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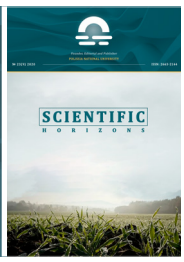

Biotechnological aspects of the molecular diagnosis of cereal crop viruses

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Abstract. The annual decline in cereal crop yields across many regions of the world due to pathogens such as Barley Yellow Dwarf Virus (BYDV), Wheat Streak Mosaic Virus (WSMV), and Soil-borne Cereal Mosaic Virus (SBCMV) underscores the importance of studying their spread and control. The aim of this study was to identify patterns in the distribution of viral infections affecting cereal crops in Ukraine and Azerbaijan. The research utilised reverse transcription followed by polymerase chain reaction (RT-PCR) and loop-mediated isothermal amplification (LAMP) to detect viruses in cereal crop samples. Results revealed a high infection rate in both countries (51.1% in Ukraine and approximately 51% in Azerbaijan), with BYDV being the dominant virus. Regional differences were identified: in Ukraine's forest-steppe zone, BYDV was most prevalent (41.5%), while in the steppe zone, WSMV was more frequently detected (25.9%). In Azerbaijan, BYDV prevalence was lower (⩽ 18.7%), but the frequency of

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SBCMV was higher (up to 20.4%). Co-infections were found in 26.1% of infected samples in Ukraine and 18.9% in Azerbaijan, with 23.7% of asymptomatic plants in Ukraine and 18.5% in Azerbaijan testing positive for viral material. The LAMP method proved effective for rapid field diagnostics, demonstrating 100% specificity and over 82% sensitivity. The findings highlighted the need for regionally adapted strategies to control viral diseases in cereal crops. The practical value of this work lies in the development of recommendations for improving plant monitoring and protection systems, taking local conditions into account, thereby contributing to increased yields and food security in the region

Keywords: polymerase chain reaction; reverse transcription; loop-mediated isothermal amplification; soil-borne cereal mosaic virus; barley yellow dwarf virus; wheat streak mosaic virus; arable land; agriculture

INTRODUCTION

Agriculture faces numerous challenges related to the spread of viral infections in cereal crops, leading to economic losses and posing risks to food security. Viral pathogens such as Barley Yellow Dwarf Virus (BYDV-PAV), Wheat Streak Mosaic Virus (WSMV), and Soil-borne Cereal Mosaic Virus (SBCMV) present a serious threat due to their high contagiousness, ability to quickly adapt to diverse agroecological conditions, and potential to develop new, aggressive strains. Therefore, developing effective methods for the diagnosis, monitoring, and control of viral infections is crucial for preventing epidemics and ensuring stable crop yields. Importantly, cereal production occupies a significant share of global arable land, making viral epidemics a serious concern not only for local but also for international agriculture (Andreychenko *et al.*, 2024).

Recent research highlights the importance of molecular diagnostic techniques such as reverse transcription followed by polymerase chain reaction (RT-PCR) and loop-mediated isothermal amplification (LAMP) for the accurate detection of viral pathogens in plants at early stages of infection. For instance, the work by V.C. Chalam *et al.* (2020) demonstrates the advantages of modern biotechnological approaches in diagnostics, including high sensitivity, specificity, and the capacity to detect latent infections. Simultaneously, the study by O.A. Kolade *et al.* (2022) emphasises the need to integrate molecular methods into virus-free plant material exchange systems and phytosanitary control schemes. However, despite progress in this field, key issues remain unresolved concerning regional patterns of virus distribution, interactions with local agroecosystems, and the variable efficacy of diagnostic methods under different climatic conditions.

In the context of Ukraine and Azerbaijan, the problem is particularly relevant, as both countries are major cereal producers in their respective regions, though they differ in agroclimatic conditions, crop rotation structures, and farming systems. The research by H. Snihur *et al.* (2025) points to the role of seed transmission in the spread of cereal crop diseases in Ukraine, where up to 30% of seed material may be infected. Meanwhile, N. Sultanova *et al.* (2024) describe the characteristics of viral infections in Azerbaijan's agricultural crops, which are influenced by the arid climate and specific local agrotechnologies. This highlights the need for similarly comprehensive studies on cereal crops. This highlights the need for similarly comprehensive studies on cereal crops, especially considering that both countries dedicate vast areas of arable land to cereal production, making the sustainability of agriculture directly dependent on effective viral disease management.

Biotechnological tools such as RNA interference and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based genome editing are being explored as promising strategies to enhance cereal crop resistance to viruses (Khassanova *et al.*, 2024). However, their practical application in Ukraine and Azerbaijan requires further investigation, considering regional virus circulation patterns, the presence of local strains, and agroecosystem specifics. Another notable issue is co-infection, where plants are simultaneously affected by multiple viruses, potentially resulting in synergistic effects and increased pathogenicity (Solomiichuk & Pikovskyi, 2025). Existing studies, such as that by M. Bhanjadeo *et al.* (2023), suggest innovative nanotechnology-based approaches to virus diagnostics, including biosensors and quantum dots. Nonetheless, their practical implementation in Ukraine and Azerbaijan remains under-researched, especially regarding economic feasibility and adaptability to local laboratory conditions. Similarly, the research by L. Aguilar-Marcelino *et al.* (2020) highlights the potential of beneficial microorganisms in viral infection biocontrol, though their effectiveness in local agroecosystems requires further study, particularly under climate change conditions.

A vital aspect is the comparative evaluation of diagnostic method effectiveness in real field conditions. As K. Dhiman *et al.* (2024) note, traditional serological methods often lag behind molecular techniques in sensitivity, especially for detecting new virus strains. At the same time, high-tech methods such as metagenomic sequencing remain inaccessible for routine use in most agricultural laboratories in Ukraine and Azerbaijan. As shown by J. Aghayev and E. Samadova (2022), the high infection pressure within agrocenoses necessitates systematic monitoring, timely diagnosis, and comprehensive phytosanitary protection that accounts for

pathogen specificity and climatic factors. Current diagnostic approaches, according to M. Raza *et al*. (2024), involve integrating molecular methods with digital monitoring platforms, enhancing pathogen detection accuracy and enabling rapid responses. Additionally, as noted by O.V. Dubrovna *et al*. (2023), RNA interference technologies offer promising strategies for developing virus-resistant crop varieties, opening new directions for plant breeding.

The identified knowledge gaps stem from the fact that current research lacks comparative analysis of agroclimatic determinants of virus spread in the contrasting regions of Ukraine and Azerbaijan, insufficiently addresses the dynamics of co-infections, and provides limited data on the efficacy of available molecular diagnostic tools under local conditions. The aim of this study was to identify regional characteristics of the spread of viral infections in cereal crops within the contrasting agroecosystems of Ukraine and Azerbaijan. The objectives included: comparing the structure of viral infections in cereal crops across different climatic zones in both countries; assessing the effectiveness of reverse transcription followed by PCR and LAMP methods for diagnostics; analysing the impact of agroclimatic factors on infection dynamics; investigating the frequency of co-infections and asymptomatic pathogen carriage.

MATERIALS AND METHODS

Sample collection and material preparation. The study was conducted in 2024 at the laboratory of the Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine, Kyiv. In Azerbaijan, the research was carried out at the Research Institute of Plant Protection and Technical Crops under the Ministry of Agriculture of the Republic of Azerbaijan. Plant samples (leaves and basal stems) were collected during the vegetation period (May-June 2024) from fields of winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) across six agricultural enterprises located in two main agro-climatic zones. In Ukraine, samples were collected from three enterprises in the forest-steppe zone: Vinnytsia region (Agricultural Enterprise "Agro-Vinnytsia"), Cherkasy region (Scientific and Production Centre "Reproductive Grain"), and Sumy region (Farm "SULA-AGRO"); and from three enterprises in the steppe zone: Dnipropetrovsk region (Dnipro Agro Group), Kherson region (LLC "Tavria-Agro"), and Zaporizhzhia region (LLC "Druzhba 5"). In Azerbaijan, sampling was also carried out in two agro-climatic zones: Steppe zone: Beylagan district (LLC "Garabag Tahil"), Ganja district (LLC "Akrolink-Ganja"), and Shirvan district (several grain-focused enterprises, though specific legal names were not confirmed in public sources); Forest-steppe zone: Jalilabad district (LLC "Gyunashli Agro"), Lankaran district (LLC "Chinar"), and Astara district (LLC "Astarachay"). Inclusion criteria: affiliation with target crops (wheat, barley, rye); presence of typical viral symptoms (yellowing, mosaic patterns, dwarfing, necrotic spots) or complete absence of visible symptoms (asymptomatic plants); sampling from various field areas to avoid bias from localised infection hotspots. Exclusion criteria: samples with mechanical damage, fungal or bacterial infection; technical artefacts during analysis (e.g. missing internal amplification control, protocol errors); samples with conflicting detection results between RT-PCR and LAMP, without possibility of verification.

From each field in Ukraine, 15 samples were collected (5 symptomatic, 5 asymptomatic, and 5 random samples selected blindly, without visual pre-assessment), totalling 270 samples (6 enterprises × 3 crops × 15 samples). In Azerbaijan, the same sampling structure was applied, with 135 samples collected in each zone, also totalling 270. Thus, the overall sample size across both countries was 540. Samples were collected by walking diagonally across fields at random. Collected material was immediately frozen in liquid nitrogen and transported to the laboratory on dry ice for analysis. Samples were stored in a -80°C freezer (Sanyo, Japan). Virus-free wheat plants grown in vitro at the institute's cell collection served as negative controls. All procedures for sampling, transportation, and storage were conducted in accordance with the Convention on Biological Diversity (1992).

Total RNA extraction. RNA was extracted from approximately 100 mg of homogenised frozen leaf/stem tissue. The commercial kit "RNeasy Plant Mini Kit" (Qiagen, Germany) was used according to the manufacturer's protocol, which includes lysis with RLT buffer containing β-mercaptoethanol, column precipitation, washing, and RNA elution. Concentration and purity of the isolated RNA were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Acceptable RNA quality was defined as A260/A280 ratio of 1.8-2.1 and A260/A230 > 2. Samples showing signs of degradation (as evaluated by 1% agarose gel electrophoresis) were excluded from further analysis. Molecular detection of viruses. Reverse Transcription (RT) and Polymerase Chain Reaction (RT-PCR). Reverse transcription was performed on 500 ng of total RNA using the RevertAid Premium Reverse Transcriptase kit (Thermo Fisher Scientific, USA) with random hexamer primers following the manufacturer's protocol (25°C – 5 min, 42°C – 60 min, 70°C – 10 min). The resulting cDNA was used as the template for PCR. PCR was performed using specific primers for the target viruses:

1. BYDV-PAV: Forward: 5′-ACCTAGACGCGCAAATCAAA-3′; expected product: 590 bp (Malmstrom and Shu, 2004).

2. WSMV: Forward: 5′-GATCAAATACCAACCGCGTG-3′; expected product: 493 bp (Jones *et al.*, 2005).

3. SBCMV: F: 5′-GGTAGTCAGCTGTTAGCGTGT-3′; expected product: 773 bp (Marra *et al.*, 2022).

Each reaction mix (25 µL) included: 2.5 µL of 10 × Taq polymerase buffer, 1.5 µL of 25 mM $MgCl_2$, 0.5 µL of 10 mM dNTPs, 0.5 µL of each primer (10 µM), 0.25 µL

(1.25 U) of Taq DNA polymerase (Thermo Fisher Scientific), 2 µL of cDNA template, and nuclease-free water. Amplification conditions were: initial denaturation at 94°C – 3 min; 35 cycles of denaturation at 94°C – 30 sec, annealing at 58°C (BYDV, SBCMV) / 60°C (WSMV) – 30 sec, extension at 72°C – 45 sec; final extension at 72°C – 5 min. Amplification was performed using a T100 Thermal Cycler (Bio-Rad, USA). PCR products were analysed by 1.5% agarose gel electrophoresis (Agarose I, Amresco, USA) with ethidium bromide (0.5 µg/mL) in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8) at 100 V for 45 min. Visualisation was carried out using a Gel Doc XR + system (Bio-Rad, USA) under UV light. A sample was considered positive if a DNA fragment of the expected size was present. Each run included a negative control (water instead of template) and a positive control (RNA from previously confirmed infected plants).

Coinfections were identified based on RT-PCR data: a sample was considered coinfected if two or all three target viruses were detected simultaneously. The coinfection rate was calculated as the percentage of infected samples carrying multiple viruses. Additionally, the distribution of infections between symptomatic and asymptomatic plants was assessed. Samples were grouped by symptom presence, and infection rates were compared using the χ^2 test. LAMP. Virus detection was also performed using the LAMP-based "Plant Virus Detection Kit" (OptiGene, UK), with primers specific to the same target viruses (BYDV-PAV, WSMV, SBCMV), according to the manufacturer's instructions. The specific LAMP primer sequences are proprietary and not disclosed. The reaction mixture (25 µL) contained: 12.5 µL of ready-to-use lyophilised reaction mix (including Bst DNA polymerase, dNTPs, buffer, and fluorescent intercalating dye), 1 µL each of outer primers (F3, B3; 10 µM), 1 µL each of inner primers (FIP, BIP; 40 µM), 2 µL of extracted RNA, and nuclease-free water. Amplification was conducted on a Genie III device (OptiGene, UK) at 65°C for 30 minutes (amplification), followed by 98°C for 5 minutes (enzyme inactivation). Results were detected in real time via fluorescence and analysed using the amplification curves generated by the instrument. The time to positivity (Tp) was recorded. A sample was considered positive if a characteristic sigmoidal amplification curve appeared with Tp < 30 minutes. Negative and positive controls were included in each run. Data were processed using IBM SPSS Statistics v.29. The virus prevalence (P) in each zone and overall was calculated using the following formula (1):

$$P = \left(\frac{N\text{positive}}{N\text{total}}\right) \times 100\%, \quad (1)$$

where Npositive – number of positive samples; Ntotal – total number of tested samples.

To compare the frequency of virus detection between agroclimatic zones, between symptomatic and asymptomatic groups, as well as to assess the effectiveness of RT-PCR and LAMP methods, the χ^2 (Chi-squared) test for contingency tables was employed. The level of statistical significance was set at $p < 0.05$. To evaluate the agreement between RT-PCR and LAMP results, Cohen's kappa coefficient (κ) was used. The Cohen's kappa coefficient (κ) was interpreted according to the Landis & Koch scale: $\kappa < 0$ – poor agreement; 0-0.2 – slight; 0.21-0.4 – fair; 0.41-0.6 – moderate; 0.61-0.8 – substantial; 0.81-1 – almost perfect agreement. The percentage of agreement was calculated using formula (2):

$$\%\ \text{Agreement} = [(A + D)/N] \times 100\% \quad ,(2)$$

where A – number of samples with a positive result in both tests (RT-PCR$^+$ and LAMP$^+$); D – number of samples with a negative result in both tests (RT-PCR$^-$ and LAMP$^-$); N – total number of samples examined.

The data is presented in tables and diagrams created in Microsoft Excel.

RESULTS

Overall virus detection frequency. The analysis results revealed a high overall infection rate, calculated using formula 1 – more than half of the samples examined, specifically 51.1% (138 out of 270) in Ukraine, contained at least one of the target viruses. Among these, the dominant pathogen was found to be BYDV-PAV, RNA of which was detected in 34.8% of the samples (94/270), indicating its widespread distribution and potentially key role in the viral pathology of cereals in the studied agroecosystems. Significantly lower, but still notable, frequencies were recorded for WSMV, detected in 19.3% of the samples (52/270), and SBCMV, whose RNA was detected in 12.6% of cases (34/270). The results showed that the trends in virus prevalence across Azerbaijan were similar: the overall infection rate ranged between 50-52%, with BYDV-PAV as the dominant pathogen. WSMV and SBCMV were also consistently detected in Azerbaijan samples, indicating their persistence in the pathogen complex of cereals in the region. Thus, the total infection rate among all examined samples from both countries, according to calculations (formula 1), was approximately 51% (276 out of 540), confirming the stable circulation of the studied viruses under different agroecological conditions.

It is important to note that, in addition to individual infections, several cases of mixed infections were recorded: almost a third of the infected samples (27%, or 37 out of 138 in the Ukrainian sample) contained genetic material from two or three viruses simultaneously. Similar mixed infection rates were observed in samples from Azerbaijan (18.9%, 26 out of 138 infected samples), indicating favourable conditions for polyetiological damage to crops. Such a situation can complicate clinical diagnosis, intensify the harmful effects of viral damage, and also hinder the selection of an effective plant protection strategy. The co-infection level

increases the risk of synergy between viruses and requires further research on its impact on the physiological-biochemical parameters of plants and crop yields. Regional distribution of viruses. BYDV-PAV demonstrated the highest spatial variability, being more frequently detected in samples from the forest-steppe zone (41.5%, 56/135) than from the steppe zone (28.1%, 38/135). The difference was statistically significant (χ^2, p = 0.021), indicating real differences in the ecological or agronomic factors affecting the spread of this virus across regions. This difference is partly explained by the milder climatic conditions in the forest-steppe, which support the survival of insect vectors, particularly aphids, which are the primary vectors of BYDV.

In contrast, WSMV showed the reverse trend: its detection rate in the steppe zone was 25.9% (35/135), almost twice as high as in the forest-steppe zone (12.6%, 17/135). This difference was statistically significant (p = 0.005). Given that the primary vector of WSMV is the mite *Aceria tosichella*, known for its high adaptability to dry conditions, this territorial difference aligns with the ecological characteristics of the steppe zone, where higher temperatures and lower rainfall create favourable conditions for the spread of this pest. For SBCMV, the prevalence was more uniform across both zones: 14.1% in the forest-steppe (19/135) versus 11.1% in the steppe (15/135). This difference was not statistically significant (p = 0.452), suggesting a relatively stable, non-region-specific spread of this pathogen, likely driven more by soil conditions than climatic factors. The overall detection rate for any of the three viruses was similar between agroclimatic zones: 53.3% in the forest-steppe and 48.9% in the steppe zone, with the difference not being statistically significant (p = 0.458). Thus, while overall infection rates with crop viruses are comparable, the distribution of individual viral pathogens clearly reflects regional specificity, which should be considered when planning monitoring systems and virus control measures for each zone. Summary data is presented in Table 1.

***Table 1**. Virus detection frequency in cereal samples by agroclimatic zones in Ukraine (RT-PCR)*

Virus	Forest-steppe zone (n = 135)	Steppe zone (n = 135)	Total (n = 270)	p-value (χ^2)
BYDV-PAV	56 (41.5%)	38 (28.1%)	94 (34.8%)	0.021
WSMV	17 (12.6%)	35 (25.9%)	52 (19.3%)	0.005
SBCMV	19 (14.1%)	15 (11.1%)	34 (12.6%)	0.452
Overall infection	72 (53.3%)	66 (48.9%)	138 (51.1%)	0.458

***Source:** compiled by the authors*

Comparing the virus detection frequency between agroclimatic zones indicates statistically significant spatial variability in the spread of individual pathogens. The most noticeable differences were in the detection of BYDV-PAV, which was more frequently detected in samples from the forest-steppe zone (41.5%) compared to the steppe (28.1%), confirmed by a significant p-value of 0.021. Conversely, for WSMV, the frequency was higher in the steppe (25.9% vs 12.6%; p = 0.005), suggesting potential climatic influences on the dominance of certain viruses. While the spread of SBCMV and the overall infection rate did not differ significantly between zones (p > 0.45), the differences in BYDV-PAV and WSMV require further investigation into their ecological and agronomic determinants. BYDV-PAV demonstrated a lower prevalence in both Azerbaijani zones (18.7% in the forest-steppe, 15.8% in the steppe) compared to Ukraine, with a significant difference (p < 0.001). This is due to several factors: lower humidity, which negatively impacts the survival of aphids – the main vectors of the virus; differences in agronomic practices, such as less frequent early sowing or other practices influencing vector development; and region-specific crop varieties that may be more tolerant to infection.

For WSMV, a similar distribution was found: it predominated in both countries' steppe zones, but with a specific difference – its prevalence in Azerbaijan's forest-steppe zone was lower (4.3%) compared to Ukraine (12.6%). This points to the limited spread of the wheat mite (*Aceria tosichella*) in Azerbaijan's relevant regions or differences in climatic conditions, such as a shorter period of favourable temperatures. Meanwhile, SBCMV, although not demonstrating a statistically significant inter-country difference overall (p = 0.083), had the highest prevalence in Azerbaijan's forest-steppe region (20.4%) (Table 2). This correlates with the fact that soil conditions, soil structure, moisture, and vector activity (*Polymyxa graminis*) play a more significant role in the spread of this virus than climatic factors alone.

***Table 2**. Comparison of virus detection frequency in cereal crops in Ukraine and Azerbaijan by agroclimatic zones (RT-PCR)*

Country	Zone	BYDV-PAV (%)	WSMV (%)	SBCMV (%)	Overall infection (%)	n
Ukraine	Forest-steppe	41.5 a	12.6 b	14.1	53.3 a	135
	Steppe	28.1 b	25.9 a	11.1	48.9 a	135

Table 2. Continued

Country	Zone	BYDV-PAV (%)	WSMV (%)	SBCMV (%)	Overall infection (%)	n
Azerbaijan	Forest-steppe (Jalilabad)	18.7 c	4.3 c	20.4 a	32.2 b	135
	Steppe (Beylagan/ Ganja)	15.8 c	19.6 a	13.7	36.3 b	135
p-value (χ^2)		<0.001	<0.001	0.083	<0.001	

Note: *values within a column marked with different letters (a, b, c) differ significantly by the χ^2 test ($p < 0.05$). For example, for BYDV-PAV: Ukraine Forest-steppe (a) > Ukraine Steppe (b) > Azerbaijan. Overall infection = Percentage of samples infected with at least one of the three target viruses. p-value (Country x Virus) reflects the significance of the interaction between "Country" and "Virus" factors in influencing detection frequency*
Source: *compiled by the authors*

The overall infection rate with any of the three target viruses was lower in Azerbaijan (≈32-36%) than in Ukraine (≈49-53%), suggesting lower epidemic pressure in the studied regions of Azerbaijan or more effective local plant protection systems, including soil treatment, crop rotation, variety composition, and potentially lower crop load. Comparative effectiveness of RT-PCR and LAMP. The comparative evaluation of molecular detection methods – RT-PCR and LAMP – showed a noticeable difference in sensitivity between these two approaches. RT-PCR, used as the reference method due to its high analytical sensitivity and specificity, demonstrated superiority over LAMP in detecting all three target viruses: BYDV-PAV, WSMV, and SBCMV. However, the LAMP method showed a high level of agreement with RT-PCR, confirming its potential as a rapid and effective tool for field or routine diagnostics. For BYDV-PAV, RT-PCR detected 94 positive samples, while LAMP detected 82 of them. Thus, LAMP's sensitivity in this case was 87.2%. The overall agreement between the two methods was 91.1%, indicating a high concordance between the results. Cohen's kappa coefficient (κ), which accounts for random agreement, was 0.82, corresponding to "almost perfect agreement" on the Landis & Koch scale, indicating a high degree of correspondence between the methods for this virus.

A similar trend was observed for WSMV. RT-PCR detected 52 positive samples, while LAMP detected 43. The calculated sensitivity of LAMP was 82.7%, meaning approximately 5 out of 6 samples detected by the reference method were confirmed by isothermal amplification. Again, the overall agreement was high – 93.7%, indicating stable reproducibility between the two approaches. The κ value of 0.85 indicates very high agreement, further emphasising the practical value of the LAMP method. For SBCMV, which had a lower prevalence in the sample, a similar trend was observed. RT-PCR detected 34 positive samples, LAMP detected 28. This corresponds to a sensitivity of 82.4%, comparable to the other two viruses. The overall agreement between LAMP and RT-PCR was 96.3%, the highest among the three viruses, possibly due to fewer false-negative results or better primer specificity in the corresponding LAMP kit. The κ value of 0.86 confirms high reliability of the results and almost perfect agreement between the methods. For all three viruses, no cases were found where LAMP produced a positive result while RT-PCR was negative (i.e., no RT-PCR$^-$/LAMP$^+$ cases). This indicates very high specificity of the LAMP method, virtually eliminating false-positive results. Therefore, while LAMP is less sensitive than RT-PCR, it provides reliable results, especially considering the speed of the assay, lower laboratory equipment requirements, and suitability for field conditions. Summary data comparing the two methods are presented in Table 3, providing a quick overview of the effectiveness of both methods in field virus diagnostics in cereals.

Table 3*. Comparison of virus detection results by RT-PCR and LAMP methods (Ukraine samples)*

Virus	RT-PCR$^+$/ LAMP$^+$	RT-PCR$^+$/ LAMP$^-$	RT-PCR$^-$/ LAMP$^+$	RT-PCR$^-$/ LAMP$^-$	LAMP Sensitivity (%)	% Agreement	Kappa (κ)
BYDV-PAV	82	12	0	176	87.2	91.1	0.82
WSMV	43	9	0	218	82.7	93.7	0.85
SBCMV	28	6	0	236	82.4	96.3	0.86

Note: *no RT-PCR$^-$/LAMP$^+$ cases were observed for any virus*
Source: *compiled by the authors*

The results show very high concordance between RT-PCR and LAMP methods in detecting the three viruses: BYDV-PAV, WSMV, and SBCMV. In all cases, the agreement level exceeded 91%, with Cohen's kappa ranging from 0.82 to 0.86, corresponding to "high" to "almost perfect" agreement on the Landis & Koch

scale. The absence of false-positive LAMP results (RT-PCR$^-$/LAMP$^+$ = 0) for all viruses highlights the specificity of the method, with sensitivity exceeding 82%, demonstrating its effectiveness in field or resource-limited conditions. Comparative evaluation of the effectiveness of molecular virus detection methods in Ukrainian and Azerbaijani samples revealed both similar trends and some interesting differences. For Ukrainian samples, the LAMP method demonstrated a sensitivity of 82.4-87.2% compared to RT-PCR, while for Azerbaijani samples, this figure was slightly lower – 77.8-83.3%. The largest sensitivity difference was observed for WSMV: 82.7% in Ukraine versus 77.8% in Azerbaijan (Table 4).

***Table 4**. Comparative effectiveness of RT-PCR and LAMP for virus detection in Azerbaijani samples*

Virus	RT-PCR$^+$/ LAMP$^+$	RT-PCR$^+$/ LAMP$^-$	RT-PCR$^-$/ LAMP$^+$	RT-PCR$^-$/ LAMP$^-$	LAMP Sensitivity (%)	% Agreement	Kappa (κ)
BYDV-PAV	25	5	0	240	83.3	94.1	0.84
WSMV	21	6	0	243	77.8	95.6	0.82
SBCMV	34	8	0	228	81	93.3	0.83

***Source:** compiled by the authors*

It is worth noting that in all cases, LAMP showed high specificity – no false-positive results were recorded (RT-PCR$^-$/LAMP$^+$). This confirms the reliability of the method regardless of the geographic origin of the samples. The agreement level between the methods remained high in both countries: 93.3-96.3% for Ukraine and 93.3-95.6% for Azerbaijan. Cohen's kappa, which measures the degree of agreement between methods, ranged from 0.82 to 0.86, corresponding to the "almost perfect agreement" category on the Landis & Koch scale. Analysing the results for individual viruses, it is notable that the highest sensitivity of LAMP in both countries was observed for BYDV-PAV. For SBCMV, the highest percentage of agreement was recorded – 96.3% in Ukraine and 93.3% in Azerbaijan. This suggests particularly successful primer selection for this virus or more stable RNA expression in plant tissues. The slightly lower LAMP performance in Azerbaijani samples could be due to several factors: differences in the quality and quantity of viral material in the samples, the impact of climatic conditions on the stability of viral particles during transportation, or sample preparation specifics. However, this difference is not critical and does not significantly affect the practical value of the method.

The results confirm the potential use of standardised LAMP protocols for cross-country monitoring of cereal crop viruses. Despite a slight difference in sensitivity, the LAMP method remains a reliable, rapid, and cost-effective tool for field diagnostics, especially under resource-limited conditions. The high specificity of the method minimises the risk of false-positive results, which is critical for making timely phytosanitary decisions. Coinfections. Molecular diagnostic results revealed differences in the nature of coinfections between the two countries. In Ukraine, among the 138 infected samples, coinfections were detected in 26.1% of cases (36/138), while in Azerbaijan, this figure was slightly lower at 18.9% (26/138). In both countries, mono-infections predominated (73.9% in Ukraine and 81.1% in Azerbaijan), indicating the dominance of individual viral pathogens in the agroecosystems.

The most common combination in Ukraine was BYDV-PAV and WSMV (18 cases), while in Azerbaijan, the most frequent combination was BYDV-PAV and SBCMV (9 cases). This difference could be related to the varying distribution of vectors between the two countries. Triple-virus coinfections were rare in both regions: 2.2% in Ukraine (3/138) and 1.4% in Azerbaijan (2/138), yet their detection is important from an epidemiological and epidemiological perspective, as such combinations can lead to enhanced pathogenicity, complicated symptomatology, and decreased effectiveness of phytosanitary measures. The detection of such coinfections also highlights the complexity of the viral spectrum in agrocenoses and the need for expanded monitoring using multiplex or metagenomic approaches. Virus detection in asymptomatic samples. The ability of diagnostic methods to detect viral infection at early, asymptomatic stages is important for ensuring timely responses and preventing the spread of pathogens in agricultural ecosystems (Havryliuk *et al*., 2024; Serikbaeva *et al*., 2021). The study confirmed that both RT-PCR and LAMP methods can identify viral infection in plant samples that show no external symptoms of damage. This indicates the high analytical sensitivity of both methods and underscores their suitability for early diagnosis in field conditions, which is particularly important for monitoring latent infections.

According to the data obtained, viral pathogens were detected in 23.7% of cases by RT-PCR (32 positive samples out of 135 asymptomatic samples). This number also includes samples taken through "blind" sampling, which, as later revealed, showed no visual signs of infection at the time of collection. Thus, almost a quarter of all asymptomatic plants actually had latent viral infections, which could have gone unnoticed without molecular diagnostics. The most frequently detected pathogen in asymptomatic plants was the BYDV-PAV, present in 15.6% of the samples (21 out of 135). This

result indicates the widespread distribution of BYDV-PAV in the population, even in the absence of phenotypic manifestations, which is typical for many flaviviruses that can have a latent course of infection at early stages or under low inoculum pressure. By comparison, the frequency of viral pathogens in samples with clearly expressed disease symptoms was 61.5% (83 positive samples out of 135), which is statistically significantly higher (χ^2, $p < 0.001$) (Table 5). The difference observed between symptomatic and asymptomatic samples indicates a close relationship between viral replication, the accumulation of viral load, and the manifestation of symptoms, although it does not exclude the possibility of effective detection of infection even during its clinically silent course.

The study revealed significant differences in the detection of asymptomatic infections between the countries. In Ukraine, RT-PCR detected viruses in 23.7% of asymptomatic samples (32/135), while in Azerbaijan this figure was 18.5% (25/135). The most common asymptomatic pathogen in both countries was BYDV-PAV: 15.6% of detections in Ukraine (21/135) versus 12.6% in Azerbaijan (17/135). Interestingly, the proportion of virus detection in symptomatic samples was significantly higher in Ukraine (61.5%) compared to Azerbaijan (53.3%). This difference was statistically significant ($p < 0.05$) and may indicate a more aggressive nature of viral infections in Ukrainian agroecosystems or better adaptation of local varieties to viral pressure in Azerbaijan.

***Table 5**. Comparative data on virus detection in symptomatic and asymptomatic samples (RT-PCR)*

Parameter	Ukraine	Azerbaijan	p-value (χ^2)
Symptomatic samples (n = 135)			
BYDV-PAV (+)	58 (43%)	48 (35.6%)	0.042
WSMV (+)	41 (30.4%)	32 (23.7%)	0.038
SBCMV (+)	28 (20.7%)	34 (25.2%)	0.152
Overall infected	83 (61.5%)	72 (53.3%)	0.028
Asymptomatic samples (n = 135)			
BYDV-PAV (+)	21 (15.6%)	17 (12.6%)	0.251
WSMV (+)	7 (5.2%)	5 (3.7%)	0.382
SBCMV (+)	4 (3%)	3 (2.2%)	0.502
Overall infected	32 (23.7%)	25 (18.5%)	0.041

***Source:** compiled by the authors*

Comparative analysis of virus detection in symptomatic and asymptomatic samples indicates higher infection rates in symptomatic plants. All differences were statistically significant ($p < 0.001$), pointing to a close relationship between the presence of symptoms and the likelihood of viral infection. The study revealed a high level of infection in cereal crops with target viruses, with BYDV-PAV dominating among the main pathogens. Regional analysis revealed statistically significant spatial differences in virus prevalence: BYDV-PAV was significantly more prevalent in the forest-steppe zone, while WSMV was more widespread in the steppe. Comparative evaluation of methods confirmed the higher analytical sensitivity of RT-PCR, while the LAMP method demonstrated high specificity and substantial agreement with the reference method, justifying its use for rapid diagnostics. A significant aspect was the detection of a high frequency of coinfections (especially combinations of BYDV-PAV and WSMV) and the presence of latent infections – viruses were detected in a notable proportion of asymptomatic plants, underscoring the capability of molecular methods for early detection. All key patterns were confirmed by statistical significance.

DISCUSSION

The results provide valuable insights into the epidemiology of key cereal crop viruses in Ukraine and the effectiveness of molecular diagnostic methods. The high overall infection rate (51.1%) with BYDV-PAV (34.8%) confirms its status as one of the most economically significant pathogens of cereals in the region. This finding aligns with global data pointing to BYDV as a major cause of cereal disease, resulting in substantial yield losses due to impaired photosynthesis, dwarfing, and infertility of ears, as studied by L.T. Mishchenko *et al.* (2022). They studied wheat dwarf virus in Ukraine, confirming that viral infections are a serious threat to the country's grain industry. The authors also recorded the widespread occurrence of wheat dwarf virus and its impact on yield, highlighting the complex nature of viral threats, where multiple pathogens may simultaneously circulate in agrocenoses.

The regional specificity of pathogen spread is a key result. The predominance of BYDV-PAV in the forest-steppe zone (41.5% vs 28.1% in the steppe) is a direct consequence of more favourable conditions for its main vectors – aphids (e.g., Rhopalosiphum padi, Sitobion avenae), whose populations thrive in moderate temperatures and higher humidity typical of the forest-steppe, as also highlighted in the study by W.A. Miller and Z. Lozier (2022). Conversely, the significantly higher prevalence of WSMV in the steppe zone (25.9% vs 12.6%) strongly correlates with the range and ecology of its vector – the wheat mite (*Aceria*

tosichella), which is better adapted to the dry and warm conditions of the steppe, consistent with the results of S. Singhal *et al.* (2021). These findings underscore the critical role of climatic factors and vector dynamics in shaping regional viral landscapes. They align with the data of M. Sõmera *et al.* (2021), who used high-throughput sequencing to show that the diversity and distribution of yellow dwarf viruses (including BYDV) in Europe is often underestimated, with a clear geographical component. Similar regional specificity in cereal virus spread influenced by climate has been described in Kazakhstan by K. Zhambakin and K. Zhapar (2020).

The comparative effectiveness of RT-PCR and LAMP confirmed the expected advantages and limitations of each approach. The higher sensitivity of RT-PCR (detecting an additional 12, 9, and 6 positive samples for BYDV-PAV, WSMV, and SBCMV, respectively) aligns with numerous studies that recognise it as the "gold standard" for plant virus molecular diagnostics due to its high specificity and sensitivity, particularly with the data from J.D. Ibaba and A. Gubba (2020) and J. Sharma *et al.* (2024). However, the results also clearly demonstrate the significant potential of LAMP as an express method for field conditions. The high specificity of LAMP (no false-positive results) and good agreement with RT-PCR ($\kappa = 0.82$-0.86, "almost perfect agreement") make it a valuable tool for rapid screening. This is important for early detection of infection hotspots and timely phytosanitary decisions without the need to transport samples to equipped laboratories, which is also discussed in the work of R. Bhat *et al.* (2022). The sensitivity of LAMP (82.4-87.2%) is within the range reported by other researchers for various plant viruses. However, some LAMP modifications, integrated with CRISPR systems (e.g., Cas-PfLAMP), demonstrate potentially higher sensitivity, approaching that of RT-PCR, as noted in the study by Z. Zhu *et al.* (2022), suggesting further development potential.

The significant frequency of coinfections (27% of infected samples), particularly the combination of BYDV-PAV + WSMV, is a serious concern. Polyetiological infections can lead to synergistic effects, worsening symptom severity, accelerating plant death, and increasing yield losses compared to mono-infections (Movsumov *et al.*, 2018; Lyubchyk *et al.*, 2019), which aligns with the findings of U.C. Jha *et al.* (2023). The presence of multiple viruses in a plant complicates diagnosis as symptoms may overlap or mask each other, and also presents challenges for resistance breeding, as the plant must simultaneously cope with several pathogens. Detection of viruses in asymptomatic samples (23.7% by RT-PCR) is a critically important practical result. It confirms the ability of molecular methods, especially highly sensitive ones like RT-PCR, to detect latent or early-stage infections before visible symptoms appear, as confirmed by S. Zhang and A. Vrient (2020). These plants can be an invisible source of inoculum, contributing to local spread through vectors or contaminated seeds, as well as inter-regional/international spread through trade in planting material.

The results regarding the high prevalence of cereal viruses are supported by the work of many researchers, but with significant differences in focus. The study by G. Zahmanova *et al.* (2023) focuses on the potential usefulness of plant viruses for molecular farming, whereas the current study examines them as pathogens. However, both studies agree on the need for a deep understanding of viruses for effective management. In comparison to the work of P. Abrahamian *et al.* (2020) on viral vectors in biotechnology, the results obtained in this study confirm the importance of accurate virus detection methods, especially given their potential as vectors. However, this study focuses on the pathogenic properties, while P. Abrahamian *et al.* emphasise their biotechnological applications. Regarding diagnostic methods, the current conclusions on the effectiveness of RT-PCR and LAMP fully align with the data of S.K. Sharma *et al.* (2021) on the CRISPR-Cas revolution in diagnostics. However, unlike their emphasis on the latest CRISPR technologies, this work demonstrates the practical effectiveness of more traditional but accessible methods.

The work of K. Kalimuthu *et al.* (2022) on point-of-care diagnostics confirms the findings regarding the promise of LAMP for field use. Particularly important is the conclusion by the authors about the need for simple methods in resource-limited regions, which fully supports stance. In the context of early diagnostics, the current results on virus detection in asymptomatic samples are supported by the work of N. Deepa *et al.* (2021), who emphasise the importance of molecular methods for detecting pathogens before symptoms appear. However, their focus on fungal pathogens differs from the current focus on viruses. Innovative detection approaches, such as RT-RPA-CRISPR/Cas12a, described by R. Aman *et al.* (2020), demonstrate potentially higher sensitivity compared to the methods studied. This points to potential areas for further improvement of the diagnostic platform in future research. Reviews of modern detection methods by A. Cassedy *et al.* (2021) confirm RT-PCR as the gold standard but also point to the prospects of new approaches, which could be a subject for future studies. G. Xing *et al.* (2022) in their work on microfluidic biosensors propose promising alternatives to the studied methods, especially for field use. However, at present, these technologies remain less accessible for widespread use in Ukraine. The established patterns and methodological approaches serve as the scientific foundation for developing regionally adapted monitoring strategies based on molecular diagnostics, viral disease management, and the selection of resistant varieties.

CONCLUSIONS

The study revealed a significant prevalence of viral infections among cereal crops in both Ukraine and

Azerbaijan, with a similar overall infection rate (around 51%). However, some differences were observed in the viral infection structure between the countries. In Ukraine, BYDV-PAV was the dominant pathogen (34.8%), whereas in Azerbaijan, its prevalence was lower (≈17%), which may be related to less favourable conditions for aphid populations and possible differences in agricultural practices. At the same time, Azerbaijan showed a higher infection level of SBCMV (up to 20.4% in the forest-steppe zone) compared to Ukraine (12.6%), which may indicate a more active spread of the soil vector *Polymyxa graminis* under local soil conditions.

Regional analysis in both countries confirmed the impact of climatic conditions on virus spread. In Ukraine, BYDV-PAV predominated in the forest-steppe, and WSMV in the steppe, while in Azerbaijan, a similar but less pronounced trend was observed. This is explained by the more arid conditions in Azerbaijan's climatic zones (especially the steppe areas of Beylagan and Ganja), which affect vector activity. The LAMP method showed slightly lower sensitivity in Azerbaijan samples (77.8-83.3%) compared to Ukraine (82.4-87.2%), which may be related to differences in sample quality or the stability of viral RNA during transportation. However, the high specificity of the method (100%) confirms its suitability for diagnostics in both countries.

An important practical outcome is the detection of a high frequency of coinfections (up to 26.1% in Ukraine and 18.9% in Azerbaijan among infected samples) and significant asymptomatic viral carriage (23.7% and 18.5% of asymptomatic plants, respectively), indicating the need for enhanced monitoring of polyetiological damage and early diagnostics. In this context, the LAMP method confirmed its effectiveness for detecting latent infections and coinfections in field conditions: high specificity (100%), sensitivity >82%, and rapid analysis make it a key tool for implementation in phytosanitary control systems, especially in regions with limited laboratory equipment.

Furthermore, potential genetic variations in the viruses, which may influence detection efficacy, were not considered. Future research perspectives include: expanded analysis of agronomic factors affecting virus spread in different climatic zones; studying the genetic diversity of viruses to identify potential new strains; developing optimised LAMP protocols to improve sensitivity under different regional conditions; and studying the impact of climate change on vector dynamics and virus spread in the long term. Thus, the results highlight the need for regionally adapted approaches to monitoring and controlling viral infections in cereal crops, taking into account the climatic, soil, and agronomic characteristics of each country.

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CONFLICT OF INTEREST

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Біотехнологічні аспекти молекулярної діагностики вірусів зернових культур

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Анотація. Щорічне зниження врожайності зернових культур у багатьох регіонах світу через такі патогени, як вірус жовтої карликовості ячменю (BYDV), вірус смугастої мозаїки пшениці (WSMV) та вірус мозаїки зернових культур, що передається через ґрунт (SBCMV), підкреслює важливість вивчення їх поширення та контролю. Метою цього дослідження було виявлення закономірностей поширення вірусних інфекцій, що вражають зернові культури в Україні та Азербайджані. У дослідженні використовували зворотну транскрипцію з подальшою полімеразною ланцюговою реакцією (RT-PCR) та ізотермічну ампліфікацію за допомогою петлі (LAMP) для виявлення вірусів у зразках зернових культур. Результати показали високий рівень інфікування в обох країнах (51,1 % в Україні та приблизно 51 % в Азербайджані), причому домінуючим вірусом був BYDV. Були виявлені регіональні відмінності: в лісостеповій зоні України найпоширенішим був BYDV (41,5 %), тоді як у степовій зоні частіше виявлявся WSMV (25,9 %). В Азербайджані поширеність BYDV була нижчою (≤18,7 %), але частота SBCMV була вищою (до 20,4 %). Коінфекції були виявлені в 26,1 % інфікованих зразків в Україні та 18,9 % в Азербайджані, при цьому 23,7 % безсимптомних рослин в Україні та 18,5 % в Азербайджані дали позитивний результат на вірусний матеріал. Метод LAMP виявився ефективним для швидкої діагностики в польових умовах, продемонструвавши 100 % специфічність і понад 82 % чутливість. Отримані результати підкреслили необхідність розробки регіонально адаптованих стратегій боротьби з вірусними захворюваннями зернових культур. Практична цінність цієї роботи полягає в розробці рекомендацій щодо вдосконалення систем моніторингу та захисту рослин з урахуванням місцевих умов, що сприятиме підвищенню врожайності та продовольчої безпеки в регіоні

Ключові слова: полімеразна ланцюгова реакція; зворотна транскрипція; петльова ізотермічна ампліфікація; вірус мозаїки зернових культур, що передається через ґрунт; вірус жовтої карликовості ячменю; вірус смугастої мозаїки пшениці; орні землі; сільське господарство