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RESEARCH ARTICLE
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Detection of Molecular Markers of Potato Virus X Resistance Genes in the Potato Gene Pool of Kazakhstan

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ABSTRACT

The study aimed to identify and characterize the presence of molecular markers associated with potato virus X resistance genes (*Rx1, Rx2*, and *Nb*) in the gene pool of Kazakhstan. During the study, 71 potato varieties and breeding lines were analyzed using the SCAR molecular markers and markers GM 637 and GM 339 for the *Nb* gene. DNA was extracted from leaf tissues and analyzed using a polymerase chain reaction to identify the presence of these markers. Artificial potato virus X inoculation was performed under controlled conditions in a phytotron followed by ELISA tests to confirm the infection status. The study identified SCAR markers for the *Rx1* gene in 25% of the samples and for the *Rx2* gene, in 77%. Markers of the *Nb* gene were found in 68% of the samples. 15 varieties showed extreme resistance to potato virus X, as evidenced by the absence of symptoms and negative ELISA results, while three varieties showed resistance similar to tolerance, where potato virus X was detected in leaf tissue without symptoms. The characterized varieties and breeding lines of the potato gene pool of Kazakhstan, which have an extreme type of resistance to potato virus X, can be included in breeding programs to create new virus-resistant potato varieties in the future.

Keywords: Potato virus X; Breeding line; Molecular marker; Infection; Resistance

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INTRODUCTION

In the context of modern agriculture, sustainable practices are becoming increasingly vital for ensuring long-term food security and environmental protection (Kamalieva et al., 2020). The highly harmful impact of viral diseases on potato plants is explained by the fact that under the influence of a viral infection, plant growth and development deteriorate, the productivity of this crop significantly decreases, and the quality of tubers suffers (Bashir et al., 2021; Ospankulova. et al., 2023). About 40 phytopathogenic viruses infecting potatoes are currently known (Voluevich & Pavlyuchuk, 2013). Potato virus X (PVX) is considered one of the most widespread potato viruses in the world (Liu et al., 2021). Plant infection with PVX often does not have pronounced manifestations. Thus, for a long time during the growing season of potato plants, the infection remains unnoticed. However, under favorable conditions for PVX, its reproduction leads to a significant decrease in potato yield, reaching 10-25% (Anisimov et al., 2009).

To minimize the consequences of the spread of pathogens of viral infections in countries with a high level of agricultural development, phytosanitary measures are maintained and improved, providing for constant monitoring of the spread of viruses and certification of planting material based on laboratory diagnostics of phytopathogens and technology for protecting potato varieties from viral pathogens. However, in the long term, it is preferable to create virus-resistant potato varieties (Makarova et al., 2017). Studies on the distribution of the supersensitivity genes *Nx* and *Nb* in the potato gene pool and the molecular markers linked to them are few.

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This research is complicated by the fact that to this day molecular markers have not been developed to provide reliable detection of the *Nx* gene using modern DNA technologies. Information on whether old potato varieties carry *Nx* or *Nb* hypersensitivity genes is currently available for varieties created in Germany (Ross & Kohler, 1953), North America (Bagnall, 1961) and the United Kingdom (Cockerham, 1943). In the study conducted by Nyalugwe et al. (2012) involving 38 varieties and one breeding line with European origin, North American, South American, and Australian origin, the presence of the *Nx* gene was detected in 10 samples (26% of the samples) and the presence of the *Nb* gene – in eight samples (21%). There are no reliable chemical means of viral infection control due to the biology of viral pathogens. The only effective and relevant approach is the selective creation of potato varieties carrying virus-resistance genes (Boris & Kochieva, 2013). There are four main types of plant resistance to phytopathogens: extreme resistance (immunity, complete immunity), hypersensitivity (HS), field (nonspecific) resistance, and tolerance (Amelyushkina & Semeshkina, 2011).

In the South region of Kazakhstan, according to Ospanova et al. (2014) the level of potato infection with PVX was 41.9% (Ospanova et al. 2014). According to Beisembina (2024), infection of potato plants with PVX in Kazakhstan reached 35.8%. The first molecular studies of PVX isolates common in potato plantings in Kazakhstan were published by (Azhimakhan et al., 2023a). The results of phylogenetic analysis showed that Kazakh PVX isolates form clusters closely related to isolates from Asia and Europe (Azhimakhan et al., 2023b). Thus, the rapid spread of PVX in Kazakhstan requires studying the resistance of the potato gene pool to PVX. Therefore this study aimed to screen samples of the Kazakh potato gene pool for the presence of DNA markers linked to PVX resistance genes and to determine the type of resistance of the samples to this virus.

MATERIALS & METHODS

The research was carried out at the Laboratory of Plant Biotechnology, part of the Department of Biology, Plant Protection, and Quarantine at the S. Seifullin Kazakh Agrotechnical Research University (S. Seifullin KATRU). This study was conducted as part of two significant scientific initiatives. Firstly, it was supported by Grant No. AP14870270 titled "Molecular genetic substantiation of domestic and foreign potato varieties and hybrids resistant to main viral, nematode, and late blight pathogens." This grant was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan for the period 2022–2024. The project aims to enhance the understanding of genetic resistance mechanisms in potato varieties and hybrids, focusing on resilience against critical pathogens such as viruses, nematodes, and *Phytophthora infestans*, the causative agent of late blight.

Secondly, the study was integrated into the framework of the International Scientific Program "Creation of promising potato lines based on the genetic resources of the People's Republic of China and Kazakhstan" (2021-2023). This program emphasizes collaboration between Kazakhstan and China to develop innovative potato lines by leveraging the genetic resources of both nations, addressing regional agricultural challenges, and enhancing potato crop resilience and productivity. The combined efforts under these programs aim to advance the scientific basis for breeding and biotechnology in potatoes, contributing to sustainable agricultural development and food security in Kazakhstan and beyond. The objectives of the study were 71 varieties and breeding lines of the Kazakh potato gene pool, maintained in the collections of the S. Seifullin KATRU (Table 1). DNA isolation from the leaf tissue of potato plants was carried out using PhytoSorb kits (Syntol, Russia). The detection of molecular markers 1Rx (*Rx1* gene), 5Rx1 (*Rx1* gene), 106 Rx2 (*Rx2* gene), GM 339 (*Nb* gene), and GM 637 (*Nb* gene) was carried out using polymerase chain reaction (PCR) performed on MasterCycler Gradient (Eppendorf, USA) and T100 (Biorad, USA).

The temperature conditions of PCR, the composition of the reaction mixture, and the detection conditions corresponded to the methods given in (Santa Cruz & Baulcombe, 1995; Gavrilenko et al., 2018; Klimenko et al., 2019; Ahmadvand et al., 2023). To assess the resistance of the samples to PVX, artificial infection was performed by mechanical inoculation of the leaves of the studied plants with a biomaterial infected with PVX using carborundum. Leaf tissue of potato plants of the Ulybka variety infected with isolate 17x/21 belonging to the X1 strain group was used as an inoculum for infection (unpublished data). The infection of the potato samples was carried out at the stage of 4-6 true leaves. The results were evaluated using laboratory diagnostic detection of PVX by enzyme-linked immunosorbent assay (ELISA) in leaf samples taken 30 days after infection with PVX. ELISA was performed using diagnostic kits for the detection of PVX by Agdia (USA) according to the manufacturer's instructions. Potato plants were grown for artificial infection under controlled phytotron conditions (temperature: 20 to 25°C, photoperiod (illumination/darkness): 16/8 h, illumination intensity: 5,000 to 7,000 lux, humidity: 60 to 70%, soil: chernozem of lumpy and granular composition). Biomaterial of the Innovator (*Nb* gene) (Nyalugwe et al., 2012), White Lady (*Rx2* gene) (Ahmadvand et al., 2013), and Sante (*Rx1* gene) varieties were used as a positive control for the detection of molecular markers using PCR (Gavrilenko et al., 2018).

Stages

At the first stage, we detected molecular markers linked to PVX resistance genes in varieties and breeding lines of the Kazakh potato gene pool. Based on the characteristics of varieties and breeding lines (Table 1), for most samples of the Kazakh potato gene pool, there is only a generalized description of resistance/susceptibility to viral infection, without specifying the available type of resistance in a genotype (variety, breeding line). There is no listing of specific viral pathogens that potato samples are resistant to. Therefore, at the next stage, we evaluated the type of resistance of potato samples to PVX. Based on

Table 1: List of studied potato samples, origin, and characteristics

Item No.	Name	Origin	Country, originator	Source of biomaterial	Sample characteristics*
1	Akkol	Mavka x 80-1sh	Kazakhstan, Kazakh Research Institute of Potato and Vegetable Growing (KazRIPVG)	KazRIPVG	Resistant to viral diseases
2	Akjar	Vilia × [digaploid S. andigenum USW 1793 × S. rybinii]	Kazakhstan, KazRIPVG	KazRIPVG	Susceptible to viral diseases
3	Auyl	Gerlinde x Senecano	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
4	Adil	7P-41 × Dobro 129/4	Kazakhstan, North-Western Scientific and Production Center for Agriculture (NWS&PCA)	Kostanay Scientific Research Institute of Agriculture (Kostanay SRIA)	Resistant to viral diseases
5	Aksor	[Savory x Olev] x Rezerv	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
6	Artem	Self-pollination of the improved Ermak variety	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Resistant to common diseases in the north of Kazakhstan
7	Alyans	Line 397077-16 (material from the International Potato Center (CIP), Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
8	Babaev	Line 720189 (material from CIP, Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
9	Bolashak	Line 720139 (Hydra x MPI-904.61)	Kazakhstan, KazRIPVG	KazRIPVG	Relatively resistant to viral diseases
10	Berkut	B298-7/2000 s/o	Kazakhstan, KazRIPVG	Kostanay SRIA	No data available
11	Vid 2	Self-pollination of seedling n-1898	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Resistant to viral diseases
12	Valery	Self-pollination of the Vesna variety	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Resistant to viral diseases
13	Didar	Adretta x Katahdin	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to the potato leafroll virus (PLRV)
14	Dunyasha	Shortandinsky × Omega	Kazakhstan and Russia, NWS&PCA and Siberian RIA	KazRIPVG	Resistant to viral diseases
15	Elaman	D834620 (Agouti x Hydra)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
15	Dihan	cellular selection of Gatchinsky-grade biomaterial with subsequent clone selection	Kazakhstan, KazRIPVG	Scientific and Production Center of the National Academy of Sciences (S&PC NAS) of Belarus for potato, fruit, and vegetable growing	A tolerant type of resistance to viral diseases
16	Janaysan	Yubel x Anoka	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
17	Jualy	K 532 x Gitte	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
18	Ilyin	Line 392781.1 (material from CIP, Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
19	Karasaisky	Poet x Priekulsky ranniy	Kazakhstan, KazRIPVG	All-Russian Institute of Plant Genetic Resources (VIR)	Resistant to viral diseases
20	Kostanayskie novosti	Self-pollination of Tamasha variety	Kazakhstan, NWS&PCA	NWS&PCA	Relatively resistant to viral diseases
21	Krasa	Clarissa x Tobol	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
22	Kurant-1	treatment of botanical seeds of the Ideal variety with superbutagen nitrosomethyl urea (NMU)	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Slightly susceptible to viral diseases
23	Kiru	Self-pollination of the Stepan variety	Kazakhstan, Kostanay SRIA	Kostanay SRIA	No data available
24	KazSIP	Serrana x XY.4 (material from CIP, Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
25	Kogaly	Cell selection based on the somaclonal variability of the Polet variety, followed by the selection of resistant cells on a selective nutrient medium with PEG – 800 and NaCl	Kazakhstan, KazRIPVG	KazRIPVG	No data available
26	Maksim	Cardia x 128-6	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
27	Nerli	138 j x Fidelio	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
28	Nur-Alem	G_3 13 x S. andigenum followed by clone selection	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
29	Pamyati Kunaeva	LR 93.120 x C 93.154 (material from CIP, Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
30	Pamyati Ligai	Somatic clone of the Express variety obtained by cell selection	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
31	Pamyati Bobrova	80-96 x Zarevo	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
32	Sofia	Salami x Axor	Kazakhstan, KazRIPVG	VIR	Resistant to common diseases in Kazakhstan
33	Stepa	No data available	Kazakhstan, Kostanay SRIA	Kostanay SRIA	No data available
34	Seyanets Stepan	Self-pollination of the Stepan variety	Kazakhstan, Kostanay SRIA	Kostanay SRIA	No data available
35	Tamasha	Ermak x Priekulsky ranniy	Kazakhstan, KazRIPVG	KazRIPVG and Karaganda Scientific Research Institute of Agriculture	Susceptible to potato virus Y (PVY)

36	Tokhtar	Cell selection based on the selection of cellular proto-clones on board the Mir orbital complex from the original form of the Gatchinsky variety	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
37	Teniz	Nevsky x Druzhnyi	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
38	Tamyr	Vostok x Pepo	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
39	Tyan-Shansky	Lvovyanka x Baksha	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
40	Terra-1	Self-pollination of a seedling from a hybrid population 19.100 n 1753× 481-362-8	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Slightly susceptible to viral diseases
41	Tustep	S 15.7 P-41 × Dobro $^{129/4}$	Kazakhstan, KazRIPVG, Kostanay SRIA	Kostanay SRIA	Resistant to viral diseases
42	Udovitsky	Self-pollination of the Spiridon variety	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Resistant to common diseases in Kazakhstan
43	Ulan	Vesna levoberezhnaya x Rezerv	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to viral diseases
44	Ushkonyr	Line 392780-1 (material from CIP, Peru)	Kazakhstan, KazRIPVG	KazRIPVG	Highly resistant to viral diseases
45	Shagalaly	Vilnya x 128-6	Kazakhstan, KazRIPVG	KazRIPVG and North Kazakhstan Scientific Research Institute of Agriculture	Resistant to viral diseases
46	Fedor	K 20422 x Axor	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
47	Edem	Axor x Nerli	Kazakhstan, KazRIPVG	KazRIPVG	Resistant to common diseases in Kazakhstan
48	Yagodny 19	multiple selections in a hybrid population n 1753×367-8	Kazakhstan, Kostanay SRIA	Kostanay SRIA	Resistant to viral diseases
49	6-02-94	Lvovyanka x Baksha	Kazakhstan, KazRIPVG	KazRIPVG	No data available
50	11-04-3	Attsimba x Dobro	Kazakhstan, KazRIPVG	KazRIPVG	No data available
51	4-08-2	Krerka x Sedov	Kazakhstan, KazRIPVG	KazRIPVG	No data available
52	2-10-15	95-7 x Axor	Kazakhstan, KazRIPVG	KazRIPVG	No data available
53	2-94-6	Belorussky krakhmalisty x Akkol	Kazakhstan, KazRIPVG	KazRIPVG	No data available
54	12-07-03	Akkol x A_3 7037	Kazakhstan, KazRIPVG	KazRIPVG	No data available
55	4-08-2	Krerka x Sedov	Kazakhstan, KazRIPVG	KazRIPVG	No data available
56	9-07-12	Franca x Teniz	Kazakhstan, KazRIPVG	KazRIPVG	No data available
57	15-08-3	Teniz x A_3 7037	Kazakhstan, KazRIPVG	KazRIPVG	No data available
58	15 seedl. 7P41 x Dobro	7P41 x Dobro	Kazakhstan, NWS&PCA	Kostanay SRIA	No data available
59	KC-17	Aladdin x Z 876-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
60	KC-26	Xisen 6 x Udovitsky	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
61	KC-27	Kurant-1 x Z 897-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
62	KC-21	Xisen 6 x Aladdin	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
63	KC-25	Aladdin x Z 872-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
64	KC-28	Xisen 6 x Udovitsky	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
65	KC-29	17 216-9 x Kostanayskie novosti	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
66	KC-30	17 250-10 x Tustep	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
67	KC-4	Udovitsky x Z897-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
68	KC-20	Xisen 6 x Aladdin	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
69	KC-24	Aladdin x Z 872-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
70	KC-18	Aladdin x Z 872-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress
71	KC-15	Aladdin x Z 872-3	Kazakhstan, S. Seifullin KATRU	S. Seifullin KATRU	Research in progress

*Here potato samples are characterized in terms of resistance/susceptibility to viral infections based on the research published in (Azhimakhan et al., 2023; Beisembina et al., 2019; Krasavin et al., 2016).

the diagnostic results, 19 virus-free samples of uninfected PVY, PVX, PVM, PVS, and PLRV were selected using the ELISA test. These virus-free samples were planted under controlled phytotron conditions and artificially infected with PVX by mechanical inoculation with PVX-infected biomaterial. The remaining 51 samples according to the results of the ELISA test, were infected with viruses and were not involved in further studies, since the presence of other viral pathogens in the leaf tissue of plants could significantly distort the results of the evaluation of the type of potato resistance to PVX.

RESULTS

The results of the detection of molecular markers linked to PVX resistance genes are shown in Table 2. As a result of the molecular screening, 17 potato samples with the 5Rx1 SCAR marker and one sample with the 1Rx1

Table 2: The presence of DNA markers linked to PVX resistance genes in samples of the potato gene pool in Kazakhstan

Gene	Marker	Varieties and breeding lines
Not detected		Kiru, Miras, Pamyati Bobrova
Rx1	5 *Rx1*	Ulan
	1 *Rx1* + 5 *Rx1*	2-94-06
Rx2	*106Rx2*	Dunyasha, Dikhan, Ilyin, KazSip, Kogaly, Terra-1, KC-17; KC-26; KC-27; KC-21; KC-25; KC-28; KC-29; KC-30; KC-20
Nb	*GM 339*	Pamyati Konaeva, Stepan, Stepan seyanets, KC-15
	GM 637	Akkol
	GM 339+ GM 637	Aksor, Elaman, Tokhtar, Tamyr, 12-07-01, KC-4
Rx1 + Rx2	5 *Rx1+106Rx2*	Didar, 6-02-94
	1 *Rx1* + 5 *Rx1+ 106Rx2*	Babaev
Rx1 + Rx2+ Nb	5 *Rx1+106Rx2+ GM 339*	Jualı, 4-08-02
	5 *Rx1+106Rx2+ GM 637*	Krasa, 15-08-03
	5 *Rx1* + *106Rx2* + *GM 339+ GM 637*	Valery, Karasaysky, Maxim, Mechta Krasavina, Tyan-Shansky, Tustep, Fedor, 4-08-02
Rx2 + Nb	*106Rx2* + *GM 339*	Artem, Alyans, Janaysan, Kostanayskie novosti, Pamyati Ligai, Tamasha, Teniz, 2-10-15
	106Rx2 + *GM 637*	Udovitsky, KC-18
	106Rx2 + *GM 339+ GM 637*	Auyl, Adil, Berkut, Vid 2, Kurant-1, Nerli, Sofia, Ushkonyr, Shagalaly, Edem, Yagodny 19, 11-04-03, 9-07-12, KC-24, 15 seedl. 7P41 x Dobro

SCAR marker were found. Thus, molecular markers developed to detect the *Rx1* gene were found in 25% (18 pcs.) of samples from the 71 studied potato varieties and breeding lines. We identified 55 samples (77% of the samples) with the 106Rx2 molecular SCAR marker developed for the molecular detection of the *Rx2* gene in their genotype (Fig. 1). 34 samples with the GM 637 marker and 43 samples with the GM 339 marker were identified. Based on the results, we concluded that a sufficiently large number of the varieties and breeding lines of the Kazakh potato gene pool possessed a complex of two or three DNA markers linked to the genes of resistance to PVX. However, none of the molecular markers diagnosed in this study were detected in the genetic material of the potato varieties Kiru, Miras, and Pamyati Bobrova.

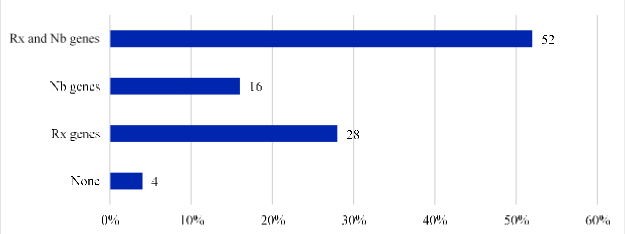

Fig. 1: The share of identified potato samples with molecular markers linked to PVX resistance genes.

Fig. 1 showed the structure of the studied potato gene pool based on the frequency of detection of DNA markers: samples with *Rx* genes: 28%, samples with *Nb* gene: 16%, samples with a complex of *Rx* and *Nb* genes: 52%, samples without molecular markers: 4%. Table 3 showed the results of the study of the type of resistance of potato samples to PVX conducted using visual observation of symptoms on PVX-inoculated potato plants and PVX detection by the ELISA test method. The absence of symptoms of PVX infection after artificial infection in isolated phytotron conditions was recorded in 15 potato samples: Alyans, Vid 2, Ilyin, Tamasha, Yagodny 19, and 15 seedl. 7P41 x Dobro, 9-07-12, 15-08-03, KC-17, KC-28, KC-26, KC-27, KC-29, and KC-30. Examination of the leaves of these samples by the ELISA test method did not show the presence of a virus, which indicates that the listed samples have an extreme type of resistance to PVX. In these potato samples, resistance to PVX was closely related to the presence of at least one molecular marker linked to the dominant alleles of the *Rx1* or *Rx2* genes in the genotype of these samples.

After artificial infection of potato plants with PVX, symptoms of virus damage, in the form of mottling and leaf spotting, were found in only one sample (Aqjar variety) with the GM339 marker (Fig. 2). The presence of the virus in the plant tissues of this sample was confirmed by a diagnostic study in the ELISA test. According to literature data, the symptoms caused by PVX can vary depending on the potato variety, virus strain, crop cultivation conditions, and weather conditions, and it can manifest as mottling. Three potato samples (Miras, Pamyati Konaeva, and KC-4 breeding line) showed a tolerant type of resistance where there were no symptoms of PVX infection on potato plants. The ELISA test reliably demonstrated the presence of PVX in the leaf tissue of these samples. The molecular markers GM 339 and GM 637 linked to the *Nb* gene were identified in the genotype of these potato samples, but there were no molecular markers associated with the *Rx1* and *Rx2* genes.

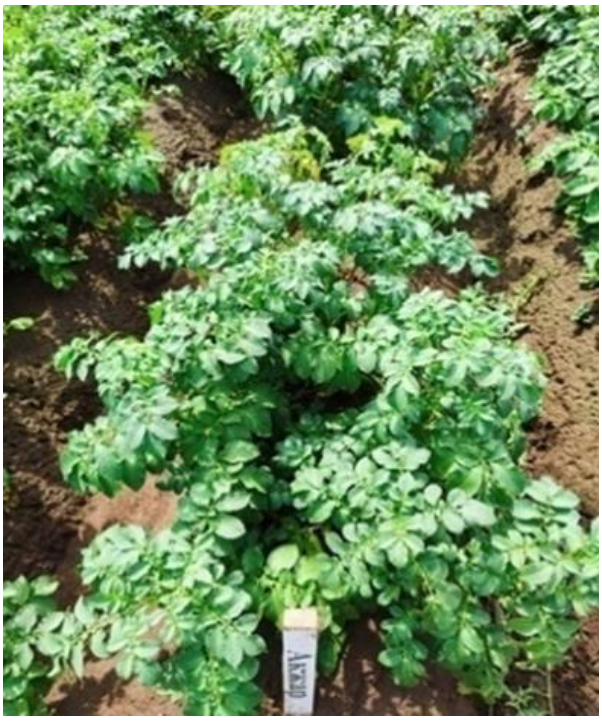

Fig. 2: Phenotype of the Akjar potato variety inoculated with PVX.

Table 3: Results of the study of the type of resistance of potato samples to PVX

Name of the potato variety or line	Plant reaction	ELISA test result			Marker presence	Type of resistance
		Extinction A_{405}, per unit	Ao/Ok	P		
Akjar	S: M	1.321	3.0	+	GM339	Non-resistant
Alyans	n\s	0.276	0.6	-	GM339, *106Rx2*	Resistant
Vid 2	n\s	0.152	0.3	-	*GM 339*, *GM 637*, 106 *Rx2*	Resistant
Ilyin	n\s	0.355	0.8	-	106 *Rx2*	Resistant
Miras	n\s	1.336	3.0	+	none	Tolerant
Pamyati Konaeva	n\s	1.325	3.0	+	*GM 339*	Tolerant
Tamasha	n\s	1.013	2.3	-	*GM 339*, 106 *Rx2*	Resistant
Yagodny 19	n\s	1.051	2.4	-	*GM 339*, *GM 637*, 106 *Rx2*	Resistant
15 seedl. 7P41 x Dobro	n\s	0.138	0.3	-	*GM 339*, *GM 637*, 106 *Rx2*	Resistant
9-07-12	n\s	0.300	0.7	-	*GM 339*, *GM 637*, 106 *Rx2*	Resistant
15-08-03	n\s	0.528	1.2	-	*5Rx1*, *GM 339*, 106 *Rx2*	Resistant
KC-17	n\s	0.429	1.0	-	106 *Rx2*	Resistant
KC-26	n\s	0.640	1.4	-	106 *Rx2*	Resistant
KC-27	n\s	0.383	0.9	-	106 *Rx2*	Resistant
KC-28	n\s	1.026	2.3	-	106 *Rx2*	Resistant
KC-29	n\s	0.581	1.3	-	106 *Rx2*	Resistant
KC-30	n\s	0.961	2.2	-	106 *Rx2*	Resistant
KC-4	n\s	1.427	3.2	+	*GM 339*, *GM 637*	Tolerant
KC-20	n\s	0.835	1.9	-	106 *Rx2*	Resistant

Note: S: systemic reaction, M: mottling and spotting, n\s: no symptoms, "+": positive reaction of the ELISA test, "-": positive reaction of the ELISA test. Ao/Ok is the ratio of the average optical density of the test sample to the average optical density of the negative sample in the ELISA test, and P is the test result (ELISA test).

DISCUSSION

Rx1 is a coiled-coil NOD-like receptor (NLR) that confers significant resistance to PVX, which can cause yield losses of up to 20% and is a common problem for potato growers. The Rx1 gene is located on chromosome XII and has been introgressed from the wild potato species *Solanum andigena* into some commercial cultivars such as cv. *Cara* and *Atlantic*. Rx1 shows high sequence complementarity to another NLR, Rx2, located on chromosome V, which was introgressed from another potato species - *Solanum acuale*. However, the functions of Rx1 are much well-understood. The Rx1 gene confers resistance to PVX through recognition of its capsid protein and promotes extreme resistance, which prevents viral replication at the cellular level without inducing a hypersensitive response. Consequently, if avirulent PVX strains infect Rx1-containing potatoes or transgenic that express this gene, no cell death is observed. The absence of capsid protein accumulation is caused by wild-type Rx1 inhibiting the translation of the appropriate mRNA rather than degradation (Richard et al., 2021; Ross et al., 2021). Identifying plants that exploit this defensive mechanism is crucial because Rx genes stop plant tissues from dying.

One of the first methods to determine resistance is to inoculate PVX strains with different plant cultivars. Nyalugwe et al. (2012) studied extreme resistance and hypersensitivity reactions in 38 varieties and one breeding line of European, North American, South American, and Australian origin using classical phytopathological analysis with various PVX differentiator strains. The *Rx* gene was detected in seven potato samples (18%). Since the methods of genetic diagnostics were not used, Nyalugwe et al. (2012) could not methodically differentiate potato genotypes containing the *Rx1* and *Rx2* genes. In addition, the hypersensitivity gene Nb was found in 32% of cultivars. Whilst our results indicated that 69% of the samples analyzed from the Kazakhstani potato gene pool included the molecular markers GM 339 and GM 637 associated with the Nb gene. PCR screening made it possible to distinguish between homologous Rx genes and more accurately assess the frequency of resistance to PVX in the population and subsequent selection. The primers used for amplification are detected depending on the method of polymorphism detection (Amplified Fragment Length Polymorphism (APFL), Restrictions Fragment Length Polymorphism (RFLP (Cleaved Amplified Polymorphic Sequence (CAPS), Relative Afferent Pupillary Defect - Sequence-characterized amplified region (RAPD-SCAR), etc.) (Bonny et al., 2020).

In the study conducted at the All-Russian Potato Center named after A.G. Lorkh, the sequence tagged site (STS) PVX marker linked to the dominant allele of the *Rx1* gene (Anisimov et al., 2009; Boris & Kochieva, 2013) was detected in 10 potato varieties created in the institution. The Rx-acl molecular marker linked to the dominant allele of the *Rx2* gene was detected only in the genotype of two varieties (Biryukova et al., 2015). Unfortunately, the data do not allow for a correct calculation of the frequency of the content of dominant alleles of the *Rx1* and *Rx2* genes in the studied population of Russian potatoes. According to Özkaynak (2023), the molecular STS PVX marker linked to the dominant allele of the *Rx1* gene was detected in the genotype of 21 promising breeding lines (34% of the studied sample) from the tested 61 samples of the Turkish potato gene pool. PCR with CAPS primers, combined with restriction, on the contrary, showed a low frequency of Rx. Among 155 South American potato samples belonging to the potato gene pool of the Austral University in Chile, only 10 samples (6%) possessed the molecular CAPS/DdeI marker CP60 linked to the resistance gene *Rx1* (López et al., 2015). Taking into account the experience of previous experiments, we chose SCAR markers - primers that allow to determine both alleles in PCR without additional manipulations. Other research have also made significant use of similar markers (Liu et al., 2020; Xu et al., 2020; Hon et al., 2021).

For instance, breeders from the League breeding company and the Belogorka Leningrad Research Institute of Agriculture developed 39 potato genotypes in the

Northwestern part of Russia and tested SCAR markers for Rx genes. Five samples, or 15% of the samples under study, had the 5Rx1 molecular SCAR marker. Eleven potato samples (33% of the samples) contained the molecular SCAR marker, 106Rx2. (Gavrilenko et al., 2018). In our study, the samples of the potato gene pool were characterized by a relatively high proportion of the dominant allele of the extreme resistance gene *Rx1* (26%) and a particularly high proportion of the allele of the *Rx2* gene (79%). A very high effectiveness of the protective action of the *Rx2* gene against PVX infection was demonstrated and verified in 15 samples of the gene pool of potatoes in Kazakhstan (Mori et al., 2011). Also, a tendency towards increased frequency of the Rx2 gene compared to the Rx1 gene was observed in the Hungarian potato population. Of the 25 studied potato genotypes, three genotypes possessed the 5Rx1 marker (12%), and 13 genotypes carried the 106Rx2 molecular marker (52%) (Shaikhaldein et al., 2018). Our findings indicate a significant role for the Rx2 gene in extreme resistance for potato varieties in Kazakhstan, which was consistent with other studies. The data set for the Rx2 gene can serve as a basis for a more in-depth study of the molecular mechanisms of Rx2 in plant antiviral «immunity», as well as the coordination of signaling pathways of extreme resistance and hypersensitivity reactions.

Conclusion

In our study, the molecular markers GM 339 and GM 637 linked to the *Nb* gene were found in 69% of the examined samples of the potato gene pool in Kazakhstan. The presence of the *Nb* hypersensitivity gene in the three studied virus-free potato samples did not lead to the development of symptoms of potato tip necrosis. Instead, it led to the development of a tolerance-like type of resistance, where there were no symptoms of PVX infection in potato plants. The ELISA test demonstrated the presence of PVX in plant tissues after their artificial PVX infection. It can be concluded that PVX-resistant varieties and breeding lines of the Kazakh potato gene pool can be included in breeding programs to create new virus-resistant potato varieties. The *Rx1* and *Rx2* genes providing extreme resistance to PVX and the hypersensitivity genes can be useful in creating potato varieties with field resistance. The incorporation of the dominant *Rx1* allele into potato breeding provides a major advantage due to its close genetic linkage with the *Gpa2* gene. This linkage enables simultaneous resistance to viruses and the *Pa2* and *Pa3* pathotypes of the cyst-forming pale nematode *Globodera pallida* (Stone) Behrens, which are highly aggressive and damaging. This dual resistance simplifies the breeding process and reduces reliance on chemical nematicides, promoting sustainable agriculture. By leveraging this genetic strategy, breeders can develop potato varieties with improved resistance to biotic stressors, higher yields, and better crop quality, supporting global efforts for resilient and sustainable farming. Samples with such complex resistance are of particular interest for potato breeding. Successful developments in this field will lead to a high level of protection of potato plants from pathogens and pests.

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