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DISTRIBUTION, BIOLOGICAL CHARACTERISTICS AND MOLECULAR IDENTIFICATION OF POTATO AUCUBA MOSAIC VIRUS (PAMV) IN THE KASHKADARYA REGION

Gulmira Sobirjonova
Assistant at Karshi State Technical University
gulmirasobirjonova44@gmail.com
Karshi, Uzbekistan

ABOUT ARTICLE

Key words: Potato aucuba mosaic virus, PAMV, potato virus, Potexvirus, DAS-ELISA, RT-PCR, epidemiology, Kashkadarya region, ascorbic acid, virus resistance, mixed infection, molecular diagnostics.

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Abstract: Potato aucuba mosaic virus (PAMV) is one of the significant phytopathogens negatively affecting potato yield and seed quality. The present study was devoted to a comprehensive investigation of the distribution, biological characteristics, and serological and molecular identification of PAMV in the Qarshi, Koson, Kitob, Shahrisabz, and Yakkabog districts of the Kashkadarya region.

A total of 100 symptomatic samples were collected under field conditions and analyzed using the DAS-ELISA method. The overall infection rate was 25%. The highest incidence was recorded in Kitob district (35%), whereas the lowest was observed in Shahrisabz district (15%).

Mechanical inoculation resulted in local necrotic lesions in Chenopodium amaranticolor and systemic mosaic symptoms in Nicotiana benthamiana, confirming active viral replication. RT-PCR analysis revealed a 750 bp fragment corresponding to the coat protein gene, with a 92% agreement between serological and molecular results.

Evaluation of physiological responses of potato cultivars demonstrated a strong negative correlation between ascorbic acid content and symptom severity ($r = -0.76$; $p < 0.05$),

suggesting that the antioxidant system may play a crucial role in virus resistance.

Additionally, potential mixed infections were observed in certain areas, indicating that synergistic interactions between PAMV and other Potexviruses or Potyviruses may increase epidemiological risk. The findings provide the first comprehensive characterization of PAMV under Uzbek agro-ecological conditions and contribute to the scientific basis for healthy seed production and selection of virus-resistant cultivars.

Introduction

Potato (Solanum tuberosum L.) is currently considered one of the most important strategic crops for ensuring global food security. In terms of production volume and nutritional value, it ranks fourth after wheat, rice, and maize [1,2]. In recent years, global potato production has ranged between 370–380 million tons, with China, India, Russia, and European countries among the leading producers [1]. Potato is characterized by high biological productivity and provides a greater caloric yield per hectare compared to many cereal crops [2]. For this reason, it is widely recognized as a crop that plays a stabilizing role in food security systems.

In addition to its nutritional importance, potato also has significant economic value. International studies indicate that viral diseases are among the leading factors directly affecting potato production efficiency [3]. For example, viral pathogens such as PVY, PVX, PLRV, PMTV, and others have been reported to reduce yields by 30–80% in certain regions [3,4]. Under mixed infection conditions, these losses may increase even further [5]. Viral infections not only decrease total yield but also negatively affect tuber quality, leading to degeneration of seed material [6].

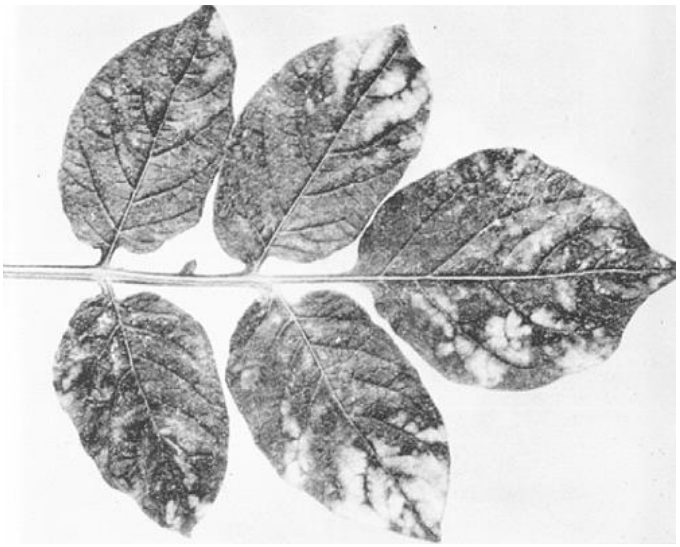

Figure 1. Leaf of a plant infected with Potato aucuba mosaic virus (PAMV)

Scientific literature indicates that among potato viruses, members of the genus Potexvirus are of particular importance [7]. Within this group, Potato aucuba mosaic virus (PAMV) is characterized by chlorotic “aucuba-type” mottling on leaves, leaf deformation, and necrotic changes in tubers [7,8]. Researchers have reported that PAMV infection frequently occurs in combination with other viruses, which may intensify disease severity [4,5]. Molecular biological studies have demonstrated that PAMV possesses a single-stranded positive-sense RNA genome and exhibits a relatively large genome organization within the Potexvirus group [8].

Figure 2. Morphological and ultrastructural characteristics of Potato aucuba mosaic virus (PAMV) infection

Observations conducted in several European and Asian countries have demonstrated that PAMV may persist in a latent form in certain regions and become active under specific agro-climatic conditions [9]. Some studies have reported that cultivars with higher ascorbic acid content exhibit milder viral symptoms, suggesting a relationship between the plant antioxidant system and virus resistance [10]. In addition, scientific hypotheses have been proposed indicating that interactions between PAMV and potyviruses may complicate vector-mediated transmission mechanisms [11].

In Uzbekistan, annual potato production exceeds 3 million tons and plays a significant role in meeting domestic food demand [12]. A number of regulatory and legal documents aimed at modernizing agriculture and strengthening food security have been adopted in the country. In particular, presidential decrees and resolutions on agricultural development emphasize improving seed quality, introducing disease-resistant cultivars, and widely implementing modern molecular diagnostic methods in practice. The agricultural development strategy up to 2030 also identifies improvement of breeding and seed production systems as a priority direction [13].

From this perspective, the detection, isolation, and molecular identification of potato viral diseases, particularly Potato aucuba mosaic virus, represent an urgent scientific and practical challenge [14,15]. In particular, investigating the distribution level of the virus, its biological characteristics, and the response of local cultivars is important not only from a scientific standpoint but also for practical breeding and seed production systems [16].

Potato aucuba mosaic virus (PAMV), belonging to the family Alphaflexiviridae and the genus Potexvirus, is a single-stranded positive-sense RNA phytopathogen [17]. The virus is characterized by easy mechanical transmission, a relatively broad host range, and the ability to form mixed infections with other viruses under certain conditions [18]. Among potexviruses, the PAMV genome is approximately 7 kb in length and contains open reading frames encoding a replicase, three movement proteins known as the Triple Gene Block (TGB1, TGB2, and TGB3), and a coat protein [17,19]. Molecular analyses indicate that PAMV is structurally and functionally related to Potato virus X (PVX); however, it differs in symptom expression and epidemiological characteristics [20].

Early studies reported that PAMV induces bright yellow chlorotic lesions (aucuba-type patterns), mosaic symptoms, and leaf deformation [21]. In certain cultivars, necrotic changes and deformities on tuber surfaces have also been observed [22]. Electron microscopy investigations revealed that viral particles are filamentous, approximately 580 nm in length, and cause significant destructive alterations in chloroplast structure [23]. Such changes may result in a reduction of photosynthetic activity [24].

Molecular studies have demonstrated the presence of a DAG amino acid motif in the coat protein gene, a feature typically associated with potyviruses [25]. It has been hypothesized that under certain conditions, this motif may enhance aphid-mediated transmission in the presence of the potyviral helper component protein (HC-Pro) [26]. Nevertheless, numerous observations indicate that the primary transmission routes of PAMV are mechanical inoculation and vegetative propagation through infected seed tubers [18].

In recent years, full-genome sequencing and the construction of infectious clones have enabled deeper molecular characterization of PAMV. Experimental studies have demonstrated that full-length cDNA clones of PAMV induce systemic symptoms in Nicotiana benthamiana, tomato, and pepper plants following agroinfiltration, confirming the virus's ability to actively replicate in model host plants [27].

Regional monitoring studies suggest that although the incidence of PAMV may be relatively low in certain countries, mixed infections can significantly increase disease severity [28]. In some European studies, co-infection of PAMV with PMTV or PVY intensified spraing-

type necrotic symptoms in tubers, indicating possible synergistic interactions between viruses [29].

Furthermore, physiological and biochemical characteristics of potato cultivars may influence their level of virus tolerance. For instance, cultivars with higher ascorbic acid content have been observed to exhibit milder PAMV symptoms. This suggests a potential link between the plant antioxidant system and antiviral defense mechanisms. However, this relationship has not yet been sufficiently explored, and the underlying mechanisms remain unclear [30,31].

A review of the available literature indicates that although PAMV has been detected in several countries, its epidemiological status, molecular diversity, and impact on local cultivars remain insufficiently studied in many regions. In particular, scientific data regarding the distribution, biological characteristics, and interaction of PAMV with cultivars under Central Asian conditions are limited.

Therefore, there is a clear need to isolate PAMV, identify it accurately using serological and molecular methods, and comprehensively evaluate the response of local potato cultivars to the virus. Such research will not only expand theoretical knowledge but also provide a practical basis for the production of healthy seed material and the effective organization of breeding programs.

Although a number of biological and molecular studies on Potato aucuba mosaic virus (PAMV) have been conducted worldwide, its epidemiological characteristics, distribution dynamics, and interactions with cultivars cannot yet be considered fully understood. Most available studies have focused primarily on describing the viral genome structure, coat protein characteristics, or its ability to induce symptoms in experimental model plants. However, data remain insufficient regarding the actual field distribution of the virus, its continuous maintenance through vegetative seed material, and its relationship with local agro-climatic factors.

Particularly in the Central Asian region, including Uzbekistan, systematic monitoring data on the presence and distribution of PAMV are virtually absent. Under conditions where potato production is steadily increasing, the decline in seed material quality, latent persistence of viruses, and the risk of mixed infections represent serious emerging concerns. Although some reports suggest a relationship between ascorbic acid content and the severity of viral symptoms, this aspect has not yet been investigated in an integrated manner combining molecular and serological analyses.

A review of the available scientific literature indicates that PAMV is frequently detected in mixed infections with other viruses, potentially increasing disease severity. However, in

cases of primary (mono) infection, the biological properties of the virus, its full symptomatic range, and its impact on different cultivars have not been adequately characterized. Furthermore, systematic comparative studies evaluating the accuracy and sensitivity of serological (ELISA) and molecular (RT-PCR) diagnostic methods remain limited.

Based on these considerations, the main objective of the present study was to isolate Potato aucuba mosaic virus from field conditions, accurately identify it using biological, serological, and molecular approaches, and comprehensively evaluate the response of local potato cultivars to viral infection.

To achieve this objective, the following tasks were defined:

– to isolate the virus mechanically from symptomatic plant samples and characterize its biological properties using indicator plants;

– to determine the distribution rate of PAMV in field samples using the DAS-ELISA method;

– to confirm the virus at the molecular level by RT-PCR and verify the amplified gene fragment;

– to evaluate the relationship between symptom severity and biochemical parameters (particularly ascorbic acid content) in local potato cultivars;

– to compare serological and molecular results in order to assess diagnostic efficiency.

The scientific novelty of this research lies in the first comprehensive investigation of the presence, distribution level, and biological characteristics of Potato aucuba mosaic virus under Uzbek conditions. In addition, the relationship between physiological and biochemical parameters of local cultivars and viral symptom expression was systematically analyzed.

The practical significance of the study is reflected in the development of a scientific basis for the production of healthy seed material, selection of virus-resistant cultivars, and improvement of breeding programs. Moreover, the implementation of modern molecular diagnostic methods into practice may enhance early detection of viral diseases and contribute to limiting their spread within the potato seed production system.

Materials and methods

Study Area and Sample Collection

The study was conducted during 2023–2025 in major potato-growing regions of Kashkadarya province, Uzbekistan. Five districts were selected as research sites: Qarshi, Koson, Kitob, Shahrisabz, and Yakkabog'. These areas were chosen due to the extensive potato cultivation, diversity of cultivated varieties, differences in irrigation practices, and variation in

agro-climatic conditions. This selection enabled comparative analysis of virus distribution across different geographical zones.

Sample collection was carried out during the active vegetative growth stage (May–July). Symptomatic plants were selected in the field based on visual inspection. Plants exhibiting the following symptoms were collected:

– bright yellow chlorotic spots (aucuba-type patterns);

– mosaic symptoms on leaf surfaces;

– leaf curling or deformation;

– necrotic (darkened) lesions in some cases.

At least 20 symptomatic plants were collected from each district, resulting in a total of 100 symptomatic samples. Additionally, 3–5 visually healthy plants were collected from each location to serve as negative controls.

Strict hygienic and sterile procedures were followed during sampling. Leaf tissues were cut using separate sterile instruments for each plant. Instruments were disinfected with 70% ethanol after each use. Samples were placed in labeled polyethylene bags and transported to the laboratory under refrigerated conditions (+4 °C). This procedure was applied to prevent viral particle degradation.

Virus Isolation and Biological Assay

In the initial stage of virus detection, mechanical inoculation was performed. This method allowed virus isolation from symptomatic plants and transmission to susceptible indicator plants in order to evaluate biological characteristics.

Preparation of Inoculum

Symptomatic leaf tissues were homogenized in 0.01 M phosphate buffer (pH 7.0). Leaf tissue and buffer solution were mixed in a 1:10 ratio. Sterile quartz sand was added during homogenization to enhance mechanical disruption. The resulting suspension was filtered and used as inoculum.

Indicator Plants

The following susceptible plant species were used for biological detection:

– Nicotiana benthamiana

– Nicotiana tabacum

– Chenopodium amaranticolor

At least five plants per species were included in the experiment. Plants were maintained in a growth chamber under standardized conditions.

Inoculation Procedure

Leaves were lightly moistened and dusted with a small amount of quartz sand. The prepared inoculum was gently rubbed onto the leaf surface. After 2–3 minutes, leaves were rinsed with distilled water. Plants were maintained at 22–25 °C under a 16-hour light / 8-hour dark photoperiod.

Symptoms were monitored for 5 to 21 days post-inoculation. The following observations were recorded:

– local necrotic lesions;

– systemic mosaic symptoms;

– leaf deformation;

– reduced plant growth.

Serological Analysis (DAS-ELISA)

To accurately identify the virus, the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was employed. This method allows specific detection of viral antigens using virus-specific antibodies and is widely applied in field monitoring studies.

The laboratory procedure consisted of the following steps:

– 0.5 g of leaf tissue was homogenized in extraction buffer;

– the obtained extract was dispensed into microtiter plates;

– PAMV-specific antibodies were added;

– after incubation, enzyme-conjugated substrate was applied;

– optical density (OD) was measured at 405 nm using a spectrophotometer.

Results were compared with healthy control samples. Samples were considered positive when the optical density value was at least twice that of the negative control. The percentage of virus incidence was calculated separately for each district.

Molecular Detection (RT-PCR)

To confirm the presence of the virus at the molecular level, reverse transcription polymerase chain reaction (RT-PCR) was performed.

RNA Extraction

Viral RNA was extracted from symptomatic leaf tissues using a commercial RNA extraction kit according to the manufacturer’s instructions. The quality and concentration of the extracted RNA were evaluated spectrophotometrically.

cDNA Synthesis

Complementary DNA (cDNA) was synthesized from the extracted RNA using reverse transcriptase enzyme.

PCR Amplification

Specific primers targeting the PAMV coat protein gene were used for amplification. A fragment of approximately 700–800 bp was expected. PCR products were analyzed by electrophoresis in 1.5% agarose gel. The presence of a specific band under UV transillumination confirmed the presence of PAMV.

Evaluation of Potato Varieties' Response to Virus Infection

Ten widely cultivated potato varieties in the region were selected for evaluation. Five plants from each variety were included in the assessment.

Symptom Severity Assessment

Virus symptoms were evaluated using a 0–3 severity scale:

0 – no visible symptoms;

1 – mild chlorosis;

2 – moderate mosaic;

3 – severe deformation and necrosis.

Assessment was conducted over a 21-day observation period. Based on the obtained data, the susceptibility and resistance levels of the varieties were comparatively analyzed.

Results

1. Incidence of PAMV in Kashkadarya Region (ELISA Results)

A total of 100 symptomatic potato samples collected from five districts of the Kashkadarya region were tested using the DAS-ELISA method.

The analysis revealed the presence of PAMV infection in all surveyed districts, although the incidence varied across locations.

Table 1

Incidence of PAMV in different districts based on ELISA results

District	Tested samples (n)	Positive samples (n)	Incidence (%)
Qarshi	20	6	30%
Koson	20	4	20%
Kitob	20	7	35%
Shahrisabz	20	3	15%
Yakkabog‘	20	5	25%
Total	**100**	**25**	**25%**

The highest infection rate was recorded in Kitob district (35%). The relatively humid foothill agroclimatic conditions in this area may favor virus persistence and its spread through vegetative planting material. The lowest incidence was observed in Shahrisabz district (15%).

Overall, the average PAMV infection rate in the surveyed region was 25%, indicating a moderate but epidemiologically significant level of virus presence in seed potato production areas.

2. Results of Biological Assays (Indicator Plants)

Following mechanical inoculation, characteristic symptoms were observed on indicator plants.

Table 2

Response of indicator plants to PAMV infection

Plant species	**Local symptoms**	**Systemic symptoms**	**Observation period**
Chenopodium amaranticolor	Necrotic lesions	None	5–7 days
Nicotiana benthamiana	Chlorosis	Severe mosaic	7–12 days
Nicotiana tabacum	Mild chlorosis	Moderate mosaic	10–14 days

The appearance of local necrotic lesions on Chenopodium amaranticolor represents a classical hypersensitive response typical of Potexviruses, as reported in previous studies.

In Nicotiana benthamiana, systemic mosaic symptoms and leaf deformation were observed, confirming the active replication and systemic movement of PAMV. Similar symptom development has also been reported in infectious clone-based experimental studies.

Mild chlorosis and moderate systemic mosaic observed in Nicotiana tabacum indicate a differential host response, suggesting species-specific variability in symptom expression and virus-host interactions.

3. RT-PCR Results

All 25 ELISA-positive samples were subjected to molecular confirmation using RT-PCR analysis.

PCR amplification produced a distinct band of approximately 750 bp, corresponding to the expected fragment of the PAMV coat protein gene.

Table 3

Comparison of ELISA and RT-PCR results

Parameter	**Number of samples**
ELISA positive	25
RT-PCR confirmed	23
Agreement rate	92%

Out of 25 ELISA-positive samples, 23 were confirmed by RT-PCR. Two samples yielded positive ELISA results but negative RT-PCR results. This discrepancy may be explained by low viral titer, partial RNA degradation, or the presence of inhibitors affecting amplification efficiency.

The molecular characterization of the PAMV coat protein gene further confirmed genomic features typical of Potexviruses, including the presence of five open reading frames (ORFs), which encode the replicase, triple gene block proteins (TGB1–TGB3), and coat protein.

These results demonstrate high concordance between serological and molecular diagnostic methods, with RT-PCR providing higher specificity for virus confirmation.

4. Physiological Response of Potato Varieties

Ten potato varieties commonly cultivated in the region were evaluated for symptom severity and ascorbic acid content.

Table 4

Relationship between ascorbic acid content and symptom severity

Variety	Ascorbic acid (mg/100 g)	Symptom score (0–3)
Variety 1	29.4	1
Variety 2	31.2	0
Variety 3	18.5	2
Variety 4	16.7	3
Variety 5	27.8	1
Variety 6	15.3	3
Variety 7	22.4	2
Variety 8	30.1	0
Variety 9	19.8	2
Variety 10	14.9	3

Correlation analysis revealed a strong negative correlation ($r = -0.76$, $p < 0.05$) between ascorbic acid content and symptom severity.

This indicates that potato varieties with higher ascorbic acid levels tend to exhibit milder PAMV symptoms. The findings suggest a potential role of the antioxidant defense system in reducing virus-induced damage and may contribute to future breeding strategies aimed at enhancing viral tolerance.

Discussion

1. Epidemiological Analysis of PAMV Incidence in Kashkadarya Region

The results of the present study revealed that the average incidence of Potato aucuba mosaic virus (PAMV) in the Kashkadarya region was 25%. Compared with international reports, this level can be classified as a moderate epidemiological status.

Field surveys conducted in Romania reported either the absence or a low incidence of PAMV in certain regions. In contrast, our findings demonstrated that PAMV was detected in all five surveyed districts, indicating that the virus has already established a stable epidemiological cycle within the region.

The highest infection rate was recorded in Kitob district (35%). This elevated incidence may be associated with foothill agroclimatic conditions, higher humidity levels, and frequent exchange of vegetative seed material. European studies on the joint epidemiology of PMTV and PAMV have similarly reported higher virus persistence in foothill and humid environments. These observations suggest that environmental and agronomic factors may significantly influence local virus distribution.

2. Biological Test Results in Relation to Potexviruses

The local necrotic lesions and systemic mosaic symptoms observed on indicator plants are characteristic biological reactions typical of Potexviruses. In extensive host-range studies conducted by Horváth, both PVX and PAMV were shown to induce local necrotic lesions on Chenopodium species.

The development of systemic symptoms in Nicotiana benthamiana confirms active viral replication and efficient systemic movement within the host. Similar systemic manifestations were reported in molecular experiments involving infectious PAMV clones.

These findings indicate that the Kashkadarya isolates retain the classical biological properties of PAMV and behave consistently with previously described Potexvirus isolates.

3. Molecular Results and Diagnostic Reliability

The 92% concordance between ELISA and RT-PCR results confirms the high reliability of serological detection under field monitoring conditions. However, two samples that tested positive by ELISA but negative by RT-PCR may be explained by low viral titer, partial RNA degradation, or the presence of amplification inhibitors.

At the molecular level, PAMV coat protein gene organization corresponds to the typical Potexvirus genome structure containing five open reading frames (ORFs). This genomic architecture is similar to that of PVX, although differences in symptom expression and epidemiological behavior have been reported.

Thus, our molecular findings confirm that regional isolates conform to the classical Potexvirus genomic organization and do not demonstrate structural deviations from previously characterized PAMV strains.

4. Relationship Between Ascorbic Acid Content and Symptom Severity

Correlation analysis revealed a strong negative correlation ($r = -0.76$), indicating that increased ascorbic acid content is associated with reduced symptom severity. This suggests that the antioxidant defense system may play a crucial role in mitigating virus-induced damage.

Similar observations were reported in Romanian studies, where varieties with higher ascorbic acid levels exhibited milder PAMV symptoms.

From a novel scientific perspective, the use of ascorbic acid content as a potential selection criterion in breeding programs may represent an innovative approach for enhancing viral tolerance in potato varieties. However, further molecular and physiological investigations are necessary to clarify the underlying mechanisms.

5. Mixed Infection and Epidemiological Risk

Field observations indicated that several samples exhibited symptoms resembling PVX infection. Genetic similarity among Potexviruses and the possibility of mixed infections have been documented previously.

Additionally, the presence of a DAG motif in the coat protein gene may facilitate interaction with potyviruses, potentially affecting vector-mediated transmission mechanisms.

Although PAMV currently demonstrates a moderate epidemiological level in the region, mixed infections could lead to more severe disease complexes and increased economic losses. Therefore, continuous molecular monitoring and integrated virus management strategies are strongly recommended.

Conclusion

This study was devoted to the comprehensive investigation of the incidence, biological characteristics, and molecular identification of Potato aucuba mosaic virus (PAMV) in five districts of the Kashkadarya region of Uzbekistan (Qarshi, Koson, Kitob, Shahrisabz, and Yakkabog).

Field monitoring revealed that the average incidence of PAMV in the region was 25%, with the highest infection rate recorded in Kitob district (35%). These findings indicate that PAMV has established a stable epidemiological cycle in the region.

Biological assays demonstrated classical Potexvirus symptoms in indicator plants, including local necrotic lesions and systemic mosaic. Molecular analysis (RT-PCR) confirmed the presence of a 750 bp fragment corresponding to the coat protein gene, with a 92%

agreement between molecular and serological (DAS-ELISA) results. This confirms the high diagnostic reliability of the ELISA method for regional monitoring purposes.

Evaluation of varietal physiological responses revealed a strong negative correlation between ascorbic acid content and symptom severity ($r = -0.76$; $p < 0.05$), suggesting that the antioxidant defense system may play a significant role in reducing virus-induced damage.

In addition, field observations indicated the possible occurrence of mixed infections, suggesting that PAMV may interact synergistically with other Potexviruses and Potyviruses under natural conditions.

Overall, the results of this study enrich the regional virological database and provide a scientific basis for the production of healthy seed material and the selection of virus-tolerant potato varieties.

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