density of the negative control; Blank –control; Positive – positive control; Negative – negative control.					

Pathogenicity test

Biological testing is an important method to investigate the pathogenicity of viruses. According to Bawden & Kassanis, pathogenicity of a virus strain depends on the host plant variety. The main indicator plants for PVX are *Gomphrena globosa*, *Datura stramonium L.*, *Nicotiana tabacum*, and *Capsicum annum* (Dowley 1973; Shvidchenko *et al.* 2021). In our research, *D. stramonium* was inoculated by identified PVX isolates, and symptoms of infection were evaluated on the 16th day. Four plants were inoculated by each isolate. Only two isolates, i.e., KZ5 and KZ7 showed symptoms on *D. stramonium* (Fig. 3).

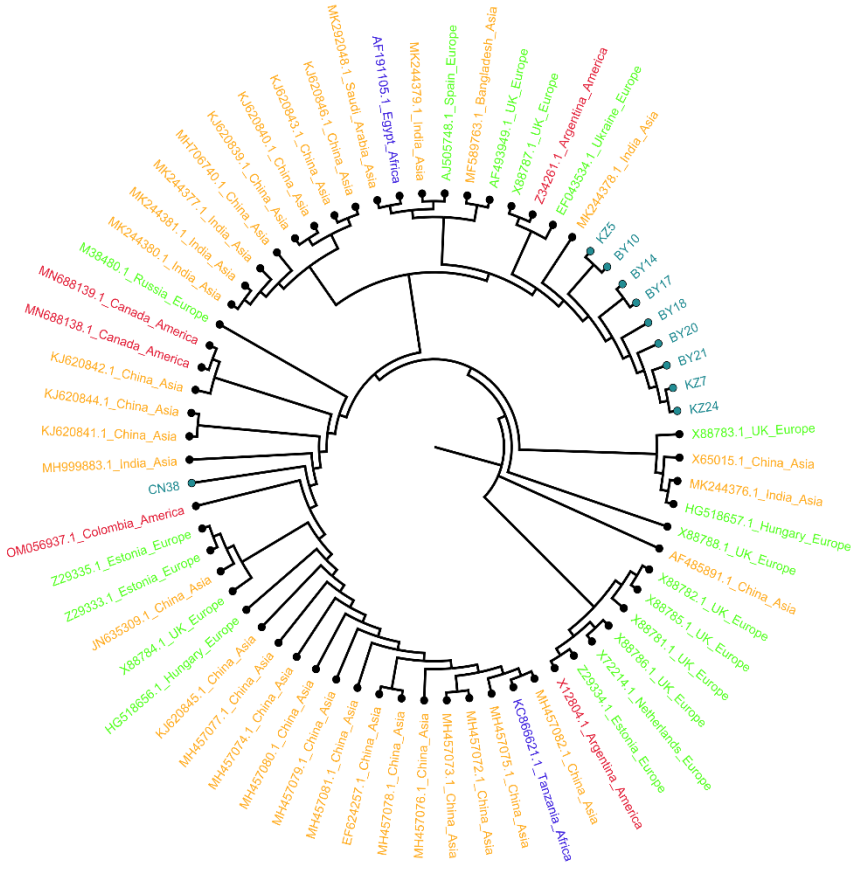

Fig. 2. Maximum likelihood cladogram of PVX isolates. Isolates from Africa are marked in blue; American in red; European in green; Asian in orange; and Kazakh in cyan.

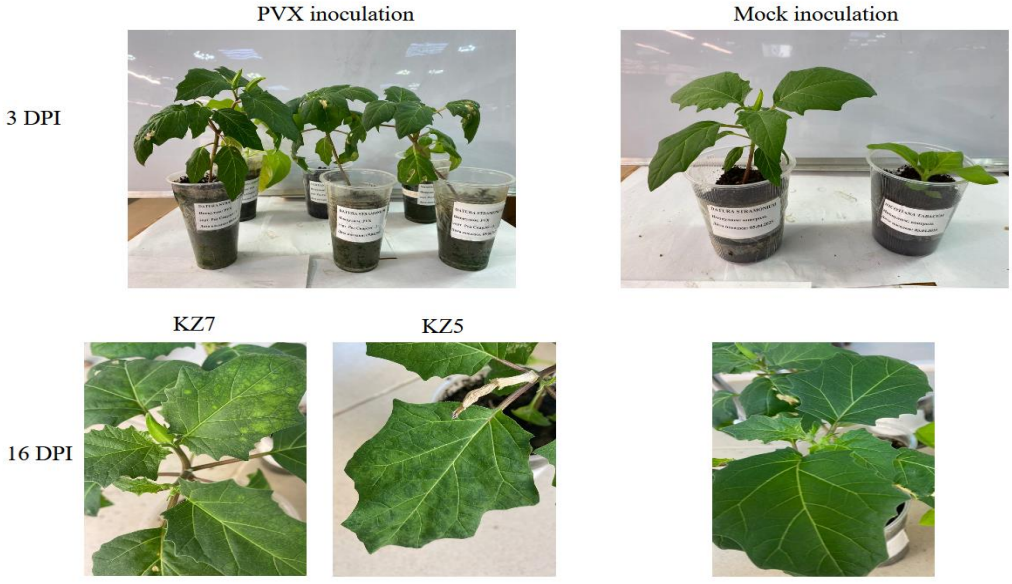

Fig. 3. *Datura stramonium* plants inoculated with PVX isolates, DPI – day post inoculation.

The isolate KZ7 showed local symptoms of soft mosaic on the upper leaves of *D. stramonium* while isolate KZ5 caused symptoms of light green speckled spots on the upper leaves, in agreement with the previous studies (Hajizadeh & Sokhandan Bashir 2017; Malko *et al*. 2023).

DISCUSSION

In this study, out of 300 potato samples, 35 and 10 were identified as positive for PVX by ELISA and RT-PCR respectively. The collecting of samples was conducted in Kazakhstan, Belarus, and China. Sequencing and phylogenetic analyses were performed to determine the geographical origin of PVX isolates. Previous studies by Kreuze & Jones (Kreuze *et al.* 2020; Jones *et al.* 2013) comparing the CP gene sequences of 13 new PVX isolates from Australia or the UK with those of 72 other isolates from GenBank as well as phylogenetic analysis revealed two major phylogroups (I and II) and two minor phylogroups (II-1 and II-2). Most of the isolates belonged to the main phylogroup I, originating from Australia, Africa, Asia, Europe, South America (not Andean), and North America, and they included the Argentine X MS isolate. Recent studies (Fuentes *et al.* 2021) show that a large number of isolates from Asia, Australia, Europe, and America formed two separate lineages: Clade I contained more than 70 isolates with over 93% nucleotide identity, which were described as the Eurasia group; while Clade II contained fewer isolates with more than 82% identity and known as the American group. In the present study, genetic analysis of potato virus X based on the sequence of the CP gene showed that the tested isolates belonged to one phylogroup consist of strains from India, Argentina, Ukraine and the UK. The pathogenicity testing revealed only two PVX isolates induced durable symptoms of mottling and soft mosaic on *Datura stramonium* plant, in agreement with the literature data (Lebenshtein *et al.* 2000). The previously identified strains cause necrotic strokes, severe mosaics, curly and wrinkled leaves, as well as significant crop losses (Kreuze *et al.* 2020).

CONCLUSION

In this study, out of 300 potato samples, 35 and 10 were identified as positive for PVX by ELISA and RT-PCR respectively. The collecting of samples was conducted in Kazakhstan, Belarus, and China. The PVY and PVS were identified in 11 samples. The identified by RT-PCR isolates of PVX were sequenced and used to construct a phylogenetic tree, which demonstrated that KZ and BY isolates formed clusters closely related to isolates from Asia and Europe, but CN isolate formed a separate clade. KZ5 and KZ7 PVX isolates showed local symptoms of mosaic and light green speckled spots on the upper leaves of *Datura stramonium* in pathogenicity test.

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