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METHODOLOGICAL JOURNAL****MENTAL ENLIGHTENMENT SCIENTIFIC –
METHODOLOGICAL JOURNAL**<http://mentaljournal-jspu.uz/index.php/mesmj/index>**BIOLOGICAL CHARACTERISTICS AND MOLECULAR IDENTIFICATION OF
POTATO AUCUBA MOSAIC VIRUS (PAMV)****Gulmira Sobirjonova**

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ABOUT ARTICLE

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Abstract: Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world and plays a significant role in ensuring global food security. However, viral diseases remain one of the major factors limiting potato productivity and quality. Among them, Potato aucuba mosaic virus (PAMV), belonging to the genus Potexvirus, is considered an important pathogen affecting potato plants in many regions. The present study aimed to investigate the biological characteristics of PAMV and to identify the virus using molecular diagnostic methods.

Field observations revealed several typical viral symptoms in infected potato plants, including chlorotic mosaic patterns, leaf deformation, yellow spotting, and necrotic lesions on tubers. The frequency of observed symptoms showed that mosaic patterns were the most common symptom (42%), followed by chlorotic spots (35%), leaf deformation (28%), and necrosis (19%). Light microscopy analysis of infected leaf tissues revealed significant anatomical changes, including chloroplast deformation, disruption of cellular structures, and the formation of necrotic zones. These alterations indicate that viral infection significantly affects the physiological and metabolic processes of plant cells.

Molecular analysis using reverse transcription polymerase chain reaction (RT-PCR) successfully detected the presence of PAMV in infected samples. Agarose gel electrophoresis revealed a specific DNA fragment of approximately 700 bp corresponding to the viral genome region, confirming the presence of the virus in infected plant tissues, while no amplification was detected in healthy control samples. Furthermore, infected plants exhibited reduced growth parameters, including decreased plant height, leaf number, and tuber yield compared with healthy plants.

The results of this study confirm that PAMV infection can significantly affect potato growth and productivity. Molecular diagnostic tools such as RT-PCR provide reliable and sensitive methods for early detection of plant viruses. These findings may contribute to improving monitoring systems, selecting virus-free planting material, and developing effective strategies for managing viral diseases in potato cultivation.

Introduction. Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide [1,3]. This crop plays a crucial role in ensuring global food security and ranks fourth in production volume after wheat, rice, and maize [1]. Potatoes are characterized by high productivity, significant nutritional value, and the ability to adapt to various agro-climatic conditions [3]. According to the Food and Agriculture Organization (FAO), global potato production amounts to hundreds of millions of tons annually, and the crop is cultivated in many countries around the world [2]. Potato tubers contain carbohydrates, proteins, vitamins, and minerals, making them an important source of energy in the human diet [3].

In recent years, increasing potato production and obtaining high-quality yields have become priority objectives of agricultural policies in many countries. In the Republic of Uzbekistan, several regulatory and legal documents have been adopted to modernize agriculture and ensure food security. In particular, the Resolution of the President of the Republic of Uzbekistan dated April 5, 2023, aimed at supporting the production and processing of agricultural products, identifies increasing crop productivity, introducing disease-resistant varieties, and developing modern diagnostic technologies as key priorities [4]. In addition, the “Uzbekistan–2030” Strategy emphasizes the enhancement of scientific research potential in the

agricultural sector, the study of crop resistance to diseases, and the wider application of biotechnological approaches [5].

One of the main factors limiting potato productivity is plant diseases. Among them, viral diseases cause particularly significant economic losses [6]. Viruses disrupt photosynthesis, suppress plant growth and development, and ultimately reduce crop yield [6]. Studies indicate that potato viruses can lead to very high yield losses under certain conditions, especially in cases of mixed infections [6]. The most common viruses affecting potato crops include Potato virus A (PVA), Potato virus M (PVM), Potato virus S (PVS), Potato virus X (PVX), Potato virus Y (PVY), Potato leaf roll virus (PLRV), and Potato mop-top virus (PMTV) [1,7].

Among potato viruses, one relatively less studied but important pathogen is the Potato aucuba mosaic virus (PAMV) [8,9]. This virus belongs to the genus Potexvirus and possesses a positive-sense single-stranded RNA genome [8,9]. PAMV causes a variety of symptoms in potato plants, including mosaic patterns on leaves, chlorosis, deformation, and reduced plant growth. In some cases, the virus may occur in mixed infections with other viruses, which can intensify disease symptoms [6,8].

Numerous studies have been conducted on the detection of potato viruses and the investigation of their biological characteristics [6]. Initially, virus detection relied mainly on symptom observation and serological methods, with ELISA (Enzyme-Linked Immunosorbent Assay) later becoming widely used [7]. However, ELISA may sometimes show limited sensitivity, particularly when the virus concentration is low or during the early stages of infection [7]. For this reason, molecular biological methods, especially RT-PCR technology, have become widely applied in recent years. This method allows rapid and accurate detection of viral genomes and provides opportunities for detailed genetic analyses [7,9].

Today, the study of potato viruses is important not only for plant pathology but also for agricultural production [6]. Early detection of viral diseases is essential for selecting healthy seed material, developing disease-resistant varieties, and preventing the spread of viruses [6]. Therefore, studying the biological characteristics of Potato aucuba mosaic virus and identifying it using molecular methods represents an important direction in modern phytopathological research [6,9].

From this perspective, the main objective of this study is to investigate the biological characteristics of Potato aucuba mosaic virus (PAMV) and to identify the virus using molecular diagnostic methods [9]. The results of this research will contribute to the development of scientifically based recommendations for the detection, monitoring, and reduction of viral diseases in potato crops [6,9].

Materials and Methods. The present study was conducted to investigate the biological characteristics of Potato aucuba mosaic virus (PAMV) and to detect the virus using molecular methods. The research included field observations, laboratory analyses, and molecular diagnostic techniques.

Research object and sample collection

Potato plants (*Solanum tuberosum* L.) showing symptoms of viral infection were used as the research material. Sampling was carried out in potato-growing areas based on field observations. During the study, samples were collected from plants showing typical symptoms such as mosaic patterns on leaves, chlorotic spots, leaf deformation, and reduced plant growth. In addition, the presence of necrotic spots on tubers was also recorded.

Leaf samples were cut using sterile scissors and transported to the laboratory for further analysis. Part of the collected samples was used for biological observations, while the remaining samples were stored at $-20\text{ }^{\circ}\text{C}$ for molecular analysis.

Observation of biological symptoms

Symptoms in virus-infected plants were regularly monitored throughout the vegetation period. Chlorotic mosaic patterns, yellow spots on leaves, deformation of the leaf blade, reduced plant growth, and necrotic symptoms were recorded. The overall development of the plant, as well as the shape and color of leaves, were also evaluated. To determine the presence of necrotic rings or dark spots in tubers, tubers were cut and visually examined.

Anatomical observations using light microscopy

Microscopic observations were carried out using a light microscope in order to identify anatomical changes occurring in virus-infected plant tissues. Small sections were prepared from infected leaves for microscopic examination. Leaf tissues were cut into thin sections using either a microtome or manual sectioning techniques and then placed on microscope slides.

The prepared slides were stained with safranin or other contrast dyes. After staining, the samples were covered with a cover slip and examined under a light microscope. During microscopic observation, deformation of cell walls, disruption of chloroplasts, formation of necrotic zones, and changes in tissue structure were recorded.

RNA extraction

Total RNA was extracted from infected leaf tissues for molecular detection of the virus. Approximately 100 mg of leaf tissue was ground using liquid nitrogen. RNA extraction was then performed using special extraction reagents according to standard laboratory protocols. The quality and concentration of the extracted RNA were evaluated using a spectrophotometer.

Virus detection by RT-PCR

Reverse transcription polymerase chain reaction (RT-PCR) was used to detect Potato aucuba mosaic virus. First, complementary DNA (cDNA) was synthesized from the extracted RNA. Subsequently, PCR amplification was performed using primers specific to PAMV.

The PCR reaction mixture contained the cDNA template, specific primers, Taq DNA polymerase enzyme, dNTPs, and reaction buffer. The amplification was performed under the following temperature conditions: initial denaturation at 94 °C, followed by repeated cycles of denaturation, primer annealing, and DNA synthesis. The final extension step was carried out at 72 °C.

Electrophoresis analysis

PCR products were analyzed by agarose gel electrophoresis. A 1.5 % agarose gel was prepared, and a nucleic acid staining dye was added to visualize DNA fragments. After electrophoresis, the gel was observed under ultraviolet light, and DNA fragments corresponding to the virus genome were identified and evaluated.

Phylogenetic analysis

To assess the genetic similarity of the identified virus isolates, their nucleotide sequences were compared with sequences available in international gene databases. Bioinformatic analysis was performed to construct a phylogenetic tree showing the relationships among the virus isolates and their genetic similarity to isolates reported in other countries.

Statistical analysis

The results obtained during the study were analyzed using statistical methods. The frequency of virus symptoms in plants was expressed as percentage values, and the results were summarized in the form of graphs and tables.

Results and discussion. Observation of Virus Symptoms in Potato Plants

During the study, various symptoms characteristic of viral diseases were observed in potato crops. In infected plants, chlorotic mosaic patterns, yellow spots on the leaf blade, leaf deformation, and reduced plant growth were recorded. In some plants, leaf curling and a reduction in leaf size were also noted. In addition, the appearance of necrotic spots on the tubers of infected plants was observed.

At the early stages of the vegetation period, symptoms appeared as small chlorotic dots on the leaves. Over time, these dots merged and formed a mosaic pattern. At more advanced stages of disease development, leaf deformation and necrotic zones became clearly visible. Due to the disruption of the photosynthesis process in infected plants, growth was suppressed and a decrease in total biomass was observed.

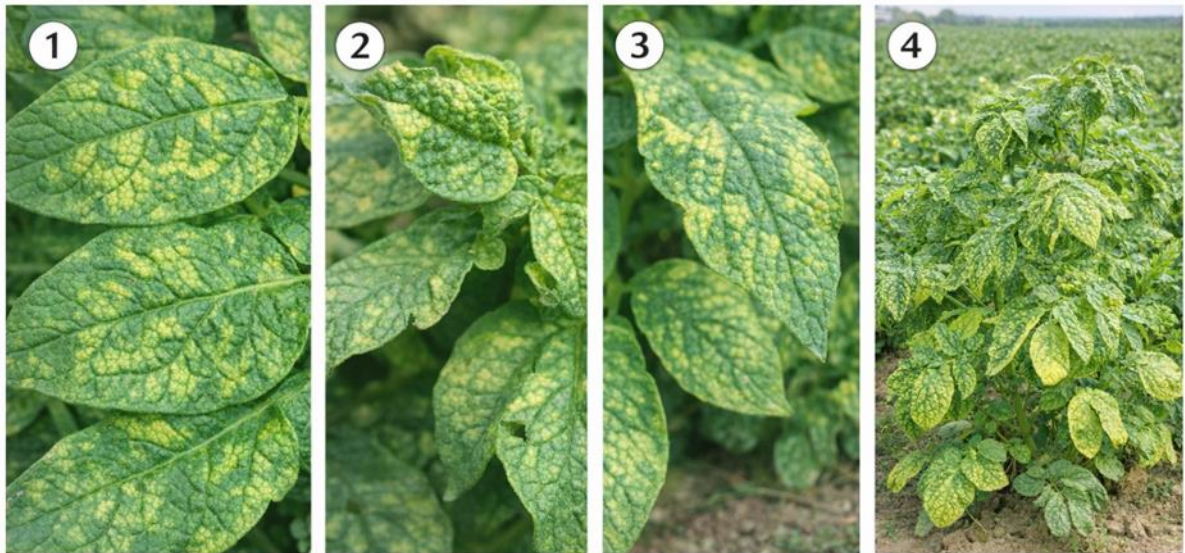


Figure 1. Symptoms of PAMV infection on potato leaves

(1) mosaic spots on the leaf, (2) leaf deformation, (3) chlorotic spots, (4) general appearance of the infected plant.

These observations are consistent with the results of previous studies on potato viral diseases. Researchers have reported that potato viruses negatively affect the photosynthetic system of plants and significantly reduce crop productivity.

Anatomical changes detected using light microscopy

Microscopic examination of infected leaf tissues using a light microscope revealed several anatomical changes. In virus-infected cells, deformation of chloroplasts, changes in cytoplasmic density, and thickening of cell walls were observed.

The formation of necrotic zones and the breakdown of cells were detected in the leaf parenchyma tissues. In some cells, a reduction in pigmentation was observed, indicating disruption of the photosynthetic process.

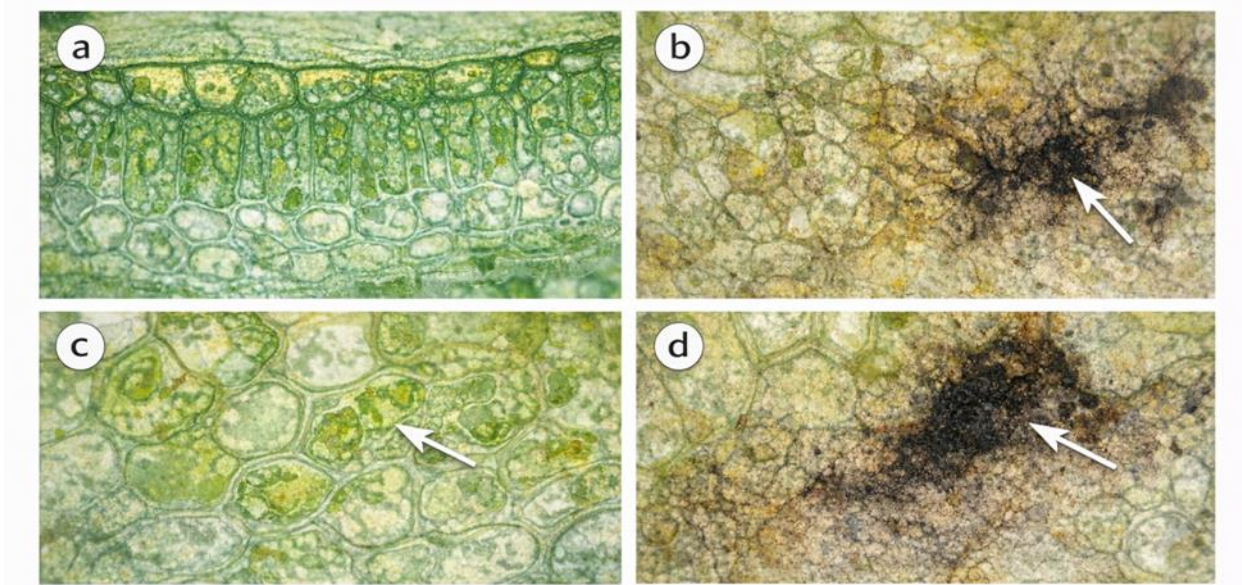


Figure 2. Changes in leaf tissues observed under a light microscope

a – healthy leaf tissue, b – virus-infected leaf cells, c – chloroplast deformation, d – necrotic zones.

RT-PCR Results

The results of molecular diagnostics confirmed the presence of Potato aucuba mosaic virus (PAMV). As a result of the RT-PCR reaction, a specific fragment of the viral genome was successfully amplified.

Electrophoresis analysis showed a DNA fragment of approximately 700 bp on the agarose gel. This fragment corresponds to the genome of PAMV. In the control samples, however, this fragment was not detected.

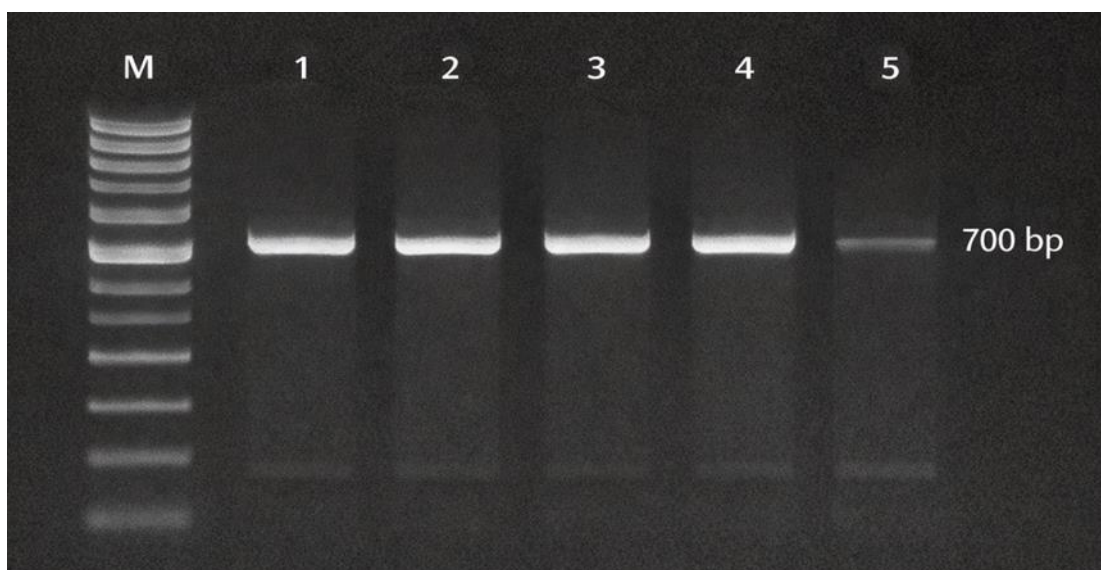


Figure 3. Agarose gel electrophoresis image of RT-PCR results

M – DNA marker, 1–4 – infected plant samples, 5 – healthy control sample.

Frequency of Virus Symptoms

During field observations, the frequency of virus symptoms was also evaluated. The obtained results are presented in the following table.

Table 1

Frequency of virus symptoms in potato plants

Symptom type	Occurrence rate (%)
Leaf mosaic	42
Chlorotic spots	35
Leaf deformation	28
Necrotic spots	19
Reduced growth	24

The results showed that the most common symptom was leaf mosaic, which was detected in nearly half of the observed plants.

Effect of Viral Infection on Plant Development

The developmental indicators of virus-infected plants were also evaluated. In infected plants, stem height and tuber formation were lower compared to healthy plants.

Table 2

Effect of viral infection on plant development

Indicator	Healthy plant	Infected plant
Stem height (cm)	65	48
Number of leaves	22	16
Number of tubers	8	5
Average tuber mass (g)	95	63

The results indicate that viral infection significantly reduces potato productivity.

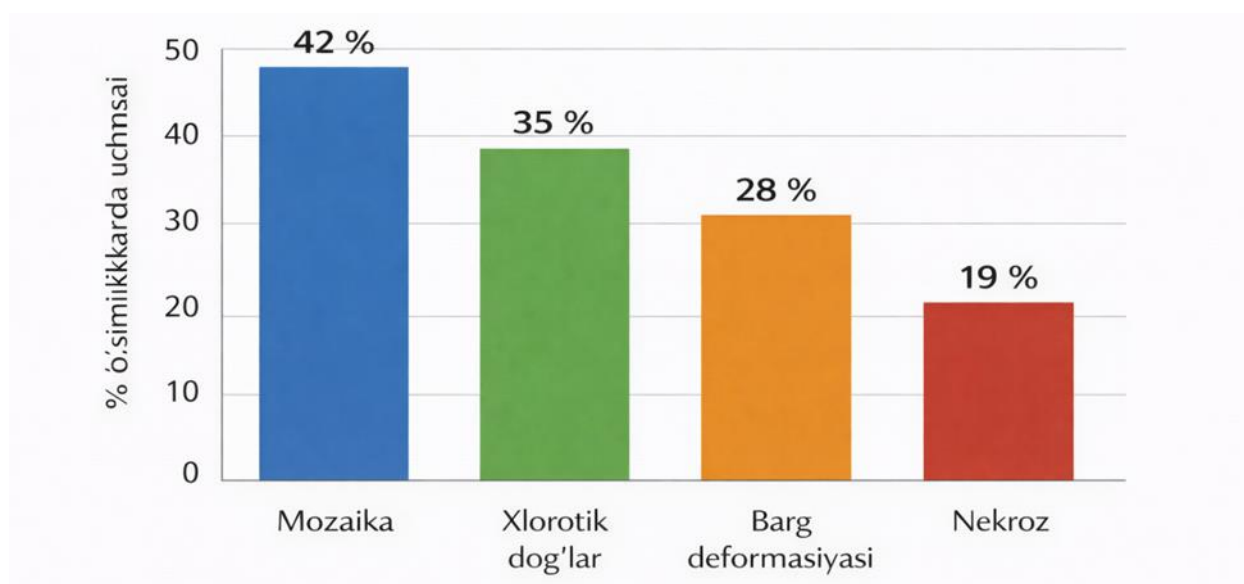


Figure 4. Distribution of observed symptoms in potato plants

Mosaic – 42%, Chlorotic spots – 35%, Leaf deformation – 28%, Necrosis – 19%.

Discussion. The results of this study indicate that Potato aucuba mosaic virus (PAMV) causes significant morphological, anatomical, and physiological changes in potato plants. Field observations revealed that infected plants exhibited symptoms such as chlorotic mosaic patterns on leaf blades, leaf deformation, necrotic spots, and reduced plant growth. These symptoms suggest that viral infection negatively affects plant metabolism and the photosynthetic process. Previous studies in phytopathology have also reported that potato viruses, particularly those belonging to the Potexvirus group, can lead to disturbances in pigment synthesis and disruption of chloroplast activity in plant cells. Therefore, the mosaic patterns and chlorotic spots observed on leaves may be associated with the degradation of photosynthetic pigments.

During the study, the frequency of symptoms observed under field conditions was also analyzed. According to the results, the most common symptom was leaf mosaic, detected in 42% of the examined plants. Chlorotic spots were observed in 35% of cases, leaf deformation in 28%, and necrotic symptoms in 19% of the plants. These findings indicate that PAMV infection is most often manifested through pigmentation changes in leaf tissues. The relatively lower occurrence of necrotic symptoms may be related to the severity of infection and the resistance level of the potato variety. Previous studies have also demonstrated that the symptoms of potato viral diseases can vary considerably depending on agro-climatic conditions and plant varieties.

Anatomical observations conducted using light microscopy showed that several structural changes occurred in infected leaf tissues. In virus-infected cells, deformation of chloroplasts, disruption of cytoplasmic structure, and the formation of necrotic zones were observed. Chloroplast deformation indicates impairment of the photosynthetic process due to viral infection. In addition, thickening of cell walls and structural alterations in cells may be associated with stress responses occurring in plant cells during viral replication. Numerous studies on plant viruses have demonstrated that viral infection alters the functional state of cellular organelles and leads to disturbances in metabolic processes.

The results of molecular diagnostics confirmed that the observed symptoms were indeed associated with PAMV infection. RT-PCR analysis revealed amplification of a DNA fragment approximately 700 bp in size. This fragment corresponds to a specific region of the viral genome and confirms the presence of the virus. The absence of this fragment in control samples demonstrates the reliability and specificity of the applied method. In recent years, RT-PCR has become one of the most sensitive and accurate diagnostic methods used in phytopathology for detecting plant viruses. This method allows detection of even very low concentrations of viral RNA and therefore plays an important role in the early diagnosis of viral diseases.

The impact of viral infection on the developmental parameters of potato plants was also evaluated in this study. The results showed that infected plants exhibited a significant reduction in stem height, number of leaves, and tuber formation compared with healthy plants. For example, the average stem height of healthy plants was 65 cm, whereas in infected plants it was only 48 cm. In addition, both the number of tubers and their average mass were considerably reduced. This phenomenon can be explained by the disruption of metabolic processes in infected plants and the limitation of nutrient transport to the tubers. Numerous scientific studies have reported that potato viruses can cause substantial yield losses, and in some cases viral infections may reduce productivity by more than 50%.

Overall, the obtained results indicate that Potato aucuba mosaic virus has a significant impact on the productivity and quality of potato crops. Therefore, early detection of viral diseases, the use of healthy seed material, and the introduction of virus-resistant varieties are essential measures for effective disease management. The application of modern molecular diagnostic methods enables rapid and accurate detection of potato viruses and provides an important scientific basis for controlling their spread.

Conclusion. This study was aimed at investigating the biological characteristics of Potato aucuba mosaic virus (PAMV) and detecting the virus using molecular diagnostic

methods. Field observations revealed several symptoms typical of viral diseases in potato plants, including leaf mosaic, chlorotic spots, leaf deformation, and necrotic signs. These symptoms were confirmed to be associated with viral infection. Among the observed symptoms, leaf mosaic was the most common, recorded in 42% of the studied plants. This finding indicates that PAMV infection primarily affects leaf pigmentation and the photosynthetic processes of potato plants.

Anatomical observations conducted using light microscopy demonstrated significant structural changes in virus-infected leaf tissues. In particular, deformation of chloroplasts, disruption of cellular structures, and the formation of necrotic zones were recorded. These changes confirm that viral infection leads to disturbances in metabolic processes within plant cells.

Molecular analyses confirmed the presence of PAMV at the molecular level. Using the RT-PCR method, a DNA fragment of approximately 700 bp corresponding to the viral genome was successfully amplified. The absence of this fragment in control samples confirmed the reliability of the applied methodology. These results further demonstrate that RT-PCR is a highly sensitive and accurate method for the detection of plant viruses.

The results of the study also showed that viral infection significantly affects the growth and development parameters of potato plants. In infected plants, stem height, number of leaves, and tuber formation were considerably reduced compared with healthy plants. This indicates that viral diseases represent an important phytopathological factor leading to reduced potato productivity.

Thus, the results of the study confirm that Potato aucuba mosaic virus is widely distributed in potato plants and significantly affects their morphological and physiological condition. The use of molecular diagnostic methods, particularly RT-PCR technology, plays an important role in the early detection and monitoring of viral diseases. The findings of this study provide a scientific basis for developing effective strategies for controlling potato viral diseases, selecting healthy seed material, and breeding virus-resistant potato varieties.

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