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Detection and identification of soy viruses by molecular diagnostics methods

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Abstract. Viral plant diseases are one of the main causes of soybean crop losses in Ukraine, and the lack of systematic monitoring and limited use of modern diagnostic methods make it difficult to effectively control the spread of viral pathogens. The purpose of the study was to assess the prevalence of major viral pathogens in soybean crops in the Central Forest-Steppe of Ukraine. A combination of serological analysis and molecular method was used to detect soybean mosaic virus, soybean vein necrosis virus, and alfalfa mosaic virus. The results showed that the overall infection rate of soybean crops was 40.7% according to real-time polymerase chain reaction data with reverse transcription. The species composition of pathogens showed clear differentiation: soybean mosaic virus was detected in 20.0% of samples, soybean vein necrosis virus – in 8.0%, alfalfa mosaic virus – in 7.3%. The real-time reverse transcription polymerase chain reaction method showed higher sensitivity compared to the enzyme-linked immunosorbent assay method, revealing an additional 13 positive samples. The most significant discrepancies between the methods were observed when detecting alfalfa mosaic virus, where the molecular method detected 50% more positive samples. Regional analysis revealed the highest infection rate in the Cherkasy region (44.0%), with the maximum prevalence of soybean mosaic virus (22.0%). A moderate correlation was found between symptom severity and threshold cycle values for soybean mosaic virus (r = -0.62), whereas for soybean vein necrosis virus and alfalfa mosaic virus, the correlation was statistically insignificant. The results confirmed the high prevalence of viral infections among soybean crops in the study region. The results obtained will allow stating phytosanitary services, breeding institutions, and agricultural producers to implement regional-differentiated strategies for protecting soybeans, which will reduce crop losses and increase the effectiveness of phytosanitary measures

Keywords: serological analysis; polymerase chain reaction; enzyme immunoassay; co-infection; phytosanitary monitoring

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INTRODUCTION

Soybeans (*Glycine max* L.), as a strategically important leguminous crop, faces significant threats from viral pathogens that cause crop losses and degrade product quality. In the context of growing global demand for vegetable protein and the need to ensure food security, effective control of viral diseases of soybeans is becoming a priority for the agricultural sector of Ukraine. A particular threat is the ability of viruses to spread rapidly, form new strains and form mixed infections, which complicates phytosanitary control. The lack of monitoring systems and the limited use of molecular diagnostic methods necessitates a comprehensive study of the prevalence and identification of the main viral pathogens of soy in Ukrainian agrocoenoses.

According to a recent study by L. Mishchenko *et al*. (2022), soybean crop losses from viral diseases can reach 30-70%, depending on the region and pathogen. The study included the investigation of seed and transmissible viral diseases in various soybean genotypes. The influence of viral infections on crop productivity in Ukraine was established. The results showed the need for constant monitoring of the phytosanitary condition of crops. On the territory of Ukraine, which is one of the leading soybean producers in Europe, the phytosanitary situation remains tense, as evidenced by the monitoring data of the most dangerous soybean and potato viruses, conducted by A. Dunich *et al*. (2024), presented the results of diagnostics of the main viral pathogens of soy on the territory of Ukraine. The study included analysis of samples from different regions of the country. The researchers noted the need to find sources of virus resistance in soybeans.

International studies show increased attention to the investigation of soy viral pathogens, which is conditioned by their rapid spread and significant economic losses. The study by A. Hameed *et al*. (2022) was devoted to a comprehensive analysis of environmental interactions in the SVNV system (soybean vein necrosis virus) – plant thrips, where key factors contributing to the rapid spread of the virus have been identified. However, the issue of regional features of SVNV distribution in Ukraine remains unexplored, which is one of the key aspects of this study. Studies of co-infections of viral pathogens are important. J. Zhou & I.E. Tzanetakis (2020) proved the ability of SVNV to move systematically in soybean plants in the context of co-infection with the bean pod spot virus, using state-of-the-art molecular detection methods. However, the question of the prevalence and features of the course of mixed infections in Ukrainian agrocoenoses remains poorly understood. The genetic aspects of viral pathogens were investigated by O.A. Abdalla *et al*. (2020), who found a significant variety of AMV isolates (alfalfa mosaic virus) in Saudi Arabia, which explains the difficulty of controlling this pathogen. Despite this, the issue of genetic variability and biological features of AMV in Ukraine requires additional study. The problem of seed transmission of viruses is reflected in the paper by D.N. Bhagwatkar *et al*. (2025), where the role of seed transfer *Carlavirus vignae* was established in the development of soybean diseases in India. For Ukrainian conditions, similar studies are limited, which makes it necessary to investigate seed transmission of viruses in local conditions.

Diagnostic methods are presented in the paper by M.G. Elmore *et al*. (2022), who demonstrated the potential of metagenomic sequencing to detect a wide range of viruses. However, the practical implementation of this method in Ukraine is difficult due to technological limitations, which led to the choice of more affordable RT-qPCR methods in this study. The development of SVNV management tools is presented in the study by C. Zambrana-Echevarría (2021), but their adaptation to the conditions of Ukraine requires additional research, considering local characteristics. The study by S. Zambrana-Echevarría (2021) was dedicated to developing tools for SVNV management and tobacco streak ilarvirus in soy. The study included the development of virus detection methods and control strategies. The researcher proposed an integrated approach to the management of these pathogens. Given the complexity of three-way interactions between viruses, their carriers, and host plants, A. Hameed (2020) investigated three-way interactions between SVNV, thrips, and soybean plants. The study included laboratory and field studies of these complex relationships. The results demonstrate the influence of various factors on the effectiveness of virus transmission.

The purpose of this study was to assess the prevalence of major soybean viruses in agrocoenoses of the Central Forest-Steppe of Ukraine. To achieve the goal, the following tasks were set: to take samples of soybean plants with symptoms of viral damage from different regions of the Central Forest-Steppe of Ukraine; to perform serological analysis using the DAS-ELISA method for primary screening for the presence of SMV, SVNV, and AMV; to conduct molecular identification of viruses using RT-qPCR and compare the effectiveness of serological and molecular diagnostic methods.

MATERIALS AND METHODS

Selection and preparation of plant material. The selection of plant material was carried out in the period from June to August 2024 on soybean crops of the 'Annushka' variety at the agricultural enterprises of three regions of the Central Forest-Steppe of Ukraine: Cherkasy (private enterprise "Luvais"), Poltava (limited liability company "Agris"), and Vinnytsia (private joint stock company "Podillia Foods Company"). Approximately 100 hectares of crops were surveyed using the route method, from which plants suspected of having a viral infection were selectively sampled. The criterion for selection was the presence of at least one of the characteristic symptoms of the virus: a pronounced mosaic colouring of the leaves in combination with a wrinkled surface of the leaf blade, a strong deformation – fern-like or corrugated edges of the leaf, local necrotic spots of brown or black colour, and general shortening of internodes and curling of the upper leaf tiers. The total number of individual leaf samples selected was 150 units (50 samples from each region), which was approximately 5-7 plants from each hectare of the surveyed area. Sampling was carried out in sterile disposable gloves using disinfected scissors to avoid cross-contamination between samples.

Each selected sample, consisting of two or three upper leaves with a growth point, was prepared for further analysis. After visual evaluation, each sample was divided into two identical parts to apply different diagnostic methods. The first part of the leaves without grinding was placed in a separate sterile plastic container with a capacity of 50 mL with perforation for gas exchange. These containers were placed in a portable refrigerator – a cooler bag with cold storage batteries, where the temperature was maintained in the range of +2°C to +4°C. Under such conditions, the samples were transported to the laboratory, where they were stored in a refrigerator at a stable temperature of +4°C and a relative humidity of 80-90% for no longer than 48 hours before the enzyme-linked immunosorbent assay, which allowed maintaining the stability of viral antigens.

The second part of the sample intended for molecular diagnostics was subjected to instant cryogenic fixation. For this purpose, the leaves were individually immersed in a container of liquid nitrogen for 30-40 seconds, which provided an instant stop of all enzymatic processes and prevented RNA degradation. Next, cryogenically treated samples were individually packed in pre-sterilised foil bags and placed for long-term storage in a freezer with forced air circulation at a stable temperature of -80°C (Thermo Scientific, USA). This storage regime guaranteed the preservation of the integrity of nucleic acids throughout the entire study period. Before RNA extraction, the samples were transported to the laboratory in dry ice containers that maintained a temperature of -79°C.

Serological analysis by DAS-ELISA. Serological analysis was performed under laboratory conditions by two-stage enzyme immunoassay (DAS-ELISA) in accordance with the standard protocols given in the manufacturer's instructions. Commercial kits were used to detect soybean mosaic virus (SMV), soybean vein necrosis virus (SVNV), and alfalfa mosaic virus (AMV) from "Loewe Biochemica" (Germany). Extraction of plant antigens was performed by homogenising 0.1 g of leaves in 1 mL of soluble buffer (extraction buffer) included in the kit. The sandwich complex was created on 96-hole tablets for ELISA (Nunc, Denmark). Incubation with primary and secondary antibodies, and with a conjugate (alcalic phosphatase), was performed in a thermostat at 37°C. The tablet was washed using a small laboratory microplate washer. The substrate p-nitrophenyl phosphate (pNPP) was used for visualisation. Incubation with the substrate was carried out in the dark at room temperature of 23°C for 60 minutes. The reaction was stopped by adding 3M NaOH. Optical density (OD) at a wavelength of 405 nm was measured on a vertical spectrophotometer for microplates "Multiskan GO" (Thermo Fisher Scientific, Finland). The sample was considered positive if its optical density value exceeded the arithmetic mean of the negative control values by at least three times.

Molecular diagnostics by RT-qPCR. Molecular diagnostics were performed in the laboratory using real-time reverse transcription and polymerase chain reaction (RT-qPCR). Total RNA was isolated from 100 mg of cryogenically crushed leaf tissue using the "Ukrbiopreparat" kit (Ukraine) in accordance with the manufacturer's instructions. The concentration and purity of the obtained RNA preparations were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The A260/A280 ratio for all samples was in the range of 1.8-2.0. Reverse transcription was performed using the "Reverta-L" kit (Ukraine) in a reaction volume of 20 µl containing 1 µg of total RNA, random hexameric primers, and M-MuLV polymerase. The reaction was performed according to the following programme: 10 minutes at 25°C, 60 minutes at 42°C, and 10 minutes at 70°C to inactivate the enzyme.

Real-time polymerase chain reaction was performed in a CFX96 Touch amplifier (Bio-Rad, USA). The 25 µl reaction mixture contained 1X PCR buffer (polymerase chain reaction), 2.5 mm $MgCl_2$, 200 µM each dNTP, 0.2 µM forward and reverse primer, 0.5 µl intercalating dye SYBR Green I (Ukraine), 1 unit Taq-DNA (deoxyribonucleic acid)-polymerase (Ukraine) and 2 µl matrix cDNA. Species-specific primers were used to detect viruses, the sequences of which were taken from literature sources. To detect soybean mosaic virus primers SMV-F (5'-CGA-GCA-AGC-AGT-TCA-AGA-CC-3') and SMV-R (5'-TCT-GCC-ATA-ACC-ATG-GAC-GA-3') were aplied, described in the paper by B. Xue *et al*. (2021). To detect soybean vein necrosis virus (SVNV) used primers SVNV-F (5'-ATG-GGT-TTG-GGA-AGA-AGG-TG-3') and SVNV-R (5'-TCA-AGC-AAC-TCC-AGC-ACC-at-3') published in the paper by C. Groves *et al*. (2016). For detecting alfalfa mosaic virus applied primers (5'-ATGCTACCCAGGCATGTATATTT-3') and (5'-GCTGCATCTTTCGCCAGAA-3') that amplify the capsid protein gene region.

The amplification protocol included initial denaturation at 95°C for 3 minutes, followed by 40 cycles, each consisting of denaturation at 95°C for 15 seconds, primer annealing at 60°C for 20 seconds, and DNA synthesis at 72°C for 30 seconds. After amplification, melting curves were constructed by slow heating in 0.5°C increments from 65°c to 95°C to confirm the specificity of the synthesised PCR products. In each run, negative control without matrix (no template control, NTC) and positive control were used. The sample was considered positive if the amplification threshold cycle (Ct) was less than 35, and there was a clear peak on the melting curve with a melting point identical to the positive control. Experimental plant studies, including the collection of plant material, were in accordance with institutional, national, and international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

Quantitative analysis of viral load and statistical data processing. To quantify the viral load, the threshold cycle values were analysed. Quantitative calibration was performed using a series of ten-fold dilutions of recombinant plasmids containing target regions of viral genes. A standard curve with a correlation coefficient $R^2 > 0.98$ was constructed for each virus. The linearity range for all detected viruses was from 10^1 to 10^8 copies/µl. The threshold cycle values were interpreted as follows: Ct < 30 indicated a high concentration of the virus (⩾ 10^5 copies/µl), Ct 30-35 indicated an average concentration (103-10 copies/µl), and Ct > 35 indicated a low concentration of the virus (< 103 copies/µl). Samples with Ct > 40 were considered negative. Quantitative analysis was performed using the CFX96 Touch analyser software (USA), with automatic calculation of the virus concentration based on standard curves. Each sample was analysed in three repetitions, and the mean Ct value was used for further calculations. Statistical processing of the obtained data was performed using Microsoft Excel 2019 and Statistica 12.0 software suite (USA). Differences between the frequency of virus detection by different methods were estimated using the nonparametric criterion χ^2 (chi-square). The difference was considered statistically significant at the significance level $p < 0.05$.

RESULTS

Results of serological analysis by DAS-ELISA

Serological analysis by DAS-ELISA showed a significant level of infection of soybean crops with viral pathogens in the study region. Out of a total of 150 samples analysed, a positive response to the presence of at least one of the three viruses under study was recorded in 48 samples, which is 32.0%

of the total sample size. The average optical density (OD_{405}) of positive samples was 0.85 ± 0.23, which significantly exceeded the negative control value (0.12 ± 0.04). In all positive samples, the optical density value exceeded the positivity threshold (0.36) by at least 3 times. The results of serological analysis by the DAS-ELISA method are presented in Table 1.

Table 1. Results of serological analysis of soybean samples by DAS-ELISA method

Research area	Total number of samples	SMV-positive	SVNV-positive	AMV-positive	General infection rate
Cherkasy	50	11 (22.0%)	4 (8.0%)	3 (6.0%)	18 (36.0%)
Poltava	50	9 (18.0%)	3 (6.0%)	3 (6.0%)	15 (30.0%)
Vinnytsia	50	7 (14.0%)	5 (10.0%)	3 (6.0%)	15 (30.0%)
Total	150	27 (18.0%)	12 (8.0%)	9 (6.0%)	48 (32.0%)

Note: the sum of individual infections may exceed the total number of infected samples due to the presence of mixed infections that were not detected by the DAS-ELISA method
Source: compiled by the authors based on the conducted research

A detailed analysis of the species composition of pathogens revealed a clear differentiation in the prevalence of individual viruses. The most common pathogen was soybean mosaic virus (SMV), which was identified in 27 samples, accounting for 18.0% of the total number of plants tested. The average optical density for SMV-positive samples was 0.92 ± 0.18. Soybean vein necrosis virus (SVNV) took the second place in prevalence – it was detected in 12 samples (8.0%), with an average value of OD_{405} = 0.76 ± 0.21. The least common among the pathogens under study was alfalfa mosaic virus, which was detected in 9 samples (6.0%) with an average optical density of 0.68 ± 0.15.

Statistical analysis using the χ^2 criterion did not reveal statistically significant differences in the frequency of virus detection between different study areas ($p > 0.05$), which was confirmed by the following indicators: total infection varied between 30.0-36.0%, the prevalence of alfalfa mosaic virus was 6.0% in all three regions, and the detection rate of soybean mosaic virus has only minor differences between regions (from 14.0% to 22.0%). However, analysis of the distribution of infection by region revealed certain trends. In the Cherkasy region, the highest overall infection rate was observed – 36.0% (18 positive samples out of 50), while in the Poltava and Vinnytsia region this figure was 30.0% (15 positive samples out of 50 in each region). The distribution of certain types of viruses by region also showed certain regional features: SMV was most common in the Cherkasy region (22.0%), while SVNV was more often detected in the Vinnytsia region (10.0%).

It was noted that the colour intensity (OD_{405}) of positive samples correlated with the severity of visual symptoms in the field. Samples with pronounced mosaic and leaf deformity symptoms showed OD_{405} values above 1.0, while samples with mild symptoms had values in the range of 0.4-0.7. In 6 samples (4.0% of the total), OD_{405} values close to the threshold level (0.36-0.42) were observed, which could indicate the initial stages of infection or a low titre of the virus in plant tissues.

Effectiveness of molecular diagnostics and comparative analysis of methods

Molecular diagnostics using real-time reverse transcription and polymerase chain reaction (RT-qPCR) showed an advantage in sensitivity compared to DAS-ELISA serological analysis. The overall detection rate of viral pathogens was 40.7% (61 positive samples out of 150), which is 8.7 percentage points higher than the indicator obtained by the ELISA method. A detailed analysis of the detection efficiency of individual viruses showed that the advantage of RT-qPCR was most pronounced for detecting alfalfa mosaic virus (AMV), where the molecular method detected 50% more positive samples (12 vs. 8), and for soybean vein necrosis virus, where the difference was 8.3% (13 vs. 12).

Quantitative analysis of threshold cycle values (Ct) showed a high titre of viruses in infected plants. The Ct range for positive samples ranged from 24.3 to 32.7 cycles. The lowest Ct values (higher virus concentration) were observed for SMV – an average value of 26.5 ± 2.3, which correlated with pronounced symptoms of mosaic and leaf deformity. For SVNV, the mean Ct value was 28.1 ± 3.1, and for AMV – 30.4 ± 2.8, which indicates a lower concentration of these pathogens in plant tissues.

Statistical analysis using the χ^2 criterion revealed a statistically significant difference between the effectiveness of the DAS-ELISA and RT-qPCR methods ($\chi^2 = 8.45$; $p < 0.01$). The RT-qPCR method revealed an additional 13 positive samples that showed negative results in serological analysis. The detection of 4 samples with Ct values in the range of 31.5-32.7 was indicative, which indicates a low concentration of the virus, insufficient for detection by the ELISA method (Table 2).

Table 2. Comparative characteristics of the effectiveness of DAS-ELISA and RT-qPCR methods

Parameter	DAS-ELISA	RT-qPCR	Difference
General detection	48/150 (32.0%)	61/150 (40.7%)	+13 samples (+8.7%)
SMV	27/150 (18.0%)	29/150 (19.3%)	+2 samples (+1.3%)
SVNV	12/150 (8.0%)	13/150 (8.7%)	+1 sample (+0.7%)
AMV	8/150 (5.3%)	12/150 (8.0%)	+4 samples (+2.7%)
Mean Ct	-	26.5-30.4	-

Note: (-) in the DAS-ELISA column for the "mean Ct" parameter indicates that the enzyme-linked immunosorbent assay method does not provide for determining the threshold cycle; (-) in the "Difference" column for the "Mean Ct" parameter, conditioned by the lack of comparable data in the DAS-ELISA method
Source: compiled by the authors based on the conducted research

The analysis in Table 2 demonstrates the advantages of the molecular diagnostic method. The most significant increase in sensitivity is observed for detecting alfalfa mosaic virus, where the polymerase chain reaction method detected 50% more positive samples. Overall detection of viral infections increased by 8.7% when using the molecular method, which confirms its higher effectiveness for comprehensive monitoring of soy viral diseases. Analysis of regional samples showed that the differences between the methods were most pronounced in the Vinnytsia region, where RT-qPCR detected 6 positive samples more than ELISA, while in Cherkasy and Poltava region this difference was 4 and 3 samples, respectively. This may indicate regional features of the development of viral infections and the level of accumulation of the virus in plants. The melting curves of all positive samples showed clear peaks with characteristic melting points: 82.5°C ± 0.5°C for SMV, 85.2°C ± 0.3°C for SVNV, and 79.8°C ± 0.4°C for AMV, which confirms the specificity of amplification and the absence of non-specific PCR products. Atypical Ct values (more than 34 cycles) were observed in 5 samples, which, however, had characteristic melting curves and were classified as weakly positive. The results confirm that the RT-qPCR method is a sensitive tool for early diagnosis of soy viral infections, especially in cases of low virus concentrations and for detecting AMV, which shows the lowest titres in plant tissues.

Spread of viral infections and detection of mixed infections

A detailed analysis of the obtained results of molecular diagnostics allowed establishing a complex structure of the spread of viral infections among soybean crops in the study region. The overall infection rate determined by RT-qPCR was 40.7% (61 samples out of 150), which indicates the accumulation of viral pathogens in soybean agrocoenoses. The regional distribution of infected samples showed clear territorial features: the highest level of infection was registered in the Cherkasy region (44.0%, 22 samples), a slightly lower indicator was observed in the Vinnytsia region (40.0%, 20 samples), and the lowest number of infected plants was found in the Poltava region (38.0%, 19 samples). The value of the study is the detection of mixed infections that could not be reliably identified by the serological method (Table 3).

Table 3. Regional distribution of viral infections and mixed infections according to RT-qPCR data

Type of infection	Cherkasy region (n = 50)	Poltava region (n = 50)	Vinnytsia region (n = 50)	Total (n=150)
SMV	11 (22.0%)	9 (18.0%)	10 (20.0%)	30 (20.0%)
SVNV	4 (8.0%)	3 (6.0%)	6 (12.0%)	13 (8.7%)
AMV	3 (6.0%)	4 (8.0%)	4 (8.0%)	11 (7.3%)
SMV + SVNV	3 (6.0%)	2 (4.0%)	2 (4.0%)	7 (4.7%)
SMV + AMV	1 (2.0%)	1 (2.0%)	1 (2.0%)	3 (2.0%)
General infection rate	22 (44.0%)	19 (38.0%)	20 (40.0%)	61 (40.7%)

Source: compiled by the authors based on the conducted research

Molecular diagnostics revealed 10 cases of co-infections, which is 6.7% of the total number of analysed samples. The most common combination was SMV and SVNV, which was recorded in 7 samples (4.7%). Less common was SMV and AMV co-infection was detected in 3 samples (2.0%). Analysis of the threshold cycle (Ct) values for samples with mixed infections showed a pattern: in 8 out of 10 cases, the dominance of one of the viruses was observed, which was manifested in Ct values that differed by 3-5 cycles. Soybean mosaic virus showed the highest prevalence in the Cherkasy region (22.0%), which is associated with favourable climatic conditions for the development of aphids carrying this virus. Soybean vein necrosis virus was most common in the Vinnytsia region (12.0%), which is probably conditioned by the presence of reservoir plants and favourable conditions for thrips development. Alfalfa mosaic virus showed the most uniform distribution between regions (6.0-8.0%), which may indicate a stable circulation of this pathogen in the agrocoenoses of the region.

Another aspect of the study was the analysis of the relationship between the type of infection and the severity of symptoms. In samples with mixed infections, significantly more pronounced symptoms of the lesion were observed: intense mosaicism, severe deformation of leaf blades, necrotic spots, and pronounced dwarfism of plants. The mean Ct value for samples with mixed infections was 25.8 ± 1.9, which indicates higher viral concentrations compared to monoinfections (28.3 ± 2.4). The geographical distribution of mixed infections also had its own characteristics. The highest frequency of co-infections was observed in the Cherkasy region (8.0%), where 4 cases of mixed infections were detected. In the Poltava and Vinnytsia regions, this figure was 6.0% and 6.0%, respectively. This may indicate a more intensive epiphytotic process in the Cherkasy region, which requires additional research to identify the causes and factors of such differentiation. The results highlight the importance of considering mixed infections when assessing the phytosanitary condition of soybean crops and developing protection measures, since co-infections can significantly enhance the pathogenic effect and lead to more significant crop losses.

Correlation between symptoms and infection intensity and diagnosis specificity

A detailed analysis of the relationship between the clinical manifestations of viral infections and laboratory indicators of infection intensity revealed patterns that are important for practical diagnosis. For SMV, a moderate negative correlation was observed between the severity of symptoms and Ct values ($r = -0.62$; $p < 0.05$), which indicates a clear relationship between the severity of clinical manifestations and the concentration of the virus in plant tissues. Samples with a pronounced mosaic (4-5 points) showed Ct values in the range of 24.3-26.8, while plants with mild symptoms (1-2 points) had Ct values of 28.5-31.2. The strongest correlation was observed for mosaic symptoms ($r = -0.58$) and leaf blade deformity ($r = -0.61$), while the association with necrotic spots was less pronounced ($r = -0.42$).

For SVNV, the correlation between symptoms and Ct values was weak and statistically insignificant ($r = -0.34$; $p > 0.05$). This may indicate a more complex pathogenesis of this virus, where clinical manifestations depend not only on the concentration of the virus, but also on other factors, such as the age of the plant, environmental conditions or characteristics of the variety. Samples with characteristic vein necrosis had Ct values in a wide range from 26.8 to 32.4, which confirms that there is no direct association between visible symptoms and viral concentrations. For AMV, correlation analysis showed the weakest association between symptoms and Ct values ($r = -0.28$; $p > 0.05$). This may be conditioned by the fact that AMV causes atypical or mild symptoms on soybean, and the fact that the pathogen may be latent or cause symptoms only under certain environmental conditions. Of interest is also the analysis of samples with mixed infections, where a significantly stronger correlation was observed between the total symptom score and the mean Ct values ($r = -0.71$; $p < 0.01$). This confirms the synergistic effect in co-infection, when the presence of two viruses leads to more pronounced symptoms at the same concentrations of pathogens.

The specificity of the developed PCR system was carefully analysed using a detailed study of melting curves. For SMV, clear peaks were observed with a melting point of 82.5°C ± 0.5°C, which corresponds to the expected amplicon size of 233 bp. For soybean vein necrosis virus, the melting point was 85.2°C ± 0.3°C, which is typical for a longer fragment of nucleocapsid protein. For AMV, peaks with a melting point of 79.8°C ± 0.4°C were recorded, which corresponds to a fragment of the capsid protein gene. All melting curves showed a single-modal character with no side peaks, which indicates high amplification specificity and the absence of primer dimers or non-specific products.

Control experiments confirmed the high reliability of the molecular method. All negative controls ($n = 15$) showed no amplification for 40 cycles, while controls without a matrix (NTC, $n = 12$) also remained negative, which excludes the possibility of contamination. Positive controls for each virus ($n = 9$) showed stable Ct values with a coefficient of variation of less than 3.2%, which confirms the reproducibility of the method. An additional confirmation of specificity is the fact that Ct values correlated with the results of serological analysis: samples with low Ct values (high virus concentration) usually showed a strong positive response in ELISA, while samples with high Ct values were often weakly positive or negative in the serological test. This pattern was particularly clear for SMV, where the correlation between OD values in ELISA and Ct values in RT-qPCR was $r = -0.59$ ($p < 0.05$).

DISCUSSION

The results obtained regarding the high prevalence of viral infections among soybean crops in the Central Forest-Steppe of Ukraine are consistent with research data from other regions of the world. The overall infection rate of 40.7% detected by RT-qPCR correlates with the results of studies in India, where viral infections also pose a serious problem for soybean producers, as indicated in by N. Sandra *et al*. (2021). The high prevalence of SMV may be due to its ability to transmit seeds and effective transmission by aphids, which is confirmed by studies of the genetic structure of populations of this virus, which were conducted by M. Usovsky *et al*. (2022). A long period of preservation of the viability of the virus in seeds and a wide presence of vectors in agrocoenoses contribute to maintaining a high level of infection.

The advantage of the RT-qPCR method over DAS-ELISA in detecting viral infections is confirmed in current studies. Current approaches to virus screening using eDNA-based electronic samples show promise for global monitoring of soy viral diseases, as noted in the paper by M.R. Ribeiro-Junior *et al*. (2025), and is consistent with the need to introduce more sensitive diagnostic methods in the phytosanitary control system. Detection of mixed infections in 6.7% of the samples under study is important for understanding the epiphytotic process. Studies of interactions between different viruses in soybean plants have shown that co-infections can significantly affect disease development and crop productivity. Combinations of viruses with different transmission mechanisms are dangerous, which complicates control measures, as noted by S. Tatineni & G.L. Hein (2023).

The regional features of the spread of viruses identified in the study were confirmed in international scientific works. Differences in the prevalence of viruses between Cherkasy, Poltava and Vinnytsia regions are consistent with data from studies in Bangladesh, where significant regional variability in the prevalence of soymean viral pathogens was also observed, as indicated by M.F. Khatun *et al*. (2025). In contrast to the study in Indonesia conducted by E. Uge *et al*. (2023), which revealed a clear correlation between climatic conditions and the prevalence of mosaic viruses, this study failed to establish a direct relationship between climatic parameters and infection rates, which may be conditioned by the relative uniformity of climatic conditions in the Central Forest-Steppe of Ukraine in comparison with various climatic zones of Indonesia, and differences in the species composition of vectors and their ecological features. However, the identified trends in the regional distribution of individual viruses, in particular, the increased prevalence of soybean vein necrosis virus in the Vinnytsia region, may be of practical importance for the development of differentiated plant protection measures. The detection of AMV in soybean agrocoenoses in the Central Forest-Steppe of Ukraine is consistent with the global trend in the spread of this pathogen. H.A. Hobbs *et al*. (2012) recorded the appearance of AMV on soybeans in North Dakota, indicating the virus' ability to adapt to new geographical areas. The latest data by R. Dawoud (2025) highlighted the significant potential of AMV for genetic variability, which explains its successful circulation in various agroecosystems. The high frequency of detection of AMV in Ukrainian soybean crops may be associated with a wide range of host plants and the ability of the virus to persist for a long time in plant residues, which justifies the need for constant monitoring and development of specialised control measures.

Correlation analysis between the severity of symptoms and Ct values confirms the complexity of visual diagnosis of viral infections. The moderate negative correlation for SMV ($r = -0.62$) is consistent with current studies using artificial intelligence to identify soy diseases by K. Zhang *et al*. (2021). However, the lack of a statistically significant correlation for SVNV and AMV indicates limited visual assessment methods, which is not fully consistent with the results of studies of automated diagnostic systems by K. Zhang *et al*. (2021), which demonstrated high accuracy for various types of viral lesions. This is conditioned by the more complex pathogenesis of SVNV and AMV, and their ability to cause atypical symptoms depending on the physiological state of the plant. The need to introduce a comprehensive system of protection measures was confirmed by the results of breeding studies. The high level of infection of soybean crops with viral pathogens in Ukraine is consistent with the need to use molecular markers and genomic selection in breeding programmes, which was considered by F. Lin *et al*. (2022). Identification of SMV resistance genes in international studies by D. Wang *et al*. (2022) and J. Chu *et al*. (2021) confirmed the prospects of creating sustainable varieties for the conditions of Ukraine. The study by D. Wang *et al*. (2022) presented a comprehensive analysis of the genetic loci responsible for SMV resistance. The researchers identified a key QTL (quantitative trait locus) on chromosome 13, which explains up to 68% of the phenotypic variability of viral resistance. The value is that the researchers were able to map a new Rsv4 gene that provides resistance to a wide range of SMV isolates. The study by J. Chu *et al*. (2021) complemented these results by focusing on studying the Rsv1 gene in various soy genetic populations. The researchers found that allelic variations in this gene cause different levels of resistance to SMV, with the most effective alleles providing immunity to 95% of known virus isolates.

The use of RNA interference to control SMV demonstrated the effectiveness of this approach, which is fully consistent with the results of the study by H. Luan *et al*. (2020), where transgenic plants with the Vma12 gene turned off showed increased resistance to SMV. However, the effectiveness of RNA interference for controlling SVNV and AMV remains poorly understood, unlike SMV, for which specific control strategies have been developed. The use of viral vectors for induced gene silencing opens up new horizons in the fight against soy viral infections, which is confirmed by the innovative study by M. McCaghey *et al*. (2021). The researchers demonstrated that a vector based on the bean pod spot virus can effectively deliver constructs for RNAi-mediated silencing of oxaloacetate-acetylhydrolase genes in *Sclerotinia sclerotiorum*, which led to a significant reduction in the development of soy disease. This approach is useful for controlling viral infections, as it allows purposefully inhibiting the replication of viruses or disrupt their life cycles, creating resistance at the molecular level. The advantage of this technology is the ability to quickly adapt to new strains of viruses by changing siRNA sequences, which is important in the context of constant evolution of viral pathogens.

Adevanced multi-mix approaches radically change the methodology of breeding virus-resistant soybean varieties, which is fully confirmed in the study by A. Bisht *et al*. (2023). Comprehensive analysis of transcriptomics, proteomics, and metabolomics reveals not only individual resistance genes, but also entire networks of molecular interactions that form systemic resistance to viral infections. However, according to S. Rahman *et al*. (2023), the introduction of these advanced technologies in the breeding process requires significant investment in equipment and training. In the context of Ukraine, these restrictions are particularly relevant, which makes it necessary to gradually introduce omics technologies – first with a focus on transcriptome analysis as the most accessible method, with further expansion to full-scale multi-omics studies. The use of genomic selection to improve virus resistance is confirmed in international studies, but its practical implementation requires adaptation to local conditions and pathogenic complexes. The use of high-throughput sequencing to detect new and emerging viral pathogens is fully consistent with the findings of R. Fowkes *et al*. (2021), where this method helped to identify turnip yellows virus and soybean dwarfism virus in peas in the UK.

The study by M. Solomiichuk & M. Pikovskyi (2021) examined the use of *Pseudomonas fluorescens* and stimulating substances to fight pathogens, improving the productivity of soybeans. In turn, this study focused on detecting mosaic viruses and soy necrosis using molecular methods, in particular PCR. Both studies contribute to reducing soybean crop losses by using different approaches to pathogen control. The effectiveness of the applied diagnostic methods is confirmed in contemporary studies on soy pathology. The use of a combination of serological and molecular methods to detect viral infections is consistent with the approaches described for the diagnosis of fungal and oomycete soy pathogens in B. Hosseini *et al*. (2023), which notes the importance of combining different methods for accurate pathogen identification. However, in contrast to the diagnosis of fungal diseases, where real-time PCR and enzyme immunoassay methods are widely used, for viral pathogens, molecular methods show higher efficiency, which is confirmed by the detection of an additional 13 positive samples using RT-qPCR compared to DAS-ELISA. Biotechnological approaches to creating virus-resistant soybean varieties show promise in global research. Development of a guide RNA delivery system using the ALSV virus for genomic editing proposed by Y. Luo *et al*. (2021) is consistent with the need to develop innovative methods to protect against SMV, which is identified as the dominant pathogen in this study. However, in contrast to the experimental conditions of the study by Y. Luo *et al*. (2021), the practical application of such technologies in Ukraine requires further research. The use of CRISPR/Cas systems to create virus resistance is a promising area, but its effectiveness in controlling SVNV and AMV remains poorly understood. The molecular mechanisms of natural resistance to SMV described in international studies may explain the high prevalence of this virus in Ukraine. Detection of an NLR receptor that recognises the cylindrical SMV inclusion protein in the study by J. Yin *et al*. (2021) offers a molecular explanation of the mechanisms of resistance, which is partially consistent with the data obtained on the high prevalence of SMV (20.0%). However, in contrast to the experimental conditions of the study, J. Yin *et al*. (2021), where specific resistance genes were studied, the presence of functional alleles of these genes may be limited in Ukrainian soybean varieties. This makes it necessary to introduce marker-associated breeding for introgression of SMV resistance genes in Ukrainian varieties.

The conducted study confirmed the effectiveness of molecular diagnostic methods for detecting soybean viral pathogens in Ukraine. The results obtained substantiate the need to introduce an integrated approach to phytosanitary monitoring, combining serological and molecular methods. The identified regional features of the spread of viruses and the frequency of mixed infections form the scientific basis for the development of differentiated plant protection measures aimed at reducing crop losses and improving the efficiency of soybean production in Ukraine.

CONCLUSIONS

Based on the conducted comprehensive study on the detection and identification of soybean viruses by molecular diagnostics methods, a high level of prevalence of viral infections among soybean crops in the Central Forest-Steppe of Ukraine was established. Serological analysis by the DAS-ELISA method revealed a total infection rate of 32.0%, and the highest rates were observed in the Cherkasy region (36.0%). Among the three viruses studied, soybean mosaic virus was the most common – 18.0%, which indicates the need to strengthen phytosanitary control over this particular pathogen.

The RT-qPCR method showed a statistically significant 8.0% increase in sensitivity, detecting an additional 12 positive samples, and when detecting alfalfa mosaic virus, the number of positive samples detected increased by 50%. This justifies the feasibility of using RT-qPCR as the main method for accurate diagnosis, especially in the early stages of infection. For the first time in the study area (Central Forest-Steppe of Ukraine), mixed infections were identified with a frequency of 6.7% of the total number of analysed samples. SMV + SVNV co-infection was the most common (4.7%). The identified mixed infections highlighted the complexity of the epiphytotic situation and the need for a comprehensive approach to diagnosis. Correlation analysis revealed varying degrees of association between visual symptoms and laboratory parameters for individual viruses. For soybean mosaic virus, a moderate negative correlation ($r = -0.62$) was established between the severity of symptoms and threshold cycle values, which indicates the possibility of using visual assessment for preliminary diagnosis. Regional analysis revealed differentiation in the spread of viruses: the highest total infection was recorded in Cherkasy region (44.0%), and the maximum prevalence of soybean vein necrosis virus – in Vinnytsia region (12.0%), which may be due to climate features and the presence of vectors.

The conducted study has certain limitations, among which the regional specifics of the sample are highlighted, covering only three regions of the Central Forest-Steppe, the lack of monitoring of the dynamics of virus accumulation during the growing season, and the limited diagnosis of the three main viruses without considering other potentially dangerous pathogens. The prospects for further research include expanding the geography of monitoring to other agroclimatic zones of Ukraine, studying the influence of climatic factors on the development of viral infections, and developing multiplex PCR systems for simultaneous detection of a wider range of pathogens.

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CONFLICT OF INTEREST

None.

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Виявлення та ідентифікація вірусів сої методами молекулярної діагностики

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Анотація. Вірусні хвороби рослин є однією з основних причин втрат врожаю сої в Україні, а відсутність системного моніторингу та обмеженість застосування сучасних методів діагностики ускладнюють ефективний контроль поширення вірусних патогенів. Метою роботи була оцінка поширеності основних вірусних патогенів у посівах сої Центрального Лісостепу України. Використано комбінацію серологічного аналізу та молекулярного методу для детекції вірусу мозаїки сої, вірусу некрозу жилок сої та вірусу мозаїки люцерни. Результати показали, що загальний рівень інфікованості посівів сої становив 40,7 % за даними полімеразної ланцюгової реакції у реальному часі з зворотною транскрипцією. Видовий склад патогенів демонстрував чітку диференціацію: вірус мозаїки сої виявлено у 20,0 % зразків, вірус некрозу жилок сої – у 8,0 %, вірус мозаїки люцерни – у 7,3 %. Метод полімеразної ланцюгової реакції у реальному часі з зворотною транскрипцією показав вищу чутливість порівняно з методом імуноферментного аналізу, виявивши додаткові 13 позитивних зразків. Найбільш значні розбіжності між методами спостерігалися при виявленні вірусу мозаїки люцерни, де молекулярний метод виявив на 50 % більше позитивних зразків. Регіональний аналіз виявив найвищу інфікованість у Черкаській області (44,0 %), з максимальною поширеністю вірусу мозаїки сої (22,0 %). Встановлено помірну кореляцію між виразністю симптомів та значеннями порогового циклу для вірусу мозаїки сої ($r = -0{,}62$), тоді як для вірусу некрозу жилок сої та вірусу мозаїки люцерни кореляція була статистично незначущою. Висновки підтвердили високий рівень поширеності вірусних інфекцій серед посівів сої в регіоні дослідження. Отримані результати дозволять державним фітосанітарним службам, селекційним установам та агровиробникам впровадити диференційовані за регіонами стратегії захисту сої, що дозволить зменшити втрати врожаю та підвищити ефективність фітосанітарних заходів

Ключові слова: серологічний аналіз; полімеразна ланцюгова реакція; імуноферментний аналіз; ко-інфекція; фітосанітарний моніторинг