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Metagenomic Characterisation of Putatively Novel Cereal Crop Virus Candidates in Ukraine and Azerbaijan: Implications for Phytosanitary Risk Assessment

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ABSTRACT

This study aimed to identify and characterise putatively novel viral genome candidates with potential phytosanitary relevance for cereal crops in Ukraine and Azerbaijan using metagenomic sequencing. The research was conducted through metagenomic sequencing of total ribonucleic acid (RNA) extracted from agrocenoses of key cereal crops, which yielded more than 1.2 gigabases of data. The results demonstrated differences in the structure of agrocenosis metagenomes between the two countries: in Ukraine, bacterial sequences predominated (65.2 ± 4.8%), whereas in Azerbaijan, a higher proportion of viral sequences was observed (15.3 ± 2.1%). Taxonomic analysis identified 247 viral genomes belonging to 21 families of RNA- and DNA-containing viruses, with the families *Virgaviridae* and *Potyviridae* being the most abundantly represented. A key finding was that 32.8% met the criteria for putatively novel viral genome candidates, representing priority targets for further validation rather than experimentally confirmed novel viral species. Comparative analysis between the countries showed that the proportion of novel viruses was higher in Azerbaijan, which may be associated with specific agroclimatic conditions. The obtained results may be used by phytosanitary organisations, agricultural enterprises, and state regulatory authorities to develop risk-based phytosanitary monitoring, preventive surveillance frameworks, and targeted control strategies for emerging viral candidates that require further pathogenicity, host-range, and yield-impact assessment.

1. INTRODUCTION

The relevance of this study is determined by the growing threat posed by viral infections to food security through their impact on the yield of major agricultural crops. In particular, viral diseases of cereal crops such as wheat, barley, and maize can reduce the productivity of agrocenoses, leading to economic losses and posing risks to food security at the global level. Since viral infections are often associated with unpredictable outbreaks, the timely detection of novel viruses and the assessment of their impact on agricultural crops are essential for the development of effective control and prevention measures.

Research on viral infections in cereal crops in Ukraine and Azerbaijan has already attracted the attention of scholars. In particular, Pozhylov and Snihur [1] investigated the distribution of viral infections in cereal crops in Ukraine during 2020-2021, identifying a high level of infection, especially in wheat. The authors emphasised the importance of monitoring such infections to maintain the sustainability of the national agri-food sector. The diversity of viruses infecting maize in Ukraine, including the genetic structure of these viruses and their impact on crop yield, was examined by Vlasova et al. [2]. The authors noted the presence of novel viral isolates that may pose a significant threat to the agricultural sector. The distribution of the major wheat viruses in Azerbaijan and their impact on crop productivity were investigated by Sultanova et al. [3]. The authors emphasised the need for systematic monitoring and the implementation of phytosanitary measures to maintain the stability of agricultural production. Antipov et al. [4] examined biotechnological aspects of the molecular diagnosis of cereal crop viruses, in particular the application of polymerase chain reaction (PCR) and other molecular methods for pathogen detection. The researchers highlighted the importance of accurate diagnostic tools for the effective control of viral infections in agrocenoses. Masenya et al. [5] investigated the application of metagenomics to the study of microbial communities of cereal crops, with an emphasis on their impact on food security. The authors stressed the importance of the metagenomic approach for exploring the diversity of viruses and microorganisms infecting cereal crops.

The metagenomic composition of the wheat viral community, including the identification of novel viral sequences associated with plants and fungi, was studied by Redila et al. [6]. Their results demonstrated the considerable potential of metagenomics for identifying new viruses that may pose a threat to agriculture. Lappe et al. [7] described the

metagenomic identification of novel viruses of maize and teosinte in North America, emphasising the importance of such approaches for the detection of emerging pathogens. The authors also discussed the evolutionary and ecological aspects of these viruses, which contribute to the development of future protection strategies. The current state of biosecurity for cereal crops in Australia and the prospects for combating viral diseases were analysed by Maina and Jones [8]. The authors discussed new approaches to virus control, particularly through the improvement of diagnostic and monitoring methods. In a separate study, Ruschel [9] focused on the use of high-throughput sequencing technologies for the detection and characterisation of viruses infecting cereal crops. This approach enables greater accuracy in virus detection and increases the effectiveness of control measures.

In the book by Golnaraghi and Gaur [10], emerging and increasing threats posed by plant viruses that can seriously affect global food security were discussed. The authors examined the mechanisms of the spread of novel viruses and provided recommendations for measures to control such threats.

Comparative metagenomic studies from other climatic regions further demonstrate that cereal crop viromes are strongly shaped by ecological and agroclimatic conditions. In tropical East Africa, metagenomic analysis of maize lethal necrosis in Kenya revealed complex viral associations in maize agroecosystems, illustrating how warm climates, vector pressure, and mixed infections can intensify phytosanitary risks. In North America, the metagenomic identification of novel viruses in maize and teosinte showed that temperate cereal systems also harbour previously undescribed viral diversity with evolutionary and ecological significance [7]. Australian grain-crop studies have additionally emphasised the need for biosecurity-oriented surveillance under conditions of climatic variability and recurrent virus disease pressure [8]. Similar concerns have been reported for rice, where newly emerging viral threats require continuous genomic monitoring and improved crop protection strategies. These global examples indicate that the comparison of Ukraine and Azerbaijan should be interpreted within a broader climatic framework, where regional virome composition may reflect the combined influence of host range, vector ecology, land use, and environmental stress.

The analysis of the scientific literature made it possible to identify several key research gaps. First, comparative studies of the complete viral spectrum of cereal crop agrocenoses in geographically and climatically differentiated regions, such as Ukraine and Azerbaijan, using a harmonised metatranscriptomic protocol, remain lacking. Second, even within metagenomic studies, the relationship between virome structure and specific agrotechnological practices (for example, irrigation type or crop rotation system) remains insufficiently explored, as does the comprehensive risk assessment of newly discovered species based on integrated criteria, including transboundary potential, the presence of vectors, and evolutionary plasticity. Addressing these gaps formed the basis for defining the aim of the present study.

This study aimed to investigate putative novel viral genome candidates with potential phytosanitary relevance for cereal crops in Ukraine and Azerbaijan, as well as to compare viral communities in the agrocenoses of these two countries. To achieve this aim, the following objectives were set: (1) to conduct a metagenomic analysis of viruses infecting the major cereal crops in both countries; (2) to identify differences in the structure of viral communities across different agroclimatic zones; and (3) to assess genomic and ecological risk indicators associated with emerging viral candidates.

2. MATERIALS AND METHODS

The study was conducted in agrocenoses of major cereal crops (winter wheat, cultivar Podolianka; barley, cultivar Deviatyi Val; and maize, cultivar Bereka) in Ukraine and Azerbaijan during 2020-2023. The selection of Ukraine and Azerbaijan was justified by the need to compare the viral complexes of cereals within the Black Sea-Caucasus macroregion under conditions of similar study objects (leading cereal crops and comparable agrotechnological approaches) but contrasting climatic and agricultural regimes, including gradients of moisture availability and aridity, temperature background, frequency of irrigation, and technological intensity. This design made it possible to disentangle the contributions of agroclimatic factors and technological practices to virome formation and to identify viral taxa with potentially increased phytosanitary risk under different cultivation conditions.

The field stage was carried out during the growing seasons from April 2020 to September 2023. Sampling was carried out during periods of maximum representativeness of phytoviral load in the above-ground plant biomass and peak activity of vectors: for winter wheat, May-June; for barley, May-June; and for maize, July-August (with an additional control sampling at the end of the season in September, if required). The research was conducted at the Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine (Kyiv) and at the Research Institute of Plant Protection and Technical Crops of the Ministry of Agriculture of the Republic of Azerbaijan (Ganja).

The selection of agroclimatic zones was based on the principle of covering key ecological gradients that determine the structure of viral communities and the circulation of vectors, including humidity, temperature regime, seasonal contrast, the need for irrigation, and the intensity of agrotechnological practices. In Ukraine, 24 composite samples were formed from three zones: the Southern Steppe (Odesa and Mykolaiv regions) as a model of warmer and drier conditions; the Forest-Steppe (Cherkasy and Vinnytsia regions) as a model of moderate conditions with a high proportion of intensive cultivation; and Polissia (Zhytomyr and Chernihiv regions) as a model of more humid conditions. In Azerbaijan, 20 composite samples were formed from the main cereal-producing regions: the Southern region (Nakhchivan) as a model of arid conditions; the Shamakhi Valley as a model of a temperate climate; and the Ganja Highlands as a model of stable cereal production conditions with a distinct altitudinal and climatic profile.

To ensure the comparability of the agrotechnological context, each field was described using a unified observation sheet that included the type of farming system (intensive monoculture systems or traditional/less intensive fields), the duration of monoculture cultivation, crop rotation schemes, the application of glyphosate-based herbicides, the irrigation system (none, surface irrigation, or other systems), as well as basic soil and climatic characteristics. The areas of the fields included in the sampling were as follows: in Ukraine, 10-120 ha (Southern Steppe: 30-120 ha; Forest-Steppe: 20-90 ha; Polissia: 10-70 ha); in Azerbaijan, 5-40 ha (Nakhchivan: 5-30

ha; Shamakhi Valley: 6-35 ha; Ganja Highlands: 8-40 ha). This approach made it possible to include both intensive production areas and less intensive fields without altering the sampling logic.

The number and distribution of crops and cultivars across the zones were as follows (in all cases, the same cultivars were used: winter wheat Podolianka, barley Deviatyi Val, and maize Bereka). In Ukraine, a total of 24 composite samples were collected: winter wheat, n = 8; barley, n = 8; maize, n = 8. In the Southern Steppe, n = 8 samples were formed (winter wheat n = 3; barley n = 2; maize n = 3); in the Forest-Steppe, n = 8 (winter wheat n = 3; barley n = 3; maize n = 2); and in Polissia, n = 8 (winter wheat n = 2; barley n = 3; maize n = 3). In Azerbaijan, a total of 20 composite samples were collected: winter wheat, n = 7; barley, n = 7; maize, n = 6. In Nakhchivan, n = 7 samples were formed (winter wheat n = 3; barley n = 2; maize n = 2); in the Shamakhi Valley, n = 7 (winter wheat n = 2; barley n = 3; maize n = 2); and in the Ganja Highlands, n = 6 (winter wheat n = 2; barley n = 2; maize n = 2). Such a distribution ensured a balance of crops within each country and adequate representation of each agroclimatic zone/region for comparative statistical procedures and the interpretation of metagenomic profiles.

Composite samples were formed by combining above-ground parts of cereal crops, small accompanying plants, plant residues, and small invertebrates present in the above-soil layer at the time of sampling. Fungal structures associated with plant residues and, where present, nematode-associated material from the near-surface plant matrix were also retained as part of the composite sample, since these organisms may contribute to soil- or residue-mediated viral circulation. To ensure the comparability of the material and to prevent systematic bias in metagenomic profiles, exclusion criteria were established. A sample was not included in further analysis if: (1) signs of external contamination were recorded during sampling or transport (contact with non-sterile surfaces or instruments outside the established procedure, substantial soil contamination, or mixing of material between fields); (2) the sample had insufficient mass or volume of biomaterial for standardised ribonucleic acid (RNA) extraction and library preparation, or was formed with a substantial disproportion of components that violated the unified principle of "compositeness"; (3) storage conditions were violated (lack of cooling or freezing, thawing during transport, or prolonged exposure to room temperature); (4) laboratory quality control revealed RNA of inadequate quality for metagenomic sequencing (pronounced degradation, low concentration or yield), which made library preparation impossible; or (5) the sample contained a disproportionately high contribution of a single dominant non-target biological component (for example, large fragments of one accompanying plant species or a high abundance of invertebrates), rendering it unrepresentative of the above-soil layer of the field. In cases of exclusion, repeat sampling was carried out within the same field and crop at the nearest available time to maintain a balanced distribution of samples across countries, zones, and crops.

Within each site, 25-point samples were collected and combined into a single composite sample. The 25-point scheme was selected to capture within-field microheterogeneity in plant condition, residue distribution, and potential vector occurrence while maintaining a comparable sampling unit across countries, crops, and agroclimatic zones. Points were distributed across each field to avoid edge effects and localised disease foci, thereby reducing the probability that the metagenomic profile would be dominated by a single infected plant or a single microhabitat. The material was frozen in liquid nitrogen at approximately -196 °C and transported to the laboratory under a maintained cold chain at a temperature not exceeding -20 °C. Prior to RNA extraction, the samples were homogenised in liquid nitrogen to obtain a uniform mass, and equal subsample weights were taken from the homogenate for RNA extraction, which ensured cellular integrity and prevented nucleic acid degradation.

Total RNA was extracted from 100-200 mg of homogenised material using a commercial kit for plant tissues with additional purification steps to remove inhibitors. RNA quality and quantity were assessed by spectrophotometry using a NanoDrop 2000 instrument and by capillary electrophoresis using an Agilent 2100 BioAnalyzer. Only samples with an RNA integrity number of at least 7.0 were accepted for further analysis.

This threshold was used as the main RNA-quality criterion before rRNA depletion and library preparation. In addition to RIN, RNA concentration, A260/280 and A260/230 absorbance ratios, electrophoretic profile, and the absence of pronounced degradation were checked for each sample. Samples that did not meet these quality requirements or provided insufficient RNA yield were excluded from sequencing or re-extracted before library construction. The retained libraries subsequently showed high sequencing quality, as confirmed by Q30 values exceeding 90%, supporting the reliability of downstream taxonomic and genome-reconstruction analyses.

To enrich the viral and non-coding fraction, protocols for the depletion of plant and bacterial ribosomal RNA were applied using the commercial QIAseq FastSelect – rRNA Plant kit (QIAGEN) [11]. Complementary deoxyribonucleic acid (DNA) libraries were prepared from the purified RNA and sequenced on the Illumina NovaSeq 6000 platform, generating paired-end reads of approximately 150 bp per sample. The target sequencing depth, defined as "hundreds of millions of paired-end reads per library", was considered sufficient to achieve two objectives simultaneously: sensitive detection of low-abundance viruses and de novo reconstruction of complete or near-complete viral genomes. In the composite matrices of agrocenoses, the predominant proportion of nucleic acids is usually derived from the host plants, accompanying vegetation, microbiota, and the eukaryotic fauna of the above-soil layer. Accordingly, the viral fraction may be represented either abundantly (for dominant taxa) or at very low levels (for rare or transient viruses). Under such conditions, an increase in the total number of sequencing reads enhances the probability of detecting low-abundance taxa after the stages of filtering, trimming, and removal of host-derived sequences and also provides sufficient coverage for the assembly of longer contigs and the reconstruction of genomes without gaps. Thus, the selected data volume was consistent with the subsequent virus identification strategy, which relied not only on the taxonomic classification of reads but also on the assembly and analysis of virus-like contigs and genomes, a process that requires adequate sequencing depth. In total, more than 1.2 gigabases of paired-end reads were generated for the entire study, and the quality metrics of the libraries (in particular, the proportion of Q30 bases) confirmed the suitability of the dataset for sensitive taxonomic analysis and subsequent genome reconstruction.

Raw sequencing data were cleaned of adaptor sequences and low-quality nucleotides using Trimmomatic (version 0.39) and FastQC (version 0.11.9); reads with quality scores below the defined threshold and excessively short fragments were removed. To eliminate host-derived sequences, the reads were mapped to the reference genomes of the main cereal crops, as well as to available reference genomes of accompanying species, using Bowtie2 (version 2.4.4) and BWA (version 0.7.17). To assess library quality, Q20 and Q30 metrics, average read length, and guanine-cytosine (GC) content were calculated using FastQC and GATK (Genome Analysis Toolkit, version 4.2.6.1). Filtered reads were then assembled de novo using metagenomic algorithms, notably SPAdes (version 3.14.0), and the resulting contigs were used as the basis for subsequent viral annotation. The taxonomic assignment of reads and contigs was performed by aligning them against reference databases of viruses, bacteria, and eukaryotes using Diamond (version 0.9.32) and BLAST+ (version 2.9.0). Virus-like contigs underwent refined annotation, and candidate genomes were selected if they covered the majority of the predicted genome length and had sufficient coverage.

To reduce the risk of false assignment caused by reagent contamination, cross-sample contamination, or misannotation of host-derived sequences, candidate viral contigs were interpreted conservatively. Contigs were retained only when they showed consistent viral-domain matches, sufficient read coverage, coherent open reading frame (ORF) organisation, and no stronger assignment to plant, fungal, bacterial, or other non-viral sequences in comparative database searches. Since external negative controls from certified virus-free plant material or sterile growth-chamber samples were not included in the sequencing run, the identified sequences were classified as putatively novel viral genome candidates rather than as experimentally confirmed novel viral species.

Viral genomes were considered complete or near-complete if their length was consistent with expectations and they contained a full complement of predicted ORFs, as determined using GeneMarkS (version 4.34) and Prodigal (version 2.6.3).

Virus assignments were not based on a single alignment output alone. The results obtained with BLAST+ and Diamond were compared for consistency at the family and genus levels, and ambiguous contigs were retained only when viral attribution was additionally supported by coherent genome organisation, predicted ORF structure, conserved protein domains, and phylogenetic placement. The <90% amino acid identity threshold was therefore used as an initial criterion for recognising putatively novel viral genome candidates, rather than as an isolated taxonomic decision. Where genome architecture allowed, additional structural signals, including tRNA-like elements or promoter-associated motifs reported for related viral groups, were considered as supportive evidence; however, these features were not treated as universal criteria because they are not present or diagnostically informative across all plant virus families.

For each genome, the genomic organisation, ORF composition, and the presence of atypical structural or functional motifs were further analysed using InterProScan (version 5.49-82.0) and Pfam (version 33). For selected virus groups, multiple alignments of the amino acid sequences of key proteins were constructed using MAFFT (version 7.490) and Clustal Omega (version 1.2.4). Optimal evolutionary models were determined with ModelTest-NG (version 0.1.7), and phylogenetic trees with bootstrap support were reconstructed using RAxML (version 8.2.12) and IQ-TREE (version 2.1.2). The selection of evolutionary models was performed separately for each alignment of key viral proteins, as different taxonomic groups and different proteins (for example, replicase domains and capsid proteins (CP)) can vary substantially in amino acid substitution frequencies, degree of conservation, and saturation levels. For this reason, a "data→model" approach was applied: based on multiple alignments of amino acid sequences, the optimal substitution model was determined in ModelTest-NG by comparing alternative candidate models and selecting the best-fitting model according to information criteria (minimising Bayesian Information Criterion (BIC)/Akaike Information Criterion (AIC)), accounting for among-site rate heterogeneity (γ-distribution) and, where necessary, additional parameters such as empirical amino acid frequencies. The selected model (or its equivalent implementation in a given programme) was then applied in both RAxML and IQ-TREE to avoid biases in topology and bootstrap support caused by model misspecification, which is critical for viral genomes with high evolutionary rates and uneven substitution patterns across sites. To detect recombination events, complete or near-complete genomes were analysed using multiple algorithms, including RDP4 (version 4.97), SimPlot (version 3.5.1), GENECONV (version 1.81), and BootScan. Recombination signals were considered only if supported statistically and consistent across methods.

For the risk-scoring procedure, recombination evidence was considered significant only when the same event was detected by at least two independent algorithms and when the inferred breakpoint positions were broadly concordant across methods. The algorithms were not treated as equally weighted single votes; instead, priority was given to events supported by complementary approaches, such as similarity-plot shifts, phylogenetic incongruence, and statistically significant recombination signals.

Alpha diversity of viral communities was assessed using the abundance of viral taxa normalised by sequencing depth, and indices of species richness and evenness were calculated using QIIME2 (version 2020.8) and the Vegan R package. Comparisons of metrics between countries, agrocenosis types, and crops were conducted using non-parametric statistical tests in R (version 4.0.5) with the Vegan package. The taxonomic structure of viromes was described at the family, genus, and species levels, highlighting a shared core as well as taxa unique to each country. For each sample, structural characteristics of the virome were compared with metadata on farming systems. The frequency of detection of individual viruses and viral families was assessed in monocultures and crop rotations, on irrigated and non-irrigated plots, and under different herbicide application regimes. Statistical associations between viral metrics and agrotechnological factors were evaluated through correlation analyses and comparison of group distributions.

To strengthen the interpretation of these associations, an observational causal-inference framework was additionally applied. Irrigation type, farming system, monoculture duration, and herbicide regime were treated as explanatory agricultural factors, while Shannon and Chao1 indices were treated as response variables reflecting virome evenness and richness. Country, crop species, agroclimatic zone, and sequencing depth were considered potential confounding variables. This framework was used to distinguish stable associations from simple bivariate correlations; however,

because the study did not involve experimental manipulation of agricultural practices, causal conclusions were interpreted cautiously.

Prior to selecting parametric or non-parametric procedures, the normality of quantitative variables was assessed using the Shapiro-Wilk test to identify deviations and outliers. For variables exhibiting an approximately normal distribution ($p > 0.05$ in the Shapiro-Wilk test) without pronounced skewness, correlations between continuous variables were calculated using Pearson's method. In cases of non-normality ($p \leq 0.05$), the presence of ordinal variables or marked non-linearity in relationships, Spearman's rank correlation was applied. To compare viral metrics between two independent groups (for example, intensive versus traditional farming, presence versus absence of irrigation, or glyphosate application versus no application), a parametric t-test was used when assumptions of normality and homogeneity of variances were met, or the Mann-Whitney U test was applied when these assumptions were violated. For comparisons involving three or more groups, analysis of variance (ANOVA) or the Kruskal-Wallis test was employed, respectively.

To identify potential vectors of novel viruses, the eukaryotic fraction of the metagenomic data was analysed using MetaPhlAn (version 3.0). Reads assigned to insects, mites, and other invertebrates were classified to the genus or species level using Kraken2 (version 2.1.2) and Centrifuge (version 1.0.4). For key novel viruses, the frequency of co-detection with individual taxa of potential vectors was calculated within the same samples. Such "virus-potential vector" pairs were treated as hypotheses regarding transmission routes for further validation.

For a systematic assessment of the risk associated with newly discovered viruses, an aggregated scoring system was developed based on four key criteria. Criterion (A), "transboundary potential", was scored as 1 if the same novel virus (or a closely related lineage within a single monophyletic cluster in the phylogenetic trees of key proteins) was detected in at least one sample from Ukraine and at least one sample from Azerbaijan. If the virus was not independently detected in both countries, a score of 0 was assigned. This approach aligned with the interpretation of "detected in both countries in similar, but not identical, variants" for priority isolates, where the risk was defined by the presence of potential transboundary spread rather than complete sequence identity. Criterion (B), "relatedness to economically important pathogens", was determined by comparing the amino acid sequences of key proteins or polyproteins of novel viruses with reference sequences of known phytopathogens that have an established economic impact (noted as causal agents of major diseases in cereals or related crops). The criterion was considered met (1 point) if the best match in amino acid identity exceeded 85% over the aligned regions, interpreted as a high degree of evolutionary relatedness to already known pathogenic lineages and, therefore, an increased likelihood of similar biological properties, including host range and epidemic potential. The >85% threshold was used solely as a "trigger" for prioritisation, not as a taxonomic criterion for species demarcation. Criterion (C), "presence of a vector", was formalised based on the taxonomic composition of the eukaryotic fraction of the metagenomic data, with a focus on insect and mite taxa known to act as vectors of plant viruses. The criterion was considered confirmed (1 point) if co-detection was observed between a virus and a taxon with known or likely vector status. The frequency of co-detection was calculated as the percentage of virus-positive samples in which sequences of the corresponding potential vector were simultaneously detected. In the absence of co-detection with taxa relevant to vector transmission, or where no biologically plausible vector status existed, a score of 0 was assigned. Criterion (D), "evidence of recombination", was assessed using complete or near-complete genomes with multiple algorithms (RDP4, SimPlot, GENECONV, BootScan), and recombination signals were considered only if statistically supported and reproducible/consistent across methods. Thus, 1 point was assigned only when recombination was confirmed as a genuine signal, rather than an artefact of a single method, whereas 0 points were assigned in the absence of statistically supported evidence. The detection of recombination events in 14 genomes resulted specifically from the application of this multi-approach procedure. Each criterion was scored either 0 or 1, with fulfilment of the criterion receiving 1 point and non-fulfilment receiving 0 points. After evaluating all criteria, the total score was used to calculate an integrated risk index, allowing the prioritisation of viruses for subsequent phytosanitary measures. Viruses with a risk index of 4 were classified as high risk and required immediate inclusion in monitoring systems and the implementation of appropriate measures. Viruses scoring 3 were considered medium risk and required further study, while those scoring 2 were regarded as low risk but still warranted observation.

Comparisons of diversity indices between groups were conducted using the non-parametric Mann-Whitney U test in R (version 4.x). Before selecting parametric or non-parametric procedures, the normality of quantitative variables (including diversity/evenness indices and other viral metrics) was assessed using the Shapiro-Wilk test, with deviations additionally checked visually via Q-Q plots. Since deviations from normality were observed for diversity indices and some related metrics, differences between groups were evaluated using the non-parametric Mann-Whitney U test (for two independent groups). Statistical associations between viral metrics and agrotechnological factors were assessed through correlation analysis. Spearman's rank correlation was used as the primary measure of association, given that agrotechnological variables included ordinal or categorical characteristics and/or did not conform to a normal distribution. In cases where both variables were approximately normally distributed ($p > 0.05$ in the Shapiro-Wilk test) and the relationship was linear, Pearson's correlation could be applied; however, for consistency across analyses, Spearman's approach was prioritised. The threshold for statistical significance was set at $p < 0.05$.

3. RESULTS

3.1 Metagenomic profile and novel viral taxa of agrocenoses

Metagenomic sequencing of total RNA extracted from composite samples of agrocenoses of key cereal crops generated a large and comprehensive dataset. Following a standard bioinformatic processing pipeline – which included adapter trimming, quality filtering, and removal of reads mapping to host plant genomes – data suitable for downstream taxonomic analysis was obtained. The total dataset comprised over 1.2 gigabases of paired-end reads. No statistically significant difference was observed in the mean read length

after trimming between the datasets from Ukraine and Azerbaijan. Quality metrics (Q20 and Q30) for all analysed libraries exceeded 95% and 90%, respectively, confirming the high reliability of the nucleotide sequences obtained for downstream analyses. A detailed comparative characterisation of the volume and quality of the metagenomic data is presented in Table 1. The number of samples that successfully passed all processing stages allowed for a representative comparison between the two regions.

Table 1. Comparative characterisation of metagenomic data volume and quality after bioinformatic processing

Country	Number of Samples (n)	Total Paired-End Reads, Millions (Post-Processing)	Average Read Length (bp)	Q30 (%)	Guanine-Cytosine (GC) Content (%)
Ukraine	24	680 ± 45	147 ± 5	92.1 ± 0.9	48.3 ± 1.2
Azerbaijan	20	520 ± 38	145 ± 6	91.5 ± 1.2	46.8 ± 1.5

Source: Compiled by the authors.

Taxonomic classification of the filtered sequences using reference databases revealed clear differences in the structural organisation of the agrocenosis metagenomes between the two countries. In samples from Ukraine, sequences classified as bacterial predominated (mean 65.2 ± 4.8%), while viral sequences accounted for 8.7 ± 1.5%. In Azerbaijani samples, there was a statistically significant increase in the proportion of viral sequences to 15.3 ± 2.1%, whereas the proportion of bacterial sequences was lower (58.1 ± 5.2%). The proportion of eukaryotic sequences, including transcripts from host plants and fungi, was similar in both datasets, comprising approximately 25-30%. These results suggest a potentially higher viral load in cereal agrocenoses under the conditions present in Azerbaijan.

Analysis of alpha diversity in viromes, assessed using the Chao1 index (estimating richness) and the Shannon index (estimating evenness), revealed a complex pattern. The Chao1 index indicated higher species richness in viral communities from Ukrainian samples compared with those from Azerbaijan, which may reflect a greater diversity of ecological niches or vectors in the Ukrainian study regions. However, the Shannon index, which accounts for the evenness of distribution, did not show statistically significant differences between the two countries overall. When samples were stratified by land-use type (intensive monoculture systems versus traditional or less intensive fields), clear patterns emerged. In intensive agrocenoses in both countries, Shannon index values were significantly lower than in traditional fields, indicating the dominance of a smaller number of viral taxa under conditions of high anthropogenic pressure. Comparisons among the major cereal crops (winter wheat, barley, maize) within each country did not reveal statistically significant differences in alpha diversity, suggesting a potential lack of host specificity for many of the detected viral communities. These results provide a quantitative basis for further analyses and validate the technical capability of the chosen approach to generate high-quality, comparable data from two geographically distinct regions. They also highlight quantitative differences in the contribution of the virome to metagenomic structure and demonstrate a consistent effect of land-use type on viral community evenness, independent of country.

Taxonomic annotation of the filtered viral sequences, followed by their assembly, enabled the reconstruction of a substantial number of complete or near-complete viral genomes. In total, 247 genomes were reconstructed, belonging to 21 families of RNA and DNA viruses. The most abundant representatives in both regions were families containing economically significant plant pathogens: *Virgaviridae*, *Potyviridae*, *Tombusviridae* and *Secoviridae*. The quantitative distribution of reconstructed genomes across major families revealed regional differences. In Ukrainian samples, the largest proportion consisted of *Virgaviridae* members (41.9% of all genomes), whereas in Azerbaijan, viruses from the *Potyviridae* family predominated (30.6% of all genomes). This distribution is summarised in Table 2, which presents the number of genomes per key family for each country. Additionally, several genomes belonging to less common or poorly studied families, such as *Partitiviridae*, *Deltaflexiviridae*, and *Geminiviridae*, were identified, indicating the complex structure of the viromes in the examined agrocenoses.

Table 2. Distribution of reconstructed complete or near-complete viral genomes by major family and country of origin, interpreted in relation to host-crop stratification

Virus Family	Number of Genomes (Ukraine)	Number of Genomes (Azerbaijan)	Total
Potyviridae	38	62	100
Virgaviridae	52	28	80
Tombusviridae	12	15	27
Secoviridae	8	10	18
Other families	14	8	22
Total	124	123	247

Note: The table summarises country-level distributions; interpretation of these patterns should be made together with the crop composition of the sampled agrocenoses (winter wheat, barley, and maize).
Source: Compiled by the authors.

To complement the country-level summary presented in Table 2, the reconstructed genomes were additionally considered in relation to the host crops from which the composite samples were derived (winter wheat, barley, and maize). This crop-oriented reading indicates that the dominant viral families, particularly *Potyviridae* and *Virgaviridae*, were detected across more than one cereal host, suggesting that the observed geographic differences were not attributable to a single crop alone. Accordingly, the country-level distribution should be interpreted together with host-crop stratification in order to avoid overgeneralising regional virome patterns.

Further analysis of the sequence identity of key viral proteins against reference strains from international databases revealed a substantial proportion of potentially novel viral species. The criterion for preliminary assignment to novel species was an amino acid sequence identity of the replicase (for RNA viruses) or the CP (for DNA viruses) below the 90% threshold established by the International Committee on Taxonomy of Viruses [12] for species in most families. This threshold was interpreted together with cross-tool annotation

consistency, ORF organisation, conserved-domain composition, and phylogenetic placement. Of the 247 reconstructed genomes, 81 (32.8%) were classified as putatively novel viral genome candidates according to this bioinformatic criterion. Comparison between countries indicated that the proportion of potentially novel species was higher in Azerbaijani samples (38.2% of all genomes from the country) than in Ukrainian samples (27.4%). The absolute number of detected putatively novel genome candidates was also greater in Azerbaijan (47 vs. 34). The largest number of novel species within families was identified among *Potyviridae* (22 genomes) and *Virgaviridae* (19 genomes), indicating these groups as the main reservoirs of previously uncharacterised diversity.

For detailed phylogenetic characterisation, two of the most representative classes of novel viruses were selected. The first analysis focused on new tobamoviruses (family *Virgaviridae*) detected in both countries. The novel isolates from Ukraine and Azerbaijan formed several distinct monophyletic clusters, positioned at considerable evolutionary distances from all officially recognised *Tobamovirus* species, such as *Tomato mosaic virus* (ToMV) and *Tobacco mosaic virus* (TMV). Notably, some of the new viruses from different countries clustered together, suggesting a close evolutionary history or the presence of transboundary viral populations. The second analysis focused on novel potyviruses (family *Potyviridae*), with the CP serving as the primary target for comparison. Phylogenetic reconstruction revealed a clear geographic structuring. Most of the novel potyviruses detected in Azerbaijan formed a distinct, well-supported clade that was sister to a cluster containing viruses previously known from the Middle East and Central Asia, such as isolates of *Wheat streak mosaic virus* from Iran. In contrast, the novel potyviruses from Ukraine clustered with known European isolates or formed separate branches within the European clade. These results suggest possible geographic isolation of viral populations and indicate differing evolutionary trajectories for pathogens of this important family in the Caucasus and Eastern Europe.

3.2 Comparative virome profile, recombination, and risk factors

Comparative analysis of the taxonomic composition of viral communities at the species and genus levels revealed a proportion of groups unique to each country, highlighting the influence of local ecological and agroclimatic factors. From the total pool of viral taxa identified with high confidence, 58% were detected exclusively in Ukrainian samples, while 32% were found only in Azerbaijani samples. Only 10% of viral taxa were shared between the agrocenoses of both countries. The shared core of viral taxa consisted primarily of cosmopolitan species with broad host ranges, such *as* TMV 7 (family *Virgaviridae*) and *Cucumber mosaic virus* (family *Bromoviridae*), confirming their widespread occurrence across diverse ecosystems. The predominance of unique taxa in Ukrainian samples may be linked to the greater diversity of ecological niches and land-use types surveyed.

The characterisation of viruses detected exclusively in Ukraine focused on groups dominant in samples from the steppe zone. Among the novel members of the family *Virgaviridae*, a tobamovirus genome was identified, provisionally named *Triticum steppe virus*. Genome annotation revealed a canonical organisation with four ORFs encoding the replicase, movement protein (MP), and CP. Within the sequence of the MP, a motif characteristic of plasmodesmal interaction was identified, which differed in amino acid composition from analogous motifs in known strains of TMV. Particular attention was drawn to a novel species from the family *Tombusviridae*, reconstructed from wheat samples. Its genome contained an additional ORF (p19) located on the subgenomic RNA. The p19 protein showed low homology (less than 40%) to known RNA interference suppressors in tombusviruses, suggesting the potential presence of an alternative immune-suppression mechanism.

In Azerbaijani samples collected under arid climatic conditions, a unique set of viruses adapted to such environments was observed. Most notable was a new potyvirus, provisionally named *Hordeum aridum virus*. Its genome, approximately 9.8 kb in length, contained the typical potyviral polyprotein, but analysis of autocatalytic proteolytic cleavage sites revealed an additional potential cleavage site between the cylindrical inclusion and VPg proteins. Motif-based functional prediction revealed the presence of an additional hydrophobic domain in the P3 protein, which may be associated with adaptation to stress conditions such as drought. Additionally, a single-segment RNA virus was detected in the same samples, classified within the family *Deltaflexiviridae*. Its genome, in addition to the conserved replicase (RdRp), contained three ORFs of unknown function, which lacked homologues in public databases, suggesting potentially unique modes of interaction with the host plant.

Table 3. Representative viral genomes exhibiting recombination detected in the study

Genome Identifier	Country	Family	Closest Known Donor 1 (Region)	Closest Known Donor 2 (Region)	Statistical Support (*p*-Value)
Poty_UA_147	Ukraine	*Potyviridae*	*Tobacco etch virus* (Europe)	*Potato virus Y* (Eastern Europe)	$<1 \times 10^{-15}$
Poty_AZ_022	Azerbaijan	*Potyviridae*	*Wheat streak mosaic virus* (Iran)	*Johnson grass mosaic virus* (India)	$<1 \times 10^{-12}$
Tobamo_UA_089	Ukraine	*Virgaviridae*	*Tomato mosaic virus* (Eastern Europe)	Not determined (unique fragment)	$<5 \times 10^{-9}$
Delta_AZ_005	Azerbaijan	*Deltaflexiviridae*	*Sclerotinia sclerotiorum* deltaflexivirus 1	*Botrytis virus F*	$<1 \times 10^{-6}$

Note: The *p*-value indicates the most significant result among the algorithms used (RDP, GENECONV, BootScan, MaxChi, or SiScan).
Source: Compiled by the authors.

Analysis for recombination events in the detected genomes using RDP4 and SimPlot identified statistically supported evidence of recombination in 14 viral genomes. The highest number of such cases (nine) was observed among potyviruses. For three novel potyviruses detected in Azerbaijan, their genomes were found to be mosaics: the 5′-terminal regions originated from a lineage closely related to *Wheat streak mosaic virus*, while the 3′-terminal regions derived from a

lineage related to *Johnson grass mosaic virus.* These recombination points corresponded to the boundaries of functional modules within the polyprotein. One novel tobamovirus detected in Ukraine showed evidence of intra-genomic recombination within the replicase gene, where a central fragment appeared to originate from an unknown donor. The results of the recombination analysis are summarised in Table 3.

The detected hybrid genomes indicate active evolutionary processes generating new combinations of genetic material within the agroecosystems of both countries, potentially capable of altering pathogen traits such as host range or aggressiveness.

To assess the influence of agrotechnical factors on the structure of viral communities, a correlation analysis was conducted between the presence and relative abundance of individual viral groups and characteristics of cropping systems. In samples from Ukraine, a statistically significant positive correlation ($p < 0.01$) was observed between the high prevalence of a novel potyvirus, provisionally named *Zea mosaic virus*, and continuous maize monoculture over areas cultivated for three or more consecutive years. This virus, belonging to the family *Potyviridae*, was present in 85% of samples from such monoculture fields, whereas its prevalence in samples from crop rotation fields did not exceed 15%. Simultaneously, a negative correlation was observed between viral species richness (Shannon index) and the intensity of glyphosate-based herbicide application in the same agroecosystems.

In Azerbaijani samples, the most pronounced associations were linked to irrigation systems. A statistically significant positive correlation ($p < 0.05$) was found between the presence and diversity of viruses in the family *Tombusviridae*, particularly a novel species provisionally named *Aridefluvial soil-borne virus*, and plots subject to surface (furrow) irrigation. The proportion of sequences belonging to this group on irrigated fields was, on average, three times higher than that observed on rainfed (non-irrigated) land. Additionally, irrigated plots exhibited an increase in overall viral load (number of viral reads per million) and in the proportion of sequences characteristic of soil-borne viruses, suggesting that moisture conditions may facilitate their accumulation and potential dissemination [13-15]. When interpreted within the observational causal-inference framework, this pattern suggests that irrigation may act as an indirect driver of virome restructuring by modifying soil moisture, residue decomposition, and the ecological conditions favourable for soil-associated vectors or reservoirs. Nevertheless, this relationship should be regarded as a supported causal pathway rather than as experimentally confirmed direct causation.

To identify potential vectors of novel viral pathogens, the taxonomic composition of the eukaryotic fraction of metagenomic data was analysed, with a focus on insect families known to act as plant virus vectors. In samples positive for specific novel viruses, sequences belonging to insect and mite orders were detected. The results are summarised in Table 4, which indicates potential vectors for key new viral species.

Table 4. Potential vectors of key novel viruses inferred from co-detection in metagenomic samples

Key Novel Virus (Provisional Name)	Country	Virus Family	Potential Vector (Taxon)	Co-Detection Frequency, %	Known Vector Status of Taxon
Hordeum aridum virus	Azerbaijan	*Potyviridae*	Aphid (*Schizaphis graminum*)	92%	Primary vector for many potyviruses
Zea mosaic virus	Ukraine	*Potyviridae*	Aphid (*Rhopalosiphum padi*)	85%	Vector for cereal viruses (e.g., BYDV)
Triticum steppe virus	Ukraine	*Virgaviridae*	Mite (*Abacarus hystrix*/*Aceria* spp.)	78%	Vector for cereal viruses (e.g., *Wheat streak mosaic virus*)
Aridefluvial soil-borne virus	Azerbaijan	*Tombusviridae*	Fungus gnats (*Bradysia* spp.)/*Plasmodiophorid fungi*	65%	Known vectors of soil-borne viruses
Unclassified *Deltaflexiviridae*	Azerbaijan	*Deltaflexiviridae*	Thrips (*Frankliniella occidentalis*)	41%	Potential vectors for some RNA viruses

Note: Co-detection frequency was calculated as the percentage of samples in which the virus was detected among those in which sequences of the specified potential vector were also present.
Source: Compiled by the authors.

For instance, the novel potyvirus *Hordeum aridum virus*, detected in Azerbaijan, was co-detected with sequences belonging to the aphid *Schizaphis graminum* – a known potyvirus vector – in 92% of cases. In Ukrainian samples where the novel tobamovirus *Triticum steppe virus* was identified, sequences from mites of the family *Eriophyidae*, which are potential vectors for certain members of the *Virgaviridae*, were present at a high frequency (78%). Co-detection of a novel deltaflexivirus with sequences from fungus gnats (*Bradysia* spp.) was also recorded, suggesting a possible alternative soil-mediated transmission pathway. Differences in the fauna of potential vectors between the two countries corresponded with differences in the dominant viral families. Alongside the virus – potential vector co-detection analysis, the detection frequency of key novel viruses was compared with specific agronomic conditions, including cropping system (monoculture versus crop rotation) and irrigation practices. Correlation analysis indicated that monoculture maize cultivation was associated with a higher detection frequency of the novel potyvirus *Zea mosaic virus*, supporting the hypothesis that prolonged dominance of a single host and a stable vector feeding base increases the likelihood of establishment for new viruses [16, 17]. Simultaneously, irrigated systems were associated with an increased proportion of soil-borne viruses, particularly *Aridefluvial soil-borne virus*, suggesting a potential amplification of soil-mediated (or indirectly soil-associated) transmission under conditions of higher moisture and more intensive water management in the agroecosystem [18].

The synthesis of comparative data allowed the identification of groups of viruses that may pose a potential threat to food security at a regional scale. Despite the high level of endemism in the viromes, the analysis revealed a set of shared taxa present in the agroecosystems of both countries. This core

included both viruses with broad host ranges and pathogens specialised in cereals. Among them were representatives of the genera *Tobamovirus* (family *Virgaviridae*), *Potyvirus* (family *Potyviridae*), and *Polerovirus* (family *Solemoviridae*). Specific species, such as TMV and *Cucumber mosaic virus*, were detected in both geographical locations. Of particular note was the detection of related yet phylogenetically distinct strains of potyviruses closely related to *Wheat streak mosaic virus* in both countries, indicating independent circulation of this economically significant pathogen in different regions. The presence of these shared or closely related viral lineages suggests broad adaptability to diverse agroclimatic conditions and the potential for further geographic spread [19-21].

For a systematic assessment of the risk associated with newly discovered viruses, an aggregated scoring system was developed based on four key criteria: (A) the presence of identified isolates in both countries (transboundary potential), (B) phylogenetic proximity (amino acid sequence identity >85%) to known economically significant pathogens, (C) confirmed co-detection with a known effective vector, and (D) the presence of statistically supported signs of recombination in the genome. Each criterion was assigned a score, the sum of which determined an overall risk index (Table 5).

Table 5. Risk assessment of key novel viruses based on aggregated criteria

Virus (Isolate)	Family	Transboundary Potential (T)	Proximity to Pathogens (P)	Vector Presence (V)	Evidence of Recombination (R)	Overall Risk Index (Sum)
Poty_AZ_022	*Potyviridae*	1	1	1	1	4
Tobamo_UA_089	*Virgaviridae*	1	1	1	1	4
Aridefluvial soil-borne virus	*Tombusviridae*	1	1	1	0	3
Hordeum aridum virus	*Potyviridae*	0	1	1	0	2
Zea mosaic virus	*Potyviridae*	0	1	1	0	2
Unclassified *Deltaflexiviridae*	*Deltaflexiviridae*	0	0	1	1	2

Note: The overall risk index was calculated as the sum of scores (0 or 1) across four criteria: transboundary potential (T), proximity to pathogens (P), vector presence (V), and evidence of recombination (R). For the recombination criterion (R), a score of 1 was assigned only to genomes with statistically supported and methodologically concordant recombination evidence. Single-algorithm signals or poorly localised breakpoints were not sufficient for assigning a positive score.
Source: Compiled by the authors.

Based on the overall scores, three priority viral candidates for phytosanitary monitoring and further pathogenicity assessment were identified. The highest-risk group comprised new recombinant potyviruses, notably the isolate Poty_AZ_022. This virus combined transboundary characteristics (detected in both countries in similar but non-identical variants), high phylogenetic similarity to pathogenic strains of *Wheat streak mosaic virus,* the presence of an effective vector (*Schizaphis graminum*), and a mosaic genome structure. The second most significant group consisted of novel tobamoviruses, represented by the isolate Tobamo_UA_089, which exhibited a unique recombination event and was co-detected with mite vectors. The third priority was a newly identified species in the family *Tombusviridae* (*Aridefluvial soil-borne virus*), which was widespread in irrigated agroecosystems in Azerbaijan and detected in isolated samples from Ukraine, indicating its potential for range expansion under irrigated conditions.

The integrated risk index should be interpreted as a genomic and ecological prioritisation tool rather than as direct evidence of actual pathogenicity, yield loss, or economic damage. The criteria used in Table 5 identify viral candidates that warrant further monitoring and experimental validation; however, they do not by themselves confirm a measurable threat to food security. Therefore, higher-scoring candidates should be regarded as priority targets for host-range assays, pathogenicity testing, and yield-impact evaluation.

For these three groups, the development of specific PCR primers was recommended to enable rapid identification within routine phytosanitary surveillance, alongside initiating studies to investigate their pathogenic properties using varietal collections of major cereal crops. In practical terms, the monitoring framework should include three operational levels: (1) targeted seasonal screening of wheat, barley, and maize fields in high-risk agrocenoses, especially irrigated plots and long-term monocultures; (2) laboratory confirmation of priority viruses using virus-specific RT-PCR/qPCR assays designed from the conserved regions of the reconstructed genomes; and (3) parallel recording of agronomic factors and potential vectors, including aphids, mites, fungi, and nematode-associated material, to support risk-based interpretation of positive detections. For policy implementation, the priority viruses identified in this study should be incorporated into national phytosanitary surveillance lists, with shared reporting protocols between plant-protection laboratories, agricultural enterprises, and regulatory authorities.

The newly identified viral species in cereal agroecosystems may affect both the economic and ecological stability of agriculture if future pathogenicity studies confirm their ability to affect yield or product quality [22, 23]. For this reason, their possible spread should be evaluated in relation to potential reductions in crop productivity, grain quality, germination rates, and technological properties only after biological validation. Viral infections are often accompanied by alterations in plant metabolism, suppression of growth, and impaired development of reproductive organs, which can further affect yield stability and quality [24, 25]. For the agricultural sector, this translates into increased costs for monitoring, vector control, adjustments in agronomic practices, and the implementation of preventative measures, all of which influence production costs and the planning of sowing structures. Climate change, intensified land use, monoculture systems, and irrigation can modify vector ranges and viral circulation dynamics, thereby affecting the spatial and temporal patterns of viral infections in cereal ecosystems [26-28]. In this context, incorporating newly detected viral taxa into phytosanitary monitoring programmes and

developing specific molecular detection methods is considered a crucial tool for the timely identification of viral threats and for planning risk management measures to safeguard food security.

Since the present study did not include direct field experiments measuring yield loss or market effects, the economic dimension of the identified threats should be interpreted as a preliminary risk projection rather than as a measured loss estimate. For stakeholder-oriented assessment, the priority viruses identified in Table 5 can be linked to potential output-loss scenarios by combining four parameters: the area of the affected crop, baseline yield, virus detection frequency, and an expected yield-reduction coefficient derived from subsequent pathogenicity trials. In economic terms, projected output reduction may then be multiplied by the average farm-gate grain price to estimate potential cost exposure. This approach provides a practical pathway for translating metagenomic detection into preliminary production-risk and market-risk assessments once regional yield, price, and infection-severity data become available.

4. DISCUSSION

Comparing the results of this study with other publications, it is evident that the data obtained on viral communities in Ukraine and Azerbaijan reflect trends similar to those reported in other countries. For instance, Gupta [29] highlighted the role of metagenomics in the detection of novel plant viruses, significantly improving diagnostic capabilities and the monitoring of viral threats. The findings of the present study, particularly the identification of new viruses within the *Potyviridae* and *Virgaviridae* families, corroborate the existence of comparable viral threats in cereal agroecosystems observed in other regions worldwide. Specifically, Yousuf et al. [30] emphasised the importance of metagenomic approaches for investigating agricultural microbiomes and viral communities, noting that advanced sequencing technologies provide new opportunities for studying such threats. These observations are consistent with the results presented here, which demonstrate that metagenomic methods not only facilitate virus identification but also reveal their potential impacts on agricultural ecosystems. This approach has enhanced understanding of viral evolution and allowed assessment of their influence on the sustainable development of agroecosystems. The application of virus-associated nucleic acids in metagenomic analyses to gain deeper insight into viral communities, as explored by Moubset et al. [31], is similarly reflected in this study, where a comparable methodology was employed for the selection and analysis of viral sequences. The detection of novel viruses in cereal agroecosystems in Ukraine and Azerbaijan aligns with patterns described in studies employing metagenomic sequencing methods for virus detection and classification.

The detection of 14 recombinant viral genomes also has important evolutionary implications. Recombination may arise when genetically related viruses co-infect the same host plant or circulate within the same agroecosystem through shared vectors, crop residues, or soil-associated reservoirs. In this study, the concentration of recombination signals among potyviruses suggests that mixed infections and vector-mediated circulation may create favourable conditions for genome exchange. The localisation of recombination points near functional regions of the polyprotein may also indicate that recombination is not merely a random event, but a possible mechanism for generating variants with altered replication efficiency, vector compatibility, or host adaptation [32, 33]. However, these mechanisms remain inferential because host-range assays and experimental infection studies were not conducted. Therefore, the recombinant genomes identified here should be interpreted as evolutionary candidates that require functional validation to determine whether recombination affects pathogenicity, host specificity, or ecological fitness.

In the study by Piombo et al. [34], the importance of metagenomic approaches for identifying emerging pathogens was also emphasised, particularly under conditions of climate change and intensive agriculture, which is directly relevant to the present research. Such approaches enable not only the identification of known viruses but also the detection of new viral pathogens that may arise through evolutionary changes in existing viral populations. Investigations of viral communities in weeds and wild host plants, conducted by Hasiów-Jaroszewska et al. [35], further demonstrated the efficacy of metagenomics in characterising the diversity and ecology of viruses in uncultivated reservoirs, which can be crucial for understanding infection sources in agroecosystems. A review by Kreuze et al. [36] specifically highlighted that those new technologies, including metagenomics, provide innovative opportunities to enhance understanding of major viral diseases threatening global food security, which fully supports the practical conclusions of the present study regarding the need to integrate such methods into national phytosanitary monitoring systems.

It is also noteworthy that the findings of this study, which identified novel viral species within the *Potyviridae* and *Virgaviridae* families, correspond with the results reported by Wamaitha et al. [37], who conducted a metagenomic analysis of viruses associated with maize lethal necrosis in Kenya, providing a relevant comparison for evaluating viral threats across different regions. These findings underscore the importance of monitoring viral infections across different climatic zones, enabling the timely detection of emerging threats. The study by Mohsin et al. [38] highlighted the potential of viral metagenomics as a monitoring tool for the early identification of novel pathogens. This reinforces the significance of the metagenomic sequencing methods employed in the present study for the rapid detection of new viruses that may pose a risk to agricultural crops. Attention to emerging viral threats in rice was emphasised in the research of Ding et al. [39], where the authors reviewed decades of discoveries in the field and their implications for crop protection. Comparing these findings with those of the current study suggests that similar challenges exist regarding novel viruses in cereal crops, as these pathogens can cause substantial damage to wheat and maize in Ukraine and Azerbaijan. A common feature across these studies is the emphasis on early detection of new viruses using metagenomic technologies, which allows for forecasting and mitigating their potential impact. In their review, Sharuddin et al. [40] highlighted the role of metatranscriptomics in agriculture, particularly in the context of sustainable farming and the improvement of crop productivity. The results of this study, which also applied metagenomic approaches, demonstrated the effectiveness of these methods for identifying emerging viral threats. Such approaches can be applied to expand current knowledge and improve control of viruses that adversely affect cereal crop yields in Ukraine and

Azerbaijan.

The application of metagenomic sequencing for the surveillance of viral diseases associated with food and water, which is relevant to the study of viral infections affecting agricultural crops, was discussed by Nieuwenhuijse and Koopmans [41]. This study reinforces the necessity of employing metagenomics for virus detection, as it enables the identification of novel pathogens that may be transmitted via the environment or other pathways, similar to the viruses identified in cereal crop agroecosystems in the present research. Giolai et al. [42] focused on measuring airborne metagenomic diversity in agricultural ecosystems, providing insights into ecological conditions that facilitate the spread of viral infections. Their findings are pertinent to this research, as they highlight the significance of environmental factors in viral dissemination and offer a foundation for further investigation into the influence of agroclimatic conditions on viral communities in cereal crops. The detection of a novel virus transmitted by aphids and infecting rice, also identified through metagenomic analysis, was reported by Yan et al. [43]. The discovery of new viruses and their transmission routes in plant systems parallels the findings of this study, in which novel viral species were detected in cereal crop agroecosystems and are likely transmitted via vectors.

Islam [44] emphasised the role of genomic monitoring in combating emerging plant diseases, including wheat viruses, which is directly relevant to the analysis of novel viral threats in cereal crops. The study provided recommendations for managing viral diseases that could be adapted to the specific conditions of Ukraine and Azerbaijan, particularly regarding the establishment of monitoring systems and the detection of new viruses in cereal agroecosystems. In the research of Smadi et al. [45], metagenomic analysis was employed to detect viruses in mixed fruit orchards, assessing viral diversity through samples collected by honeybees (*Apis mellifera*). This study demonstrated the application of metagenomics for investigating viral communities not only in agroecosystems but also in natural ecosystems, highlighting the importance of virus monitoring across different ecological levels. Similar to the present research, the detection of vector-transmitted viruses underlines the role of biological agents in the spread of viral diseases [46, 47]. Viral threats to wheat and innovative approaches to their diagnosis and management were reviewed by Tanu et al. [48], which is also relevant to this study, as the focus here is on viruses of cereal crops. The authors emphasised the importance of integrated diagnostic approaches that combine metagenomic technologies with novel methods for rapid detection of viral diseases, aligning closely with the findings of the current research. The study by Muruu [49] focused on the detection of taro viruses in Kenya using next-generation sequencing, demonstrating the effectiveness of modern approaches for identifying novel plant pathogens under variable climatic conditions. This methodology could be adapted for similar research in Ukraine and Azerbaijan, where local climate and specific pathogens play a key role in shaping the diversity of viral species in cereal agroecosystems. Scott et al. [50] highlighted the impact of plant diseases on food security in the twenty-first century, emphasising the growing threat posed by viral infections to global food systems. This aligns with the findings of the present study, which demonstrated that viral pathogens can significantly compromise the stability of food systems by affecting the yield and quality of cereal crops.

Thus, a comparison of the results of this study with existing scientific literature confirms the value of a metagenomic approach for investigating viral communities in cereal crops and demonstrates the effectiveness of contemporary methods for identifying emerging viral threats. The data obtained from Ukraine and Azerbaijan correspond with findings from other studies that underscore the high diversity of viruses in cereal agroecosystems and the critical importance of monitoring these threats to safeguard food security. Given the identification of new viral species and potentially hazardous pathogens, it is evident that a systematic metagenomic approach is essential for the effective management of viral threats in agriculture. These results provide a basis for outlining subsequent steps in the development of strategies for monitoring and managing viral infections.

5. CONCLUSIONS

The study demonstrated that the applied metagenomic approach yielded representative and comparable datasets for the cereal agroecosystems of Ukraine and Azerbaijan. Following bioinformatic processing, over 1.2 gigabases of reads were obtained. For Ukraine, 24 samples were analysed (680 ± 45 million read pairs; mean read length 147 ± 5 bp; Q30 92.1 ± 0.9%; GC content 48.3 ± 1.2%), while for Azerbaijan, 20 samples were processed (520 ± 38 million read pairs; 145 ± 6 bp; Q30 91.5 ± 1.2%; GC 46.8 ± 1.5%). These metrics confirm the high quality and comparability of the data between the two countries. Comparative analysis of the metagenomic composition revealed differences in the relative proportions of taxonomic fractions. In Ukrainian samples, bacterial sequences predominated (65.2 ± 4.8%) with a viral fraction of 8.7 ± 1.5%, whereas in Azerbaijani samples, the proportion of viral sequences was significantly higher (15.3 ± 2.1%) and the bacterial fraction lower (58.1 ± 5.2%). The contribution of the eukaryotic fraction remained similar in both datasets, accounting for approximately 25-30%.

Taxonomic analysis enabled the reconstruction of 247 complete or near-complete viral genomes, representing 21 families, with a comparable total number of genomes reconstructed in each country (Ukraine – 124; Azerbaijan – 123). Economically significant groups dominated, namely *Potyviridae* and *Virgaviridae*: a total of 100 genomes were recovered for *Potyviridae* (38 from Ukraine; 62 from Azerbaijan) and 80 genomes for *Virgaviridae* (52 from Ukraine; 28 from Azerbaijan), while other families accounted for smaller proportions of the overall pool (e.g., *Tombusviridae* – 27; *Secoviridae* – 18). It was determined that 81 of the 247 genomes (32.8%) met the criteria for potentially novel species, defined as having key protein amino acid identities below the 90% threshold, with the relative proportion of such viruses being higher in Azerbaijani agroecosystems. The discovery of new taxa, including *Triticum steppe virus*, *Hordeum aridum virus*, *Zea mosaic virus*, *Aridefluvial soil-borne virus*, and a member of the Deltaflexiviridae family with unique ORFs, highlighted previously undescribed viral diversity in cereal ecosystems. Analysis of recombination events in 14 genomes, along with the presence of phylogenetically related lineages shared between the two countries, confirmed active evolutionary processes and the potential for the emergence of novel pathogen variants with expanded traits.

Co-detection of viral sequences with taxa of potential vectors provided quantitatively supported grounds for

formulating hypotheses regarding transmission pathways: for *Hordeum aridum virus*, co-detection with the aphid *Schizaphis graminum* was 92%; for *Zea mosaic virus* with *Rhopalosiphum padi*, 85%; for *Triticum steppe virus* with the mites *Abacarus hystrix/Aceria* spp., 78%; for *Aridefluvial soil-borne virus* with *Bradysia* spp./*plasmodiophorid fungi*, 65%; and for unclassified Deltaflexiviridae with the thrips *Frankliniella occidentalis*, 41%. The constructed integrated risk scale enabled the identification of three priority viral candidates for phytosanitary monitoring and further pathogenicity assessment: a group of new recombinant potyviruses (isolate Poty_AZ_022), a group of new tobamoviruses (isolate Tobamo_UA_089), and *Aridefluvial soil-borne virus*, a soil-associated virus with the potential for range expansion under irrigated conditions.

Therefore, the main applied outcome of the study is a technically implementable monitoring pathway that links metagenomic discovery, targeted molecular diagnostics, field-level risk stratification, and regulatory reporting for emerging cereal viruses.

The study was limited by the geographic scope of the two countries, the limited number of crops, and the time-bound nature of sample collection, as well as the absence of direct experimental investigations into pathogenicity and transmission. Although sampling was standardised by crop and growing-season period, the comparison between Ukraine and Azerbaijan may still have been influenced by seasonal variation, interannual climatic fluctuations, and local weather anomalies during 2020-2023. These factors could affect vector activity, plant physiological status, viral load, and the detectability of low-abundance viral sequences. Therefore, the observed country-level differences should be interpreted as geographically structured patterns within the sampled seasons rather than as fully generalisable long-term national virome profiles. Another limitation is that the study did not include external negative sequencing controls or orthogonal experimental confirmation of the putatively novel viral genomes by RT-PCR, Sanger sequencing, or in situ hybridisation. Accordingly, these genomes should be interpreted as high-priority candidates for validation rather than as fully confirmed novel viral species. Although the causal-inference framework strengthened the interpretation of associations between agricultural practices and virome diversity, controlled validation under irrigation, crop-rotation, and vector-exclusion conditions remains necessary.

Future economic assessments should integrate virus detection data with crop-yield statistics, grain-quality indicators, and farm-gate price data to quantify the potential production and market consequences of priority viral candidates. Future research should also focus on experimentally confirming the host range and pathogenicity of new viruses, expanding the geographic and seasonal coverage of sampling, and applying models that assess the long-term consequences of viral infections for the sustainability of cereal production.

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