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Innovative methods for diagnosing RNA-containing plant viruses: New approaches to control and prevention

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Abstract. This study aimed to conduct a comparative analysis of traditional methods (enzyme-linked immunosorbent assay, polymerase chain reaction) and innovative techniques for diagnosing ribonucleic acid-containing viruses in plants, in order to assess their effectiveness in detecting pathogens at early stages of infection. Experimental trials were carried out under laboratory conditions using wheat, tomato, and cucumber samples infected with typical viral agents. The detection limit, specificity, analysis duration, cost, and scalability of each method were examined. It was found that modern molecular approaches – particularly targeted cleavage of genetic material using clustered regularly interspaced short palindromic repeats with associated proteins, as well as next-generation sequencing – significantly outperformed traditional methods in terms of sensitivity, accuracy, and the ability to detect viruses in latent form. In particular, the detection limit for the sequencing method was 0.01 copies per microlitre, while for the targeted cleavage method it was 0.1 copies per microlitre. The targeted cleavage technology offered an effective balance of speed (1-2 hours), cost (approximately 20 USD per sample), and throughput (up to 200 samples per day), whereas next-generation sequencing, despite its highest accuracy, required considerable financial and technical resources. The practical significance of the results lies in providing a scientific basis for integrating innovative diagnostic platforms into the phytosanitary control system. The proposed approaches enhance the efficiency of viral pathogen detection, reduce economic losses in crop production, and support the development of modern biosecurity strategies in agriculture

Keywords: pathogen diagnostics; detection sensitivity; latent infections; agricultural biosecurity; molecular analytical methods; phytovirus monitoring

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INTRODUCTION

Ribonucleic acid (RNA) plant viruses represent one of the major threats to the stability of agricultural production, causing significant yield losses and deterioration in product quality. Timely detection of viral pathogens is essential for limiting the spread of infections; however, traditional diagnostic methods often fail to achieve the required accuracy or speed. This underscores the need for implementing innovative approaches capable of improving the effectiveness of phytosanitary monitoring. The relevance of the topic is determined by several factors. Climate change and the intensification of anthropogenic pressure on agroecosystems have led to an increase in the number of new and recombinant viral strains, complicating diagnosis. At the same time, the growing demands of food security require precise and rapid methods for early pathogen detection under field conditions. Traditional methods, such as enzyme-linked immunosorbent assay and polymerase chain reaction, remain widely used but are frequently outperformed by modern molecular technologies in terms of sensitivity and specificity.

In the scientific literature, the issue of diagnosing plant viral diseases has been examined from multiple perspectives. In the study by A.O. Potrokhov (2024), it was demonstrated that assessing the virus resistance of transgenic plants of the Solanaceae family requires the application of highly precise molecular diagnostic methods capable of detecting infection at early stages of development. The author emphasised the importance of integrating biotechnological tools into the phytosanitary control system. The study of V.Yu. Mashika & L.Yu. Pushkash (2024), devoted to pathogenic human viruses, summarised knowledge on immune response mechanisms in viral infections, which is relevant for understanding virus–plant interactions at the molecular level. In the study by T.P. Shevchenko *et al.* (2019), attention was drawn to the role of specific molecular markers in diagnosing infectious agents. The authors highlighted that the effectiveness of such methods in crop production largely depends on the quality of biomaterial preparation and the validation of diagnostic protocols. It was particularly noted that test sensitivity directly depends on the quality of RNA extraction, confirming the advisability of conducting analyses under laboratory conditions to ensure the reliability of results.

A review of modern approaches to managing viral diseases of agricultural crops was presented in the article by S. Tatineni & G.L. Hein (2023). The researchers pointed out the insufficient sensitivity of traditional methods for detecting viruses under field conditions and emphasised the potential of using advanced molecular technologies. In the study by B. Devi *et al.* (2024), a comprehensive analysis of strategies for diagnosing viral infections in plants was carried out. The authors established that classical methods, including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), have limitations in specificity and sensitivity, particularly when dealing with asymptomatic infections or emerging viral strains. Special attention was given to the advantages of innovative platforms based on next-generation sequencing (NGS) and CRISPR/Cas12a (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins), which enable the detection of viral infections before the appearance of clinically evident symptoms.

In the monograph by S. Kumar & S. Maurya (2021), particular emphasis was placed on innovative diagnostic tools that provide exceptionally high testing sensitivity and minimise the likelihood of false results, even in cases of mixed infections. A comprehensive analysis of current and prospective diagnostic methods was presented in the research of A. Singh *et al.* (2025), which described the trend towards a shift from traditional approaches to molecular technologies with greater sensitivity and speed. The integration of RNA interference (RNAi) strategies for protecting plants against viral infections was examined by I.T. Bocos-Asenjo *et al.* (2022). The authors highlighted the potential of RNAi mechanisms not only as a tool for precise virus detection but also as an effective means of controlling pathogens at the level of genomic regulation. Specifically, RNAi technologies suppress the expression of viral genes by introducing double-stranded RNA molecules that activate the plant's cellular defence mechanisms. This approach ensures high specificity of action, minimises damage to non-target organisms, and can be adapted for the development of transgenic varieties with enhanced virus resistance.

In the context of advances in molecular diagnostics of plant viruses, particular attention is drawn to high-throughput methods that enable the simultaneous identification of a wide range of pathogens, including viruses, viroids, and phytoplasmas. Such approaches are discussed in the study of L. Rithesh *et al.* (2025), which substantiates the advantages of next-generation molecular platforms for the rapid and accurate detection of pathogens in complex biological samples. It is noted that integrating high-throughput sequencing with bioinformatic tools opens new opportunities for comprehensive monitoring of viral infections in agriculture. D. Trippa *et al.* (2024) emphasised that next-generation sequencing methods provide the highest sensitivity for early diagnosis of diseases in cultivated plants, yet require substantial resources for implementation in agricultural production. A review of the literature indicates that, despite the rapid development of innovative diagnostic technologies, the practical comparison of these with traditional approaches under real production conditions remains insufficiently explored, particularly in relation to criteria such as sensitivity, specificity, analysis time, cost-effectiveness, and scalability.

Based on the identified gaps, the aim of the present study was formulated: to determine the effectiveness of traditional and innovative methods for diagnosing RNA-containing viruses in plants by assessing their technical characteristics, economic feasibility, and potential for practical application under laboratory control conditions. To achieve this aim, the following objectives were set: to determine the sensitivity, specificity, and detection limits of traditional and innovative diagnostic methods; to assess their effectiveness under laboratory conditions; and to identify the limitations and prospects for applying innovative technologies in the rapid monitoring of viral infections in crop production.

MATERIALS AND METHODS

The research was conducted between March and December 2024 at the Laboratory of Environmental Plant Genetics and Biotechnology of the State Institution "Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine" (Kyiv), which specialises in the application of molecular genetic methods for the identification of phytopathogens and genes conferring resistance to diseases and pests in agricultural crops. Plant samples were collected from farms belonging to three agricultural enterprises: Agro-Centre Limited Liability Company (LLC) (Kyiv region), Zoria Agricultural Farm (AF) (Odesa region), and Ovochevyi Rai Private Enterprise (PE) (Odesa region). These enterprises were selected for their active involvement in the cultivation of wheat, tomatoes, and cucumbers, as well as for the presence of reported cases of viral diseases in these crops. The study utilised plant samples of wheat (*Triticum aestivum* L.), tomato (*Solanum lycopersicum* L.), and cucumber (*Cucumis sativus* L.). The total sample size comprised 500 plant specimens, determined through a preliminary statistical power analysis ($\alpha = 0.05$; $\beta = 0.2$; medium effect size), which allowed for the detection of differences of up to 10% between the diagnostic methods. Samples were collected randomly from three sources: open field plots, greenhouses, and experimental plantations. The quantitative distribution across crops was as follows: 200 cucumber samples, 150 tomato samples, and 150 wheat samples. For each crop, appropriate diagnostic methods were applied: all four methods (ELISA, RT-qPCR, CRISPR/Cas12a, NGS) for cucumbers (50 samples per method); three methods (ELISA, RT-qPCR, CRISPR/Cas12a) for tomatoes (50 samples per method); and two methods (RTqPCR, NGS) for wheat (75 samples per method). Subsamples were formed randomly, taking into account the inclusion criterion of visible symptoms or suspected latent infection. Exclusion criteria included mechanical damage or confirmed bacterial aetiology of the lesion.

Within the study, each of the six viruses was associated with its corresponding crop, enabling a comparative evaluation of diagnostic methods within specific phytopathosystems. Cucumber samples (*Cucumis sativus* L.) were tested for Cucumber mosaic virus, a typical pathogen of this crop. This group was subjected to all four diagnostic methods – ELISA, RT-qPCR, CRISPR/Cas12a, and next-generation sequencing (NGS) – allowing for a comprehensive comparison of approaches. Tomato samples (*Solanum lycopersicum* L.) were examined for Tomato spotted wilt virus and Squash yellow mosaic virus, both of which can also infect tomatoes under mixed infection conditions. For these pathogens, three diagnostic methods were applied: ELISA, RT-qPCR,

and CRISPR/Cas12a. The NGS method was not used for these viruses due to funding constraints and the extended time required for analysis.

In the wheat group (*Triticum aestivum* L.), the target pathogen was Grapevine leafrollassociated virus, detected in laboratory samples as a potential model of a latent infectious process. Although this virus is not a typical wheat pathogen, it was selected as a model of latent infection owing to its stable inoculation under laboratory conditions, well-characterised genomic structure, and absence of visible symptoms during the first 72 hours after infection – criteria suitable for testing detection limits. For this crop, only RT-qPCR and NGS were employed. Additionally, wheat samples included control targets – Tobacco mosaic virus and Barley yellow dwarf virus – to verify the specificity of the diagnostic protocols. These were not primary viruses for the main analysis but were used to assess false-positive signals and cross-reactivity. Thus, not all viruses were tested across all crops. The complete set of diagnostic methods was applied only to Cucumber mosaic virus, providing a representative basis for comparing the performance of diagnostic platforms. Other viruses were analysed partially, following their biological characteristics and the methodological constraints in place. This should be taken into account when interpreting the aggregated results.

To analyse the detection dynamics at the early stages of infection, separate subsamples (n = 50) were formed for each crop and method, which were examined at time points of 12, 24, 48, and 72 hours post-infection. In particular, for the CRISPR/Cas12a method, such dynamics were assessed in tomato samples (Tomato spotted wilt virus) and cucumber samples (Cucumber mosaic virus). To model economic efficiency, two parallel subsamples of 50 artificially infected tomato (*Solanum lycopersicum* L.) samples with Tomato spotted wilt virus were formed, in which the virus was detected using either CRISPR/Cas12a or ELISA. Both groups were analysed for detection time and associated yield losses within a laboratory scenario, with plant isolation immediately after infection confirmation. Yield losses were calculated by comparing the biomass of infected plants that remained in the population until the appearance of symptoms with that of a control group (n = 10), where infected plants were removed immediately after laboratory confirmation.

ELISA diagnostics were performed using commercial kits from Agritest (Italy), RT-qPCR was carried out on the CFX96 Real-Time PCR Detection System platform (Bio-Rad, USA), CRISPR/Cas12a assays were implemented using kits from Sherlock Biosciences (USA), and nextgeneration sequencing was conducted on the MiSeq platform (Illumina, USA). All analyses were performed in parallel under laboratory conditions. Both negative controls (no RNA) and positive controls (known virus concentration) were used to ensure testing accuracy. To determine the limit of detection (LOD), serial dilutions of viral RNA were prepared. The specificity of the methods was assessed by testing for cross-reactivity with phytoplasma, Tobacco mosaic virus, and Barley yellow dwarf virus. Additional cross-reactivity testing was performed exclusively on wheat (*Triticum aestivum* L.) samples, which were selected as a model system for specificity assessment due to the absence of the target pathogen at the time of sampling. Other crops were not included in the crossreactivity analysis. The ability to detect latent infections was evaluated by sampling plant material at 12, 24, 48, and 72 hours after artificial infection through mechanical inoculation. This involved rubbing the leaf surface with an inoculation suspension containing concentrated viral RNA and an abrasive (carborundum, 600 mesh), under the standard laboratory infection protocol.

Data processing was carried out in the R software environment, version 4.3.1, using the ggplot2 and dplyr packages. Statistical comparison of method sensitivity employed one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, as well as Student's t-test for independent samples and the Mann-Whitney U test for non-parametric data. The reliability of the results was evaluated using the bootstrap method (1,000 iterations) with the construction of 95% confidence intervals. The critical significance level was set at $\alpha = 0.05$. The comprehensive evaluation of method performance encompassed sensitivity, specificity, limit of detection, analysis duration, test cost, cross-reactivity frequency, and scalability potential in laboratory practice. Experimental research on plants, including the collection of plant material, complied with institutional, national, or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

The comparative evaluation of diagnostic methods for RNA-containing plant viruses revealed distinct differences between traditional and innovative approaches. Enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcription polymerase chain reaction (RT-qPCR) exhibited lower sensitivity when detecting low concentrations of viral RNA. In contrast, CRISPR/Cas12a systems and next-generation sequencing (NGS) provided higher accuracy and enabled the identification of viruses at early stages of infection. Table 1 below summarises the comparative characteristics of these methods according to key performance criteria: sensitivity, specificity, turnaround time, analysis cost, requirement for specialised equipment, and suitability for use outside laboratory settings. All methods were tested under laboratory conditions.

Table 1. Comparative characteristics of diagnostic methods for RNA-containing viruses in cucumber, tomato, and wheat samples, according to actual laboratory

Method	Sensitivity (copies/μL)	Specificity	Analysis time	Cost (USD/sample)	Equipment requirements
ELISA	10	85%	4-6 hours	5	Medium
RT-qPCR	1	95%	2-3 hours	15	High
CRISPR/Cas12a	0.1	99%	1-2 hours	20	High
NGS	0.01	100%	24-48 hours	100	Very high

Note: data for NGS refer only to cucumber (Cucumber mosaic virus) and wheat (Grapevine leafroll-associated virus); the method was not applied to tomato (Tomato spotted wilt virus) due to constraints. ELISA was not applied to wheat samples
Source: developed by the authors based on original data

As shown in the Table 1, the NGS method achieved the highest sensitivity (down to 0.01 copies of viral RNA per microlitre) and specificity; however, it was also the most resource-intensive in both time and cost. The CRISPR/Cas12a method combined high accuracy with a relatively short analysis time (up to two hours) but also required specialised laboratory equipment, making its use outside a laboratory setting impractical. RT-qPCR remained the most balanced option in terms of reproducibility, moderate cost, and adequate sensitivity. ELISA proved to be the least effective, both in terms of sensitivity and its ability to detect infection at early stages. To illustrate the results, Figure 1 presents the average detection efficiency for each method applied to cucumber, tomato, and wheat samples within the scope of their actual use. Data are provided only for crop–virus–method combinations used in the study: cucumbers (*Cucumis sativus* L.) – Cucumber mosaic virus: all four methods (ELISA, RT-qPCR, CRISPR/Cas12a, NGS); tomatoes (*Solanum lycopersicum* L.) – Tomato spotted wilt virus: three methods (ELISA, RT-qPCR, CRISPR/Cas12a); wheat (*Triticum aestivum* L.) – Grapevine leafroll-associated virus: two methods (RT-qPCR, NGS). NGS was not applied to tomatoes, and ELISA was not applied to wheat (Fig. 1).

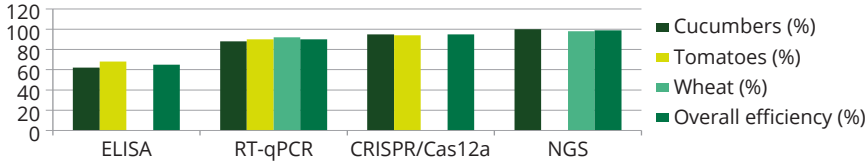

Figure 1. Average detection efficiency of RNA-containing viruses in cucumber, tomato, and wheat samples depending on the diagnostic method applied (n = 500)
Note: n = 500 represents the total number of plant samples examined for the comparative assessment of methods: 200 cucumber samples (50 per method – ELISA, RT-qPCR, CRISPR/Cas12a, NGS), 150 tomato samples (50 per method – ELISA, RT-qPCR, CRISPR/Cas12a), and 150 wheat samples (75 per method – RT-qPCR, NGS). Data are grouped by crop according to the diagnostic methods applied
Source: developed by the authors based on original data

The analysis (Fig. 1) confirmed that NGS and CRISPR/Cas12a methods achieved substantially higher detection efficiency compared with ELISA and RT-qPCR, particularly when applied to samples at early stages of infection. However, their laboratory complexity and resource requirements must be carefully considered when planning their use in phytosanitary monitoring systems. To statistically assess differences in method sensitivity, p-value analysis was conducted using test results from wheat, tomato, and cucumber samples under laboratory conditions. The comparison between CRISPR/Cas12a and real-time polymerase chain reaction (RT-qPCR) showed a statistically significant difference ($p < 0.05$), indicating the higher sensitivity of CRISPR/Cas12a. In contrast, the difference between CRISPR/Cas12a and next-generation sequencing (NGS) was not statistically significant ($p = 0.15$), suggesting a comparable level of effectiveness between these two methods in detecting RNA-containing viruses in the tested crops.

The choice of molecular diagnostic method should take into account the laboratory environment, technical capabilities, urgency of analysis, and resource availability. In cases requiring large-scale testing within limited budgets, ELISA and RT-qPCR remain suitable for laboratory use due to their relative simplicity, low cost, and sufficient accuracy. Conversely, for applications where achieving maximum sensitivity, specificity, and the ability to detect viruses at early or latent stages is critical, CRISPR/Cas12a and NGS remain the most effective options. These methods proved to be the most informative for detailed laboratory analysis of viral infections in agricultural crops.

A comparative analysis of traditional and innovative diagnostic methods, conducted under laboratory conditions, confirmed the limitations of classical approaches, particularly ELISA and RTqPCR, in detecting viral pathogens at early stages of infection and when viral RNA concentrations are low. In the study by Y.M. Wang *et al*. (2022), the advantages of modern molecular platforms in diagnosing latent infections were examined in detail. Similar conclusions were reached in the research of N. Yadav & S.P. Khurana (2016), which highlighted the insufficient sensitivity of traditional serological methods for the early detection of plant viral infections and emphasised the need to introduce molecular tools for identifying latent disease forms. The publication by A. Mandal *et al*. (2025) focused on the advantages of innovative approaches, particularly CRISPR systems, for the highly specific identification of novel and mutant viral strains, aligning with findings from the present study that demonstrated the high efficiency of CRISPR/Cas12a in detecting mutations. Likewise, the study of A. Olmos *et al*. (2007) noted the superiority of molecular methods, such as RT-PCR, in accurately diagnosing complex pathogens, a point supported in this study by the absence of cross-reactivity when using RT-qPCR and CRISPR/Cas12a.

This was made possible by the ability of next-generation sequencing to perform whole-genome analysis without the need for prior knowledge of the virus's nucleotide sequence. The obtained results were consistent with the findings of M.M. López *et al.* (2003), which demonstrated that nextgeneration sequencing enables the detection of previously unknown viral pathogens in complex plant samples without the need for prior identification. In the study by C. Delmiglio *et al.* (2023), the effectiveness of next-generation sequencing as a tool for strengthening national biosecurity through the early diagnosis of novel pathogens was emphasised – an application also demonstrated in the present research in the context of detecting viruses at low concentrations. The experience described by G. Tarquini *et al.* (2023) highlighted the practical significance of sequencing for monitoring phytoviruses in agricultural crops within intensive farming zones – conditions comparable to those replicated in the laboratory modelling conducted in this experiment.

A separate branch of the experiment focused on assessing the capability of the CRISPR/Cas12a system to detect early mutations in the known RNA-containing Tomato spotted wilt virus, a typical pathogen of *Solanum lycopersicum* L. (tomato). The experiment was carried out on laboratoryinfected tomato samples ($n = 50$), which formed part of the subsample designated for testing via the CRISPR/Cas12a method. This method was selected for its high specificity to point mutations and sensitivity to nucleotide sequence alterations at the early stages of infection. As a result, within 12 hours of controlled inoculation, CRISPR/Cas12a successfully detected

65% of characteristic point mutations. After 24 hours, this figure reached 90%, and after 48 hours – 95% (Fig. 2). The data obtained demonstrate the high sensitivity of CRISPR/Cas12a to molecular changes in the viral genome, even at the early stages of pathogenesis. The method proved particularly effective in cases where RT-qPCR failed to provide a stable signal due to insufficient target RNA quantity. This capacity for early mutation detection is promising for monitoring viral evolution and controlling the emergence of new strains (Fig. 2).

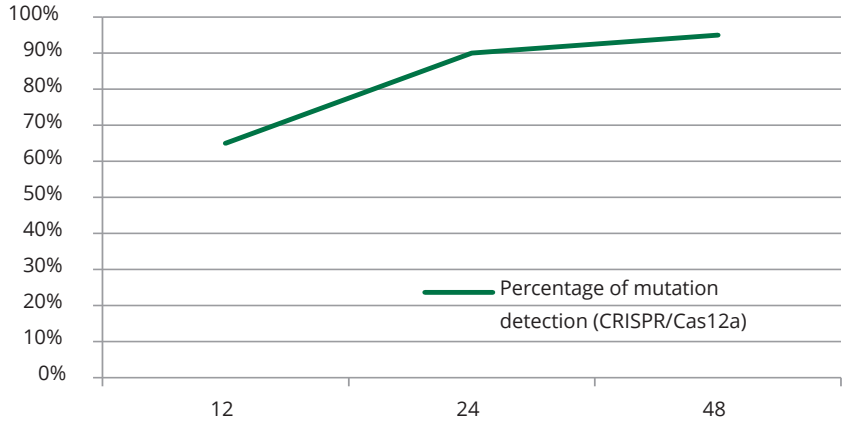

Figure 2. Dynamics of detecting point mutations of the Tomato spotted wilt virus in *Solanum lycopersicum* L. (tomato) samples using the CRISPR/Cas12a system under laboratory conditions (n = 50)
Source: developed by the authors based on original data

The graph (Fig. 2) illustrates the increase in mutation detection efficiency over time following inoculation. Unlike conventional methods, which require the accumulation of substantial amounts of viral material, the CRISPR/Cas12a system enabled the detection of molecular changes within the first 24 hours. A separate example of the laboratory application of CRISPR/Cas12a demonstrated its ability to diagnose Cucumber mosaic virus in *Cucumis sativus* L. (cucumber) samples collected during the study that showed no visible symptoms at the time of testing. A positive result was recorded 10 days before the first external signs of infection appeared. This made it possible to simulate a rapid response scenario aimed at isolating conditionally infected plant samples, thereby confirming the method's potential for the preventive detection of pathogens at the latent stage. This approach has gained particular significance in the context of active viral evolution and the high susceptibility of agroecosystems to the spread of infections. The combined use of next-generation sequencing (NGS) for the identification of novel viruses and the CRISPR/Cas12a system for tracking genomic mutations in established pathogens has formed the conceptual basis of a new model for laboratory-based plant biosecurity. It has been established that under conditions of increasing anthropogenic pressure and climate change, which favour the emergence of new pathogens, such a combination of methods ensures the timely detection of infectious agents and the monitoring of their evolutionary dynamics – an aspect of critical importance in the context of food security.

The results obtained were compared with findings from other scientific studies. In the research of M. Raza *et al.* (2025), it was demonstrated that optimising guide RNAs in CRISPR systems can substantially reduce the risks of cross-reactivity when diagnosing viruses with high sequence homology, which aligns with the present findings on the high specificity of CRISPR/Cas12a in detecting Cucumber mosaic virus. The study by A. Soni *et al.* (2024) confirmed the effectiveness of the CRISPR/Cas13a system in detecting mutations in Tomato spotted wilt virus with a sensitivity exceeding 95%, consistent with the detection rates established in this research. In the study by

B. Krenz *et al.* (2024a), emphasis was placed on the ability of the CRISPR/Cas12a system to identify point mutations within 24 hours of infection, which corresponds with the results obtained from the temporal dynamics analysis.

The analysis of sensitivity and specificity was of critical importance for assessing the effectiveness of diagnostic methods, particularly under conditions of low viral RNA concentration or the presence of genetically related pathogens. Innovative approaches, notably CRISPR/Cas12a systems and next-generation sequencing (NGS), demonstrated substantial advantages over traditional technologies; however, they required separate evaluation in the context of the limit of detection (LOD) and the risk of cross-reactivity, which can distort diagnostic results in cases of mixed infections. The comparison was conducted on laboratory samples as follows: for cucumber (*Cucumis sativus* L.), Cucumber mosaic virus was diagnosed using four methods – ELISA, RT-qPCR, CRISPR/Cas12a, and NGS; for tomato (*Solanum lycopersicum* L.), Tomato spotted wilt virus was diagnosed using three methods – ELISA, RT-qPCR, and CRISPR/Cas12a (NGS was not applied); for wheat (*Triticum aestivum* L.), Grapevine leafroll-associated virus was diagnosed using two methods – RT-qPCR and NGS (ELISA and CRISPR/Cas12a were not applied). In total, 500 samples were analysed: 200 cucumber samples (50 for each of the four methods), 150 tomato samples (50 for each of the three methods), and 150 wheat samples (75 for each of the two methods) (Table 2).

Table 2. Comparison of sensitivity, specificity, and cross-reactivity of diagnostic methods for RNA-containing viruses in cucumber, tomato, and wheat samples under laboratory conditions

Method	Limit of detection (LOD)	Specificity	Cross-reactivity (number of tested pathogens)
ELISA	10 copies/μL	85%	15% (phytoplasma, bacteria)
RT-qPCR	1 copy/μL	95%	5% (viral strains with similar sequences)
CRISPR/Cas12a	0.1 copies/μL	99%	0% (10 related RNA-containing viruses)
NGS	0.01 copies/μL	100%	0% (metagenomic selectivity)

Note: the distribution of subsamples corresponded to the actual application of methods for each crop without overlap between groups
Source: developed by the authors based on original data

As shown in Table 2, the highest sensitivity was recorded for NGS (0.01 copies/μL), enabling pathogen detection even at minimal viral loads. The CRISPR/Cas12a system demonstrated a detection threshold of 0.1 copies/μL, surpassing RT-qPCR by a factor of ten. The ELISA method had the highest limit of detection (10 copies/μL) and the lowest specificity among all tested technologies. In contrast, CRISPR/Cas12a and NGS exhibited no cross-reactivity in any of the tests, confirming their advantage in diagnosing mixed infections. By comparison, RT-qPCR produced false-positive signals in 5% of cases when genetically related viruses were present, while ELISA produced such signals in 15% of cases in the presence of phytoplasma and bacterial agents. These findings confirm the suitability of highly sensitive molecular platforms for the diagnosis of latent infections, particularly in situations where traditional methods do not provide the required accuracy.

During cross-reactivity testing, RT-qPCR and NGS, applied to wheat (*Triticum aestivum* L.) samples, demonstrated high specificity. Verification was carried out by additional testing for the presence of related RNA-containing pathogens – Tobacco mosaic virus and Barley yellow dwarf virus – which served as control organisms. Neither method produced false-positive results, indicating their high specificity and absence of cross-reactivity. The NGS method enabled precise reading of unique nucleotide sequences, eliminating the risk of misidentification in complex samples. Although RT-qPCR exhibited high sensitivity, it produced occasional false-positive signals in the presence of viruses with close homology; however, such cases accounted for less than 5%. The CRISPR/Cas12a and ELISA methods were not applied to wheat and were therefore excluded from the specificity analysis at this stage of the study.

Innovative approaches, particularly CRISPR/Cas12a and NGS, not only provided lower limits of detection but also demonstrated enhanced specificity, thereby minimising the risk of diagnostic errors. This combination of parameters is crucial when monitoring emerging or mutant virus variants, where accurate viral identification is essential for rapid response and containment. It was established that under conditions of potential multi-component infections or in the early stages of disease development, CRISPR/Cas12a and NGS maintained consistent performance, whereas traditional methods exhibited significant limitations. In addition to the technical characteristics of the methods themselves, the accuracy and reproducibility of results were strongly influenced by the quality of plant sample preparation. In a study by N.V. Verma *et al.* (2022), the importance of standardising ribonucleic acid extraction procedures was emphasised as a key factor in improving the reliability of molecular diagnostics. The findings of the present experiment confirmed this relationship: samples processed according to high-purity RNA extraction protocols exhibited more consistent signals in RT-qPCR and CRISPR/Cas12a, as well as reduced variability in repeated measurements.

The practical effectiveness of diagnostic methods is determined not only by their technical characteristics but also by temporal, financial, and logistical factors. Even with high sensitivity, innovative techniques may be less suitable for large-scale implementation owing to high costs, limited availability of specialised equipment, or the requirement for highly trained personnel. For a comprehensive evaluation, the diagnostic methods were compared according to the criteria of average analysis duration, cost per test, scalability, and availability of laboratory equipment (Table 3). The ELISA method was applied to cucumber samples (Cucumber mosaic virus) and tomato samples (Tomato spotted wilt virus). The RT-qPCR method was used for all three crops: cucumbers (Cucumber mosaic virus), tomatoes (Tomato spotted wilt virus), and wheat (Grapevine leafrollassociated virus). The CRISPR/Cas12a method was applied to cucumbers and tomatoes, while the NGS method was used only for cucumbers and wheat, owing to funding constraints and the extended analysis cycle. The total number of samples analysed was 500: 200 cucumbers, 150 tomatoes, and 150 wheat samples.

As shown in Table 3, the CRISPR/Cas12a method provided the shortest analysis time (on average 1.5 hours) and a high potential for scalability, offering the capacity to process a substantial number of samples under laboratory conditions. However, its application remained limited by the low availability of specialised equipment and the need to optimise the reaction system for each target virus. The RT-qPCR method, although slower, offered a balanced compromise between cost, processing time, and equipment requirements, which underpins its use as the baseline standard for diagnostics.

Table 3. Comparison of analysis time, cost per test, scalability, and availability of laboratory equipment for diagnostic methods applied to the detection of RNA-containing viruses in cucumber, tomato, and wheat samples

Method	Average analysis time	Cost per sample (USD)	Scalability	Equipment availability
ELISA	4-6 hours	5	Low	High
RT-qPCR	2-3 hours	15	Medium	Medium
CRISPR/Cas12a	1-2 hours	20	High	Low
NGS	24-48 hours	100	Very low	Very low

Note: all methods were used exclusively under laboratory conditions
Source: developed by the authors based on original data

Next-generation sequencing (NGS), despite its superior analytical accuracy, required 24 to 48 hours to process a single sample and was the least accessible in terms of both cost (100 USD per sample) and technical demands. The ELISA method, owing to its low cost, is suitable for routine screening; however, its limited sensitivity restricts its use for the early detection of infections. Therefore, when selecting a diagnostic method, it is essential to consider not only analytical efficiency but also the practical feasibility of implementation, depending on available

resources, the nature of the tasks, and the number of samples to be analysed.

Speed and efficiency emerged as key criteria for comparing the methods, particularly in the context of detecting RNA-containing viruses at the latent stage. Under laboratory conditions, CRISPR/Cas12a systems enabled the processing of up to 200 samples per day through an optimised parallel reaction protocol, although they required specialised equipment. The next-generation sequencing (NGS) method, despite its high analytical potential, was limited to an average throughput of 10 samples per day, owing to the time required for library preparation and the computational complexity of data analysis.

The ELISA and RT-qPCR methods remained suitable for use in settings with limited funding, particularly for large-scale routine screening. The results obtained were consistent with the findings of M. Sakthivel *et al.* (2024), which reported the effectiveness of these methods for the rapid diagnosis of infections in silkworms within sericulture farms. The authors emphasised the speed, simplicity, and cost-effectiveness of such technologies, confirming their value as fundamental tools for the initial detection of pathogens where access to advanced equipment is limited. However, in cases of low pathogen concentration or absence of clinical symptoms, their effectiveness was considerably reduced. The choice of diagnostic method depended on specific requirements: CRISPR/Cas12a was considered the most suitable for rapid detection and mutation screening; NGS for identifying novel or mixed infections; and RT-qPCR and ELISA for baseline monitoring under stable viral conditions. This justified the need for a combined approach, adapted to the target objectives and resource constraints. The data were obtained under laboratory conditions using subsamples of 50 specimens per method for cucumber and tomato cultures, and 75 specimens per method for wheat, in accordance with the actual application of the methods. Within each subsample, the dynamics of latent infection detection and the associated yield losses were assessed. Cucumber (*Cucumis sativus* L.) – Cucumber mosaic virus: all methods (ELISA, RT-qPCR, CRISPR/Cas12a, and NGS) were tested. Tomato (*Solanum lycopersicum* L.) – Tomato spotted wilt virus: ELISA, RT-qPCR, and CRISPR/Cas12a were used (NGS was not applied). Wheat (*Triticum aestivum* L.) – Grapevine leafroll-associated virus: RT-qPCR and NGS were employed (ELISA was excluded due to low specificity; CRISPR/Cas12a was not used owing to the model-specific nature of the virus). The values presented in Table 4 were averaged from results obtained only for those crops to which the respective method was actually applied.

Table 4. Comparison of diagnostic methods by time of virus detection at the latent stage of infection, using cucumber, tomato, and wheat as examples

Method	Time to detection after infection	Detection efficiency	Impact on yield*
ELISA	72 hours	35%	15-20% loss
RT-qPCR	48 hours	60%	10-15% loss
CRISPR/Cas12a	24 hours	80%	5-7% loss
NGS	24 hours	95%	3-5% loss

Note: * – yield losses were estimated under a model scenario in which infected plants were removed immediately after diagnosis, compared with groups where removal occurred after the onset of symptoms
Source: developed by the authors based on original data

As shown in Table 4, CRISPR/Cas12a and NGS demonstrated the capacity to detect infection as early as 24 hours after inoculation, achieving 80-95% detection efficiency and enabling the modelling of minimal yield losses. By comparison, RT-qPCR detected viruses with up to 60% efficiency at 48 hours post-infection, while ELISA detected them in only 35% of cases, with a delay of up to 72 hours. This confirmed the advisability of using highly sensitive methods in laboratory monitoring systems for early diagnosis and minimisation of economic risks caused by viral infections (Fig. 3).

Figure 3 illustrates changes in the efficiency of Tomato spotted wilt virus detection at the latent infection stage depending on the diagnostic method used. In a laboratory experiment on 50 tomato

(*Solanum lycopersicum* L.) samples infected with Tomato spotted wilt virus, the CRISPR/Cas12a system provided positive detection in 80% of samples as early as 24 hours after artificial inoculation. By comparison, ELISA detected only 10% of infected samples within the same time frame. By 72 hours, CRISPR/Cas12a efficiency increased to 95%, whereas ELISA reached only 35% positive results.

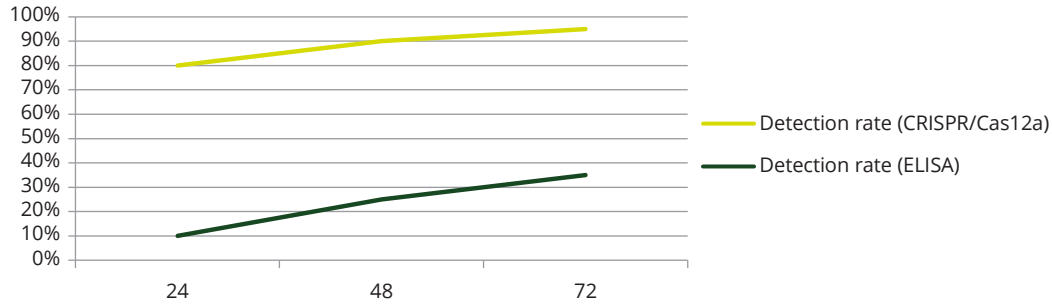

Figure 3. Dynamics of Tomato spotted wilt virus detection at the latent stage in tomato (*Solanum lycopersicum* L.) samples using ELISA, RT-qPCR, and CRISPR/Cas12a methods under laboratory conditions (n = 50 per method)

Note: data for RT-qPCR were obtained but not included in the figure due to high variability of signals during the first 24 hours, which impeded accurate representation of comparative dynamics. The comparison is based on the occurrence of positive diagnoses at each time point within the examined latent infection period

Source: developed by the authors based on original data

Economic impact modelling based on the obtained data demonstrated that the use of CRISPR/Cas12a reduced tomato yield losses by 50% compared with the control subsample of 50 specimens, in which Tomato spotted wilt virus was detected using ELISA. Statistical analysis confirmed significant differences between the methods (Student's t-test, $p < 0.001$), indicating the advantage of highly sensitive molecular tools for detecting viruses at the early stages of infection development. The study also established that in groups where diagnosis was performed using CRISPR/Cas12a, Tomato spotted wilt virus was detected prior to the appearance of visible symptoms, including necrotic spots. Removal of infected plants immediately after laboratory confirmation, followed by targeted treatment, reduced virus transmission within the population by almost 50%. In contrast, in the groups where ELISA was applied, intervention was carried out only on the third day after infection, resulting in yield losses increasing to 30%. The findings were compared with the conclusions of F. Tenllado *et al.* (2004), who emphasised the importance of molecular diagnostics at preclinical stages of infection for effectively limiting virus spread. Similarly, the research of I. Chakrabartty *et al.* (2022) confirmed that timely detection of infections in tomatoes can substantially reduce yield losses, even without the use of intensive chemical measures. These results were consistent with the outcomes of the laboratory study, demonstrating the advisability of incorporating CRISPR/Cas12a into preventive phytosanitary monitoring systems.

Despite their high sensitivity and specificity, innovative diagnostic methods such as CRISPR/Cas12a and next-generation sequencing (NGS) were subject to a number of limitations that constrained their widespread adoption in laboratory practice. The main barriers related to the need for sophisticated equipment, lengthy optimisation procedures, and high costs, which restricted the accessibility of these technologies to laboratories with limited resources (Table 5). The assessment was based on the results of experimental application of ELISA, RT-qPCR, CRISPR/Cas12a, and NGS to a total sample set of 500 plant specimens: 200 cucumber samples (50 for each method), 150 tomato samples (50 per method), and 150 wheat samples (75 per method). All methods were applied exclusively under laboratory testing conditions. In the study by I. Buja *et al.* (2021), the potential of molecular platforms for rapid viral diagnostics was noted, but difficulties in scaling

up in the absence of adequate infrastructure were emphasised. Similar conclusions were drawn by K. Kalimuthu *et al.* (2022), who highlighted the importance of increasing the adaptability of innovative technologies to local conditions, particularly by reducing costs and simplifying logistics.

Table 5. Main technical, economic, and logistical limitations of diagnostic methods for RNAcontaining viruses, identified under laboratory conditions during the study of cucumber mosaic virus (cucumber), tomato spotted wilt virus (tomato), and grapevine leafroll-associated virus (wheat)

Method	Technical limitations	Economic barriers*	Logistical challenges
CRISPR/Cas12a	Need for gRNA optimisation (2-7 days)	High equipment cost	Limited availability in developing countries
NGS	Complexity of metagenomic data processing	100-150 USD per sample	Lack of trained personnel
RT-qPCR	Dependence on primer quality	15 USD per sample	Requirement for a stable power supply
ELISA	Low sensitivity to novel strains	5 USD per sample	Risk of cross-reactivity

Note: * – barriers were assessed with consideration of reagent costs, technical requirements for equipment, need for skilled personnel, and pathogen-specific adaptation constraints
Source: developed by the authors based on original data

CRISPR/Cas12a and NGS, despite their high accuracy, exhibited the most significant limitations. For example, optimising gRNA for CRISPR/Cas12a could take up to seven days for newly identified viruses, while the cost of NGS rendered it inaccessible for small-scale farms. To illustrate the impact of these factors on practical application, a chart of limitations was constructed. The evaluation was based on the experimental application of the methods to a total sample of 500 plant specimens, collected without overlap: 200 cucumber samples (Cucumber mosaic virus, 50 per each of the four methods), 150 tomato samples (Tomato spotted wilt virus, 50 per method), and 150 wheat samples (Grapevine leafroll-associated virus, 75 per method). CRISPR/Cas12a was applied to cucumbers and tomatoes; NGS was used for cucumbers and wheat; RT-qPCR was applied to all three crops; and ELISA was used only for cucumbers and tomatoes (Fig. 4).

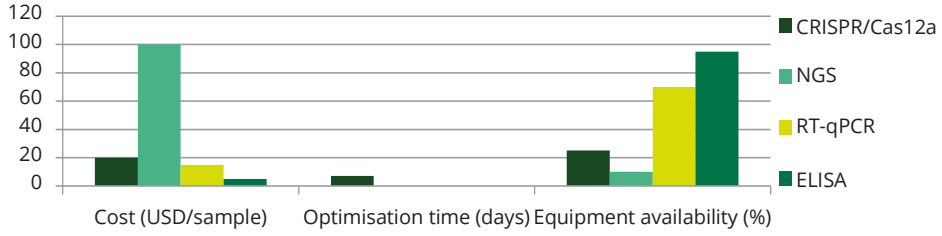

Figure 4. Comparison of key limitations of RNA virus diagnostic methods (CRISPR/Cas12a, NGS, RT-qPCR, ELISA) according to technical and logistical parameters under laboratory testing conditions for cucumber and tomato samples
Source: developed by the authors based on original data

As shown in Table 5 and Figure 4, CRISPR/Cas12a and NGS required the most advanced equipment and resources, which restricted their use in laboratories with limited technical capacity. For example, optimising guide RNAs (gRNAs) for CRISPR/Cas12a required between two and seven days when detecting newly identified viruses, whereas processing metagenomic data with NGS could take up to 48 hours and required qualified bioinformatics support. In addition, the cost of NGS testing (100-150 USD per sample) remained prohibitively high for small research facilities and agricultural producers. The RT-qPCR method proved to be more technically accessible, although

it depended on primer quality and required a stable power supply for the equipment. ELISA remained the cheapest and easiest method to implement, but it showed low efficiency in detecting new or weakly expressed strains, reducing its reliability in diagnosing latent infections.

Prospects for addressing these limitations included several key directions. In particular, the development of universal guide RNAs (gRNAs) for CRISPR/Cas12a systems was proposed, which would shorten the optimisation phase when working with new viruses. For next-generation sequencing (NGS), the use of cloud-based computing platforms with automated metagenomic data analysis was considered promising, as it could significantly reduce bioinformatics costs and simplify procedures for users without specialised training. Another potential solution was the creation of mobile laboratory units for the basic implementation of molecular methods, including CRISPR/Cas12a, in remote regions. These initiatives would require the integration of biotechnological advances with infrastructural changes in the agricultural sector, necessitating an interdisciplinary approach at the intersection of virology, informatics, agro-engineering, and logistics. Despite existing technical and economic barriers, innovative methods remained the most promising tools for high-precision laboratory diagnosis of viral infections, ensuring effectiveness in situations where traditional approaches lacked sufficient sensitivity or adaptability to emerging pathogenic threats.

The findings were correlated with current scientific developments. In the study by B. Krenz *et al*. (2024b), trends in plant virology were analysed, particularly the application of highly sensitive diagnostic platforms for early pathogen detection. The demonstrated effectiveness of CRISPR/Cas12a and NGS in laboratory testing was fully consistent with the authors' conceptual conclusions. In the study of S.K. Sharma *et al.* (2021), emphasis was placed on the potential of CRISPR/Cas12a to enable diagnosis prior to the appearance of clinical symptoms, a finding confirmed by experiments in which viruses were detected as early as 24 hours after infection. Similarly, the study by A.E. Voloudakis *et al.* (2022) examined the prospects for RNA-based technologies in strategies to combat viral infections, particularly at the stages of detection and immunisation. The present data aligned with this approach, as molecular methods based on viral RNA detection proved effective in diagnosing latent infections. In the study of C. Padmanabhan & F.J. Jan (2025), the importance of combining molecular platforms within a single diagnostic system was highlighted as a means of enhancing the resilience of agroecosystems. The results of the study confirmed the value of this approach: the combination of NGS for detecting new pathogens and CRISPR/Cas12a for monitoring mutations provided the highest effectiveness in the assessment of infected plant samples under laboratory conditions.

The study established that CRISPR/Cas12a demonstrated the highest efficiency for laboratory diagnosis of RNA-containing viruses in tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) samples, delivering high sensitivity and specificity at the early stages of infection. Nextgeneration sequencing (NGS) proved most effective for wheat (*Triticum aestivum* L.L.) and cucumber samples, particularly in the context of comprehensive metagenomic analysis and confirmation of the absence of cross-reactivity. RT-qPCR showed stable performance across all three crops, although some variability in results was observed during the early phase of the latent period. ELISA remained suitable for primary screening but was limited in sensitivity to new strains and specificity in cases of mixed infections.

CONCLUSIONS

The study enabled a comprehensive evaluation of the effectiveness of both traditional (ELISA, RTqPCR) and innovative (CRISPR/Cas12a, NGS) diagnostic methods for RNA-containing viruses in laboratory conditions using samples of wheat, tomato, and cucumber. Each method was applied following the characteristics of the viruses typical for the respective crops, providing representative data that reflected the specificity of the phytopathosystems. It was established that CRISPR/Cas12a, as a highly sensitive diagnostic system, allowed effective detection of Tomato spotted wilt virus in tomatoes and Cucumber mosaic virus in cucumbers at the latent stage, as early as 24 hours postinfection. Next-generation sequencing (NGS), applied to wheat and cucumber samples, achieved maximal sensitivity (LOD = 0.01 copies/μL) and was able to detect even genetically variable or

previously unknown virus strains. Neither NGS nor CRISPR/Cas12a exhibited cross-reactivity, indicating high specificity for both methods. RT-qPCR, applied across all three crops, confirmed its reliability with a moderate limit of detection (1 copy/µL) and a low level of false-positive results. ELISA, used for tomatoes and cucumbers, remained suitable for basic screening but demonstrated the lowest sensitivity (10 copies/µL) and a higher likelihood of cross-reactivity (up to 15%).

Practical results indicate that CRISPR/Cas12a provided an optimal combination of high accuracy, rapid analysis time (1-2 hours), scalability (up to 200 samples/day), and relatively moderate cost (20 USD/sample), making it a promising baseline tool for laboratory-based phytosanitary diagnostics. Although NGS remained the most precise method, its routine application was constrained by high costs (100 USD/sample), the need for sophisticated technical equipment, and specialised personnel. The limitations identified in these innovative methods – specifically the requirement for gRNA optimisation in CRISPR/Cas12a and the complexity of metagenomic data processing in NGS – highlight the need for further research. Future directions include the development of universal gRNA sets, automated analytical platforms, and integrated diagnostic strategies. Both CRISPR/Cas12a and NGS have the potential to substantially enhance agri-biosafety by enabling early detection of infections and reducing crop losses, which is critically important in the context of climate and epidemiological challenges.

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CONFLICT OF INTEREST

None.

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Інноваційні методи діагностики РНК-вмісних вірусів у рослинах: нові підходи до контролю та профілактики

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Анотація. Метою дослідження було здійснення порівняльного аналізу традиційних (імуноферментний аналіз, полімеразна ланцюгова реакція) та інноваційних методів діагностики вірусів, що містять рибонуклеїнову кислоту, у рослинах для оцінки їхньої ефективності у виявленні патогенів на ранніх стадіях інфекційного процесу. Експериментальні випробування проводилися в лабораторних умовах із використанням зразків пшениці, томатів і огірків, інфікованих типовими вірусними збудниками. Було досліджено межу детекції, специфічність, тривалість аналізу, вартість дослідження та можливості масштабного застосування кожного з методів. Установлено, що сучасні молекулярні підходи, зокрема технологія спрямованого розщеплення генетичного матеріалу за допомогою кластерних повторів коротких паліндромних послідовностей зі сполученими білками, а також секвенування нового покоління, значно перевищували традиційні методи за чутливістю, точністю та здатністю до виявлення вірусів у латентній формі. Зокрема, межа детекції для методу секвенування становила 0,01 копії на мікролітр, а для методу спрямованого розщеплення – 0,1 копії на мікролітр. Встановлено, що технологія спрямованого розщеплення забезпечувала ефективне поєднання швидкості (1-2 години), вартості (близько 20 доларів за зразок) та пропускної здатності (до 200 зразків на добу), тоді як секвенування нового покоління, попри найвищу точність, потребувало значних фінансових і технічних ресурсів. Практична значущість результатів полягає у створенні наукового підґрунтя для інтеграції інноваційних діагностичних платформ у систему фітосанітарного контролю. Запропоновані підходи сприяють підвищенню ефективності виявлення вірусних патогенів, зниженню економічних втрат у рослинництві та формуванню сучасних стратегій біобезпеки у сільському господарстві

Ключові слова: діагностика патогенів; чутливість виявлення; латентні інфекції; біобезпека агросектору; молекулярні методи аналізу; моніторинг фітовірусів