International Journal of Plant & Soil Science

Volume 37, Issue 11, Page 240-250, 2025; Article no.IJPSS.147810
ISSN: 2320-7035

Metagenomics of Plant Viruses: Tools and Applications

Vibha Gupta [a*]

[a] *Department of Botany, Guru Nanak Khalsa College, Matunga, Mumbai-400019, Maharashtra, India.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2025/v37i115839

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/147810

Review Article

Received: 18/09/2025
Published: 15/11/2025

ABSTRACT

Metagenomics which has gained considerable attention in recent years, offers a powerful tool for detecting plant viruses which possess a serious threat to agriculture, food security, and biodiversity globally. Traditionally, methods of diagnosis such as serological techniques, often fail to detect unknown, divergent or emergent type of viruses. However, metagenomic approaches have made it possible to characterize such viruses on a larger scale. This review provides an overview of metagenomic strategies used for plant virological studies with major emphasis on next generation sequencing technologies and bioinformatic tools used for data processing and analysis. Furthermore, applications with reference to biotechnology, nanotechnology, agriculture, and environmental studies emphasizing its role in plant virus management are also mentioned. Though significant progress made, several knowledge gaps still persist, which requires attention to enhance the utility of metagenomics for studying plant viruses, are also discussed in this review.

Keywords: Plant viruses; virus metagenomics; biotechnology; agriculture; environmental studies.

**Corresponding author: E-mail: vibha.gupta@gnkhalsa.edu.in;*

Cite as: *Vibha Gupta. 2025. "Metagenomics of Plant Viruses: Tools and Applications". International Journal of Plant & Soil Science 37 (11):240–250. https://doi.org/10.9734/ijpss/2025/v37i115839.*

1. INTRODUCTION

Plant viruses are well known catastrophic pathogens, affecting crop yields, food quality and biodiversity (Savary et al., 2019). Conventionally, methods of detection of plant viruses were based on symptomatic observations and laboratory-based methods (Fang and Ramasamy 2015, Sankaran et al., 2010) such as ELISA (Enzyme linked immunosorbent assay), PCR (Polymerase chain reaction), SEM (Scanning electron microscopy). However, these traditional techniques depends on prior knowledge of viral sequences or morphological characteristics, which limits their ability in detecting novel or highly divergent viruses.

The plant viral metagenomics has revolutionized the plant pathology discipline, enabling the direct analysis of nucleic acids from the infected tissue. It eliminates the need of culturing and helps in unbiased identification of all viruses present in a sample, including novel and uncharacterized types (Martinelli, 2015). Despite these advancements, several challenges persist in plant viral metagenomics. These includes low abundance of viral sequences relative to host and microbial DNA, structural complexity of plant tissues and the presence of plant-derived inhibitors that interfere with efficient recovery of viral genetic material and downstream analysis. Moreover, understanding the virus-host relationships remains difficult due to limited knowledge of host specificity and dependence on bioinformatic predictions (Roux et al., 2021).

According to the International Committee on Taxonomy of Viruses (2019), nearly 6,590 viruses infecting around 1,400 plant species have been characterized till date but complete information of plant virus diversity remains unexplored. Traditionally, identification methods included microscopic examinations, serological assays, nucleic acid hybridization techniques, have provided limited genomic insights and is inadequate for detecting novel viral variants (Liu et al., 2011). In contrast, viral metagenomics, which utilizes next generation sequencing (NGS) technologies, facilitates the comprehensive exploration of viral diversity, facilitates the discovery of novel viruses, and advances understanding of virus-host interactions and evolutionary relationships (Roossinck et al., 2015; Balcı et al., 2024).

This review discusses the various approaches used in plant virus metagenomics, their applications, and the key limitations associated with each technique.

2. PLANT VIRUS METAGENOMICS

It involves the study of viral diversity within a plant’s virome including both known and newly discovered viruses (Prabha et al., 2020). This field has revealed the vast and complex diversity of plant viruses and their intricate interactions with their hosts, thereby contributing to a deeper insight of their ecological and evolutionary relationship (Chitra et al., 2022, Shakir et al., 2023). It can be broadly classified into the following categories:

i) Shotgun Metagenomics (Whole Genome Sequencing): It involves sequencing of all nucleic acids (DNA and RNA viruses) extracted from a sample, thereby providing a detailed information of the viral and microbial communities. This offers an unbiased identification of both known and novel viruses but is often limited by high cost, the complexity of data interpretation, and the need for advanced bioinformatic analysis (Tremblay et al., 2022).

ii) Targeted Metagenomics (Enrichment based): It employs enrichment techniques to specifically isolate and amplify viral particles or nucleic acids prior to sequencing. Since viral nucleic acids are present in low abundance, enrichment enhances sensitivity and reduces the presence of non-viral sequences (Marios et al., 2025). Common enrichment strategies include amplicon sequencing, hybrid capture and spiked primer enrichment. However, challenges such as the detection of false positives, difficulties in result interpretation, and the high cost of analysis can limit its widespread use for virus detection.

iii) Amplicon based Metagenomics: It is a technique that focuses on amplifying and sequencing specific genetic regions of DNA (especially rRNA genes: 16S or 18S). It is used to study microbial community composition in detail. Although this approach is less commonly used to detect plant viruses as they lack universal marker genes but can be useful for certain viral families (Babalola et al., 2025). Compared with shot gun metagenomics, amplicon sequencing is more cost effective, faster, and generates less data making analysis simpler. However, its narrow focus on genetic regions might limit viral discovery.

iv) Functional Metagenomics (Vargas-Albores et al., 2018): This powerful technique helps in understanding the functions of genes within a microbial community. It uses expression or metagenomic libraries which are combined with bioinformatic tools for analysis. Although rarely used for plant virus detection but this approach offers potential to explore viral gene functions and their correlation with plant defence mechanisms, thereby further improving our understanding of host-virus interactions.

v) Meta-transcriptomics (RNA seq): This deals with the study of the total RNA content of a microbial or plant associated community, providing insights into gene expression and functional activities (Destras et al., 2024). Unlike DNA-based metagenomics, which explores the genetic potential of a community, mete-transcriptomics deals with the actively transcribed genes leading to the study of gene functions, metabolic pathways and their regulatory mechanisms. (Destras et al., 2024). This is very powerful technique as majority of plant viruses constitutes RNA as a genetic material. However, RNA stability and challenges in sample handling remain major limitations that needs to be carefully managed.

3. PLANT VIRAL NUCLEIC ACID ANALYSIS

3.1 Sample Sources

Plant viruses metagenomic studies involves samples from diverse sources to capture the entire diversity of viral communities. These include plant tissues such as aerial parts (leaves, roots, stems) and conducting element (phloem) from both wild and cultivated plant species. In addition, insect vectors such as nematodes, flies and fungi- associated organisms, as well as environmental samples like soil and irrigation water from agriculture fields, serve as important reservoirs of plant viruses (Adams et al., 2009; Al Rwahnih et al.,2018).

3.2 Targets in Plant Virus Metagenomics (Chitra et al., 2022, Roossinck, 2011)

Depending on the virus type, different nucleic acid can be targeted in metagenomic studies:

i) Total Nucleic Acids: This type of extraction can capture broad range of viral sequences, including both RNA and DNA viruses. It provides comprehensive knowledge about the viral population present within the sample.

ii) Virion Associated Nucleic Acids (VANA): This method involves purifying viral particles and extracting nucleic acids directly. It provides a targeted approach by focusing specifically on encapsulated viral genomes, thereby reducing contamination from host or microbial nucleic acids.

iii) Double Stranded RNA (ds RNA): It is a product of RNA silencing, an important host response to viral infection and helps in knowing viruses involved actively in infecting plants. Their extraction and sequencing help in identification of actively replicating RNA viruses, providing valuable insights about viral infections within the host plant.

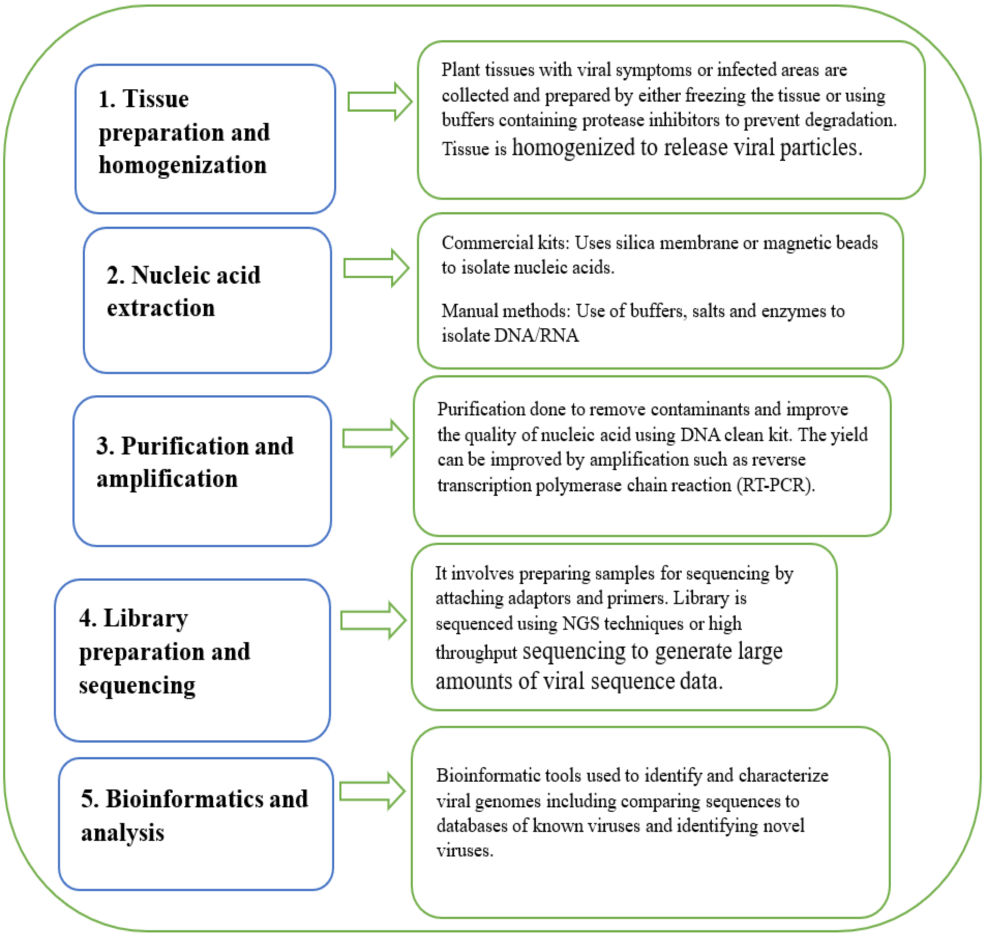
iv) Small Interfering RNAs (siRNAs): These type of RNAs is produced as a part of the host defense mechanism against viral infection. Sequencing siRNAs offers valuable information on virus-host interactions and can be used to infer the presence and identity of infecting viruses.

3.3 Nucleic Acid Extraction and Analysis

Fig. 1 illustrates the workflow of plant viral nucleic acid analysis, encompassing the extraction of viral nucleic acid, preparation of library, sequencing, and subsequent bioinformatic analysis.

3.4 Bioinformatic Analysis

Metagenomic data analysis relies on several bioinformatic tools designed to process and interpret large and complex datasets for diverse purposes such as viral identification, characterization, accurate detection. According to Pirovano et al., (2015), commonly used bioinformatic tools in metagenomics support various analytical tasks including taxonomic classification, sequence assembly, functional annotations, and binning, depending on the specific goals of the study. A combination of these tools can be used to address viral diversity and community composition. By using these computational tools, plant viral nucleic acid analysis facilitates the discovery and characterization of novel plant viruses and improves our understanding of their genomic organization and gene function (Fancello et al., 2012). Table 1 lists the common bioinformatic softwares categorized according to their applications in plant viral metagenomic research.



Plant tissues with viral symptoms or infected areas are collected and prepared by either freezing the tissue or using buffers containing protease inhibitors to prevent degradation. Tissue is homogenized to release viral particles.

Commercial kits: Uses silica membrane or magnetic beads to isolate nucleic acids.

Manual methods: Use of buffers, salts and enzymes to isolate DNA/RNA

Purification done to remove contaminants and improve the quality of nucleic acid using DNA clean kit. The yield can be improved by amplification such as reverse transcription polymerase chain reaction (RT-PCR).

It involves preparing samples for sequencing by attaching adaptors and primers. Library is sequenced using NGS techniques or high throughput sequencing to generate large amounts of viral sequence data.

Bioinformatic tools used to identify and characterize viral genomes including comparing sequences to databases of known viruses and identifying novel viruses.

Fig. 1. Steps for viral nucleic acid extraction for metagenomic study

Table 1. Bioinformatic tools used for Plant Virus metagenomic analysis

Type of metagenomic data	Bioinformatic tool	Applications
1. Assembly	MEGAHIT (Dinghua et al., 2014)	De novo assembler for metagenomic sequencing data efficient for large data sets
	metaSPADES (Nurk et al., 2017)	De novo assembler known for its accuracy and scalability, especially useful for short read data
	IDBA-UD (Peng et al., 2012)	De Bruijn graph-based assembler suitable for metagenomic data offering balance between speed and accuracy
	NECAT, metaFLYe, and Canu (Ibañez-Lligoña et al., 2023)	These tools designed for assembling long read metagenomic data generated form nanopore and PacBio sequencing
2. Taxonomic classification	Kraken (Wood, 2014)	Utilizes k-meters to identify and classify microbial species from metagenomic reads
	MetaPhlAn (Blanco-Míguez et al., 2023)	Provides species level analysis of microbial composition from shotgun method
	Centrifuge	Utilizing custom database for more accurate and sensitive classification
3. Functional analysis	MG-RAST (Keegan et al., 2016)	Popular tool for functional and taxonomic profiling metagenomic data

Type of metagenomic data	Bioinformatic tool	Applications
	HUMAnN2 (Yadav et al., 2022)	It identifies and quantifies functional pathways and genes from data
4. Binning	TETRA, S-GSOM, Phylopythia and ClaMS (Oulas et al., 2015)	These are used to group sequences into different taxonomic units
	CARMA, MetaPhyler and Sort-ITEMS (Liu et al., 2011)	These similarity based tools group sequences based on sequence similarity
	PhymmBL and MetaCluster (Oulas et al., 2015)	These tools employ a hybrid approach, combining both composition and similarity based information for binning
5. Other tools	BLAST (Bazinet et al., 2018)	Fundamental tool used for sequence alignment and homology searching, identifying similar sequences
	QIIME (Oulas et al., 2015)	Offers a wide range of analysis for quality control, OUT clustering, taxonomic classification and phylogenetic analysis
	AmpliconNoise, ChimeraSlayer and UCHIME (Hiraoka et al., 2016)	Tools for removing chimeric sequences ensuring accurate taxonomic classification

4. APPLICATIONS

4.1 Biotechnology and Nanotechnology

Plant viruses owing to their unique structural properties and ability of being genetically engineered holds immense potential in the field of biotechnology and nanotechnology.

Plant viruses are increasingly recognized as reliable biomolecular tool for gene expression and functional genomics (Abrahamian et al., 2020). One of the major applications of plant viruses lies in plant molecular farming, where viral vectors are used for high gene expression of recombinant proteins. The integration of viral vectors with *Agrobacterium tumefaciens* mediated delivery systems, has improved the production of complex therapeutic proteins and peptides including antibodies (Nunes-Leite et al., 2019). This advancement has enabled large scale production of bioactive compounds with significant pharmaceutical importance. Another major biotechnological application involves in crop biofortification and metabolic engineering through gene silencing approaches and overexpression strategies. Techniques such as virus induced gene silencing (VIGS) are widely used to suppress undesirable or enhance enzyme activity for metabolic pathways (Majer et al., 2017).

Next to biotechnological applications, these viruses emerged as flexible scaffolds in nanotechnology, with wide range of applications in vaccine development, gene therapy and nanomaterial designing (Schoenfeld et al., 2010). Their uniform and highly organized capsid structures serve as templates for synthesizing of variety of nanoparticles, developing drug delivery systems and fabricating nanosensors. Studies by Aumiller et al., 2018; Nkanga and Steinmetz, 2021, demonstrated the potential of plant viruses as efficient encapsulating agents and drug carriers for therapeutic molecules to desired tissues. Furthermore, viral nanoparticles (VNPs) are being utilized to construct highly sensitive nanosensors capable of detecting specific molecules such toxins and hormones. They have also been explored for improving the bioavailability and targeted delivery of small molecules such as insecticides, herbicides offering novel methods for pest control (Bonning et al., 2014; Cao et al., 2015).

Thus, the plant viruses represent a significant molecular tool in the field of biotechnology, offering wide applications ranging from molecular biology to pharmaceutical industry, functional genomics to bio-nanotechnology. Table 2 summarizes the biotechnological and bionanotechnological applications of metagenomic studies of plant viruses.

Table 2. Applications of plant viruses in biotechnology and nanotechnology

Name of the Plant virus	Application	References
Tobacco mosaic virus	High density data storage, imaging, sensing, catalysis and drug delivery	Balci et al., 2012
Cowpea mosaic virus	Biosensing, cancer immunotherapy	Beiss et al., 2020
Potato Virus X	Gene silencing, Protein expression	Faivre-Rampant et al., 2004
Tomato bushy stunt virus	Targeted delivery systems	Lico et al., 2021
Pea enation mosaic virus	Pest control management	Chaudhary et al., 2025
Alalfa mosaic virus	Gene delivery	Baris et al., 2024
Barley stripe mosaic virus	Gene silencing in cereals	Lee et al., 2012
Foxtail mosaic virus	Gene silencing	Tiedge et al., 2022
Cabbage leaf curl virus	Functional Genomics	Xiao et al., 2020
Bean yellow dwarf virus	Vaccine and antibody production	Chen et al., 2011

Table 3. Agricultural and environmental applications of plant virus metagenomics

Approaches	Applications of metagenomic sequencing
A) Agriculture applications	
1. Early detection and diagnosis of viral diseases	Unbiased detection helping early identification of emerging or latent viruses. This is crucial for timely control measures to minimizes crop losses.
2. Monitoring outbreak	Helps in epidemiological studies by identifying viral diversity, mutation patterns or emergence of new viral strains.
3. Identification of novel and mixed infections	Understanding these complex infections provides valuable information about synergistic interactions responsible for disease severity or alter host range
4. Crop breeding	Helps in developing disease resistant varieties via marker assisted selection and genetic engineering strategies
5. Quarantine measures	Strengthens quarantine regulations by detecting exotic or invasive viruses imported plant materials, seeds or propagation stocks.
6. Integrated pest and disease management	Sequencing viral communities in vectors (aphids, whiteflies, thirps) helps in understanding transmission and host virus adaptation mechanisms. Helps farmers in making decisions about crop rotation, vector control, sanitation measures.
B) Environmental studies	
1. Ecosystem indicators Viral diversity in natural and agricultural ecosystems	Identification of plant viruses in soil, water, air and plant associated microbiomes helps in studying viral diversity in natural and agricultural ecosystems. Act as bioindicators for pollution, deforestation, climate change etc.
2. Disease emergence	Changing virus host dynamics, emerging viral pathogens aids in early warning and prevention systems
3. Soil virome studies	Diversity of viruses in soil affecting root associated plants influencing nutrient cycling, carbon storage and plant growth. Information is helpful in monitoring soil health.
4. Pollution impact	Metagenomic profiling shows effect of pollution altering soil microbe and viral communities; useful in tracing contamination routes of viruses through waste and debris.
5. Environmental management	Rapid detection of invasive viral species, studying of virome of rare or endemic plants threatening conservation efforts, integrating metagenomics data into sustainable practices supports eco-friendly environmental management issues.

Next generation sequencing (NGS), is a powerful tool in biotechnology and nanotechnology that has revolutionized metagenomic studies, particularly in the characterization of plant viruses. Illumina sequencing provides valuable information for short read data whereas Oxford

Nanopore and PacBio provide long read data helpful in understanding the complex viral genome organization. These approaches allow the identification and characterization of plant viruses and viroids without prior knowledge of the pathogens and provides high throughput and sensitive, and comprehensive insights into viral communities (Al Rwahnih et al., 2009). Furthermore, NGS facilitates the analysis of viral co-infections, viral load estimation, and host range determination, providing useful information about virus-host interactions, pathogenicity, and transmission potential (Roossinck, 2011, Simmonds et al., 2017).

4.2 Agriculture and Environmental Applications

Metagenomic studies have become useful tools in detecting viral diseases at an early stage. This early detection is useful in quarantine and biosecurity programs, preventing disease outbreaks and minimize crop yield losses. It also plays vital role in identifying viruses that are responsible for significant threat to economically important crops (Chitra et al., 2022; Roossinck et al., 2015; Abrahamian et al., 2020; Sadvakasova et al., 2025) thereby guiding breeding programs to develop resistant varieties. Additionally, these approaches aids in epidemiological studies of viruses by tracking diversity of viruses and distribution in cultivated and wild plants, which aids in bio-surveillance and the development of effective disease management strategies (Villamor et al., 2019). In the environment context, viral metagenomics studies contributes in understanding the diversity and distribution of viruses in cultivated and natural ecosystems (Lefeuvre et al., 2019) , adaptation and transmission of viruses across ecological boundaries, virus evolution in relation to climate change (Roossinck, 2012, Sommers et al., 2019, Lopez-Roblero, 2023). The Table 3 summarizes the major applications in agricultural and environmental studies of plant virus metagenomic studies.

5. CHALLENGES AND FUTURE ASPECTS OF METAGENOMIC STUDIES

Though viral metagenomics had given promising results, but several challenges continue to restrict its full potential. Major limitations include absence of standardized protocols for sample collection, processing, and data analysis; incomplete reference databases for accurate identification and characterization, difficulties in assembling viral genomes present at low abundance within plant samples (Zhao et al., 2019). Additionally, the high cost of sequencing and computational analyses remains a significant barrier to its large-scale implementation.

However, advancements in real time sequencing, machine learning and global virome projects are expected to overcome these challenges, enhancing virus detection, prediction and expanding viral reference databases. Integration of metagenomics with functional genomics and synthetic biology holds great potential for providing practical applications in plant health management. Such interdisciplinary approaches facilitate the designing of novel biotechnological and diagnostic strategies (Prabha et al., 2020) leading to sustainable agricultural practices.

6. CONCLUSION

Plant virology has undergone a major transformation with the advancements made in the field of metagenomics. It offers a unique and précise approach for identification of the vast and previously hidden plant associated viruses. It has enhanced our understanding of viral diversity, evolution, ecology and role of viruses in agriculture and environmental systems, thereby overcoming many limitations of traditional diagnostic methods. These developments have strengthened the plant disease management, biosecurity measures, and early disease prediction systems for emerging viral threats. However, challenges persist which continue to impede progress. Bridging these knowledge gaps through coordinated efforts and improved bioinformatics tools, is crucial in plant virus management. Advancements in NGS technologies along with innovations in machine learning and artificial intelligence tools can further improve and revolutionize metagenomics of plant viruses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Author has declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Abrahamian, P., Hammond, R. W., & Hammond, J. (2020). Plant virus–derived vectors: Applications in agricultural and medical biotechnology. *Annual Review of Virology, 7*, 513–535.

Adams, I. P., Glover, R. H., Monger, W. A., et al. (2009). Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. *Molecular Plant Pathology, 10*(4), 537–545.

Al Rwahnih, M., Alabi, O. J., Westrick, N. M., & Golino, D. (2018). *Prunus geminivirus A*: A novel Grablovirus infecting *Prunus* spp. *Plant Disease, 102*, 1246–1253. https://doi.org/10.1094/PDIS-09-17-1486-RE

Al Rwahnih, M., Daubert, S., Úrbez-Torres, J. R., Cordero, F., & Rowhani, A. (2009). Deep sequencing evidence from single grapevine plants reveals a virome dominated by mycoviruses. *Virology, 387*(2), 395–401.

Aumiller, W. M., Uchida, M., & Douglas, T. (2018). Protein cage assembly across multiple length scales. *Chemical Society Reviews, 47*(10), 3433–3469.

Babalola, O. O., Osuji, I. E., & Akanmu, A. O. (2025). Amplicon-based metagenomic survey of microbes associated with the organic and inorganic rhizosphere soil of *Glycine max* L. *BMC Genomic Data, 26*, 40. https://doi.org/10.1186/s12863-025-01333-2

Balcı, M., Arpacı, B. B., Koç, M., & Erol, Ü. H. (2024). Response of some pepper genotypes to cucumber mosaic virus (CMV) and discrimination of Kilis isolates using high-resolution melting (HRM) method. *Turkish Journal of Agriculture-Food Science and Technology, 12*(8), 1435–1441.

Barış, A., Morca, A., & Alkan, M. (2024). Transmission of alfalfa mosaic virus through *Subcoccinella vigintiquatuorpunctata* (L.) (Coleoptera: Coccinellidae) in alfalfa growing areas in Türkiye. *Journal of Animal & Plant Sciences, 34*. https://doi.org/10.36899/japs.2024.1.0699

Bazinet, A. L., Ondov, B. D., Sommer, D. D., & Ratnayake, S. (2018). BLAST-based validation of metagenomic sequence assignments. *PeerJ, 6*, e4892. https://doi.org/10.7717/peerj.4892

Beiss, V., Mao, C., Fiering, S. N., & Steinmetz, N. F. (2022). Cowpea mosaic virus outperforms other members of the Secoviridae as in situ vaccine for cancer immunotherapy. *Molecular Pharmaceutics, 19*(5), 1573–1585.

Blanco-Míguez, A., Beghini, F., Cumbo, F., McIver, L., Thompson, K., Zolfo, M., et al. (2023). Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology, 41*, 1–12. https://doi.org/10.1038/s41587-023-01688-w

Bonning, B. C., Pal, N., Liu, S., Wang, Z., Sivakumar, S., Dixon, P. M., et al. (2014). Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids. *Nature Biotechnology, 32*(1), 102–105.

Cao, J., Guenther, R. H., Sit, T. L., Lommel, S. A., Opperman, C. H., & Willoughby, J. A. (2015). Development of abamectin loaded plant virus nanoparticles for efficacious plant parasitic nematode control. *ACS Applied Materials & Interfaces, 7*(18), 9546–9553.

Chaudhary, A., Naik, A., & Mistry, S. (2025). *Pea enation mosaic virus*. In *Compendium of Phytopathogenic Microbes in Agro-Ecology* (pp. 221–242). https://doi.org/10.1007/978-3-031-81884-4_15

Chen, Q., He, J., Phoolcharoen, W., & Mason, H. S. (2011). Geminiviral vectors based on bean yellow dwarf virus for production of vaccine antigens and monoclonal antibodies in plants. *Human Vaccines, 7*(3), 331–338. https://doi.org/10.4161/hv.7.3.14262

Chitra, N., Verma, R., Dhingra, N., Kumar, P., & Gaur, R. (2022). *Plant viral metagenomics: Current and future prospective for the characterization of genetic reservoir*. Agrica, 11, 41–53.

Destras, G., Sabatier, M., Bal, A., Simon, B., Semanas, Q., Regue, H., et al. (2024). *Comparison of metatranscriptomics and targeted-sequencing methods for integrative analysis of the whole microbiome*. https://doi.org/10.21203/rs.3.rs-3961672/v1

Dinghua, L., Chi-Man, L., Ruibang, L., Kunihiko, S., & Tak-Wah, L. (2014). *MEGAHIT: An ultra-fast single-node solution for large and* *complex metagenomics assembly via succinct de Bruijn graph*. Bioinformatics (Oxford, England), 31. https://doi.org/10.1093/bioinformatics/btv033

Faivre-Rampant, O., Gilroy, E. M., Hrubikova, K., Hein, I., Millam, S., Loake, et al. (2004). *Potato virus X-induced gene silencing in leaves and tubers of potato*. Plant Physiology, 34(4), 1308–1316. https://doi.org/10.1104/pp.103.037507

Fancello, L., Raoult, D., & Desnues, C. (2012). *Computational tools for viral metagenomics and their application in clinical research*. Virology, 434(2), 162–174. https://doi.org/10.1016/j.virol.2012.09.025

Fang, Y., & Ramasamy, R. P. (2015). *Current and prospective methods for plant disease detection*. Biosensors, 5, 537–561.

Hiraoka, S., Yang, C. C., & Iwasaki, W. (2016). *Metagenomics and bioinformatics in microbial ecology: Current status and beyond*. Microbes and Environment, 31(3), 204–212. https://doi.org/10.1264/jsme2.ME16024

Ibañez-Lligoña, M., Colomer-Castell, S., González-Sánchez, A., Gregori, J., Campos, C., Garcia-Cehic, D., et al. (2023). *Bioinformatic tools for NGS-based metagenomics to improve the clinical diagnosis of emerging, re-emerging and new viruses*. Viruses, 15, 587. https://doi.org/10.3390/v15020587

Keegan, K., Glass, E., & Meyer, F. (2016). *MG-RAST, a metagenomics service for analysis of microbial community structure and function*. https://doi.org/10.1007/978-1-4939-3369-3_13

Khan, J., & Dijkstra, J. (2006). *Handbook of plant virology* (1st ed.). CRC Press. ISBN not provided

Lee, W. S., Hammond-Kosack, K. E., & Kanyuka, K. (2012). *Barley stripe mosaic virus-mediated tools for investigating gene function in cereal plants and their pathogens: Virus-induced gene silencing, host-mediated gene silencing, and virus-mediated overexpression of heterologous protein*. Plant Physiology, 160(2), 582–590. https://doi.org/10.1104/pp.112.203489

Lefeuvre, P., Martin, D. P., Elena, S. F., Shepherd, D. N., Roumagnac, P., & Varsani, A. (2019). *Evolution and ecology of plant viruses*. Nature Reviews Microbiology, 17(10), 632–644.

Lico, C., Tanno, B., Marchetti, L., Novelli, F., Giardullo, P., Arcangeli, C., et al. (2021). *Tomato bushy stunt virus nanoparticles as a platform for drug delivery to Shh-dependent medulloblastoma*. https://doi.org/10.21203/rs.3.rs-703187/v1

Liu, B., Gibbons, T., Ghodsi, M., & Pop, M. (2011). *MetaPhyler: Taxonomic profiling for metagenomic sequences*. In Bioinformatics and Biomedicine (BIBM), 2010 IEEE International Conference (pp. 95–100).

Lopez-Roblero, A., Martínez Cano, D., Diego-García, E., Guillén-Navarro, G., Isa, P., & Zarza, E. (2023). *Metagenomic analysis of plant viruses in tropical fresh and wastewater*. Environmental DNA, 6. https://doi.org/10.1002/edn3.416

Majer, E., Llorente, B., Rodríguez-Concepción, M., & Daròs, J. A. (2017). Rewiring carotenoid biosynthesis in plants using a viral vector. *Scientific Reports, 7*(1), 41645.

Marios, V., Konstantinos, K., Dimitrios, K., Konstantinos, K., Svjetlana, R., Nikolina, U., Mitja, R., & Sotirios, V. (2025). Targeted analysis of metagenomes: Divide and conquer. *Biotechnology Advances, 83*, 108619. https://doi.org/10.1016/j.biotechadv.2025.108619

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, et al. (2015). Advanced methods of plant disease detection: A review. *Agronomy for Sustainable Development, 35*, 1–25.

Nkanga, C. I., & Steinmetz, N. F. (2021). The pharmacology of plant virus nanoparticles. *Virology, 556*, 39–61.

Nunes-Leite, L., Liefting, L. W., Waite, D. W., Khan, S., & Thompson, J. R. (2024). High-throughput sequencing methods for the detection of two *strawberry* viruses in post-entry quarantine. *Viruses, 16*(10), 1550.

Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. (2017). MetaSPAdes: A new versatile metagenomic assembler. *Genome Research, 27*, gr.213959.116. https://doi.org/10.1101/gr.213959.116

Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G. A., Papanikolaou, N., Kotoulas, G., et al. (2015). Metagenomics: Tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. *Bioinform Biol Insights, 5*(9), 75–88. https://doi.org/10.4137/BBI.S12462

Peng, Y., Henry, C. M., Leung, S. M., Yiu, F., & Chin, Y. L. (2012). IDBA-UD: A de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics, 28*(11), 1420–1428. https://doi.org/10.1093/bioinformatics/bts174

Pirovano, W., Miozzi, L., Boetzer, M., & Pantaleo, V. (2015). Bioinformatics approaches for viral metagenomics in plants using short RNAs: Model case of study and application to a *Cicer arietinum* population. *Frontiers in Microbiology, 27*(5), 790. https://doi.org/10.3389/fmicb.2014.00790

Prabha, K., Baranwal, V. K., & Jain, R. K. (2020). Applications of next generation high throughput sequencing technologies in characterization, discovery and molecular interaction of plant viruses. *Indian Journal of Virology, 31*(1), 24–35.

Roossinck, M. J. (2011). The good viruses: Viral mutualistic symbioses. *Nature Reviews Microbiology, 9*(2), 99–108.

Roossinck, M. J. (2012). Plant virus metagenomics: Biodiversity and ecology. *Annual Review of Genetics, 46*, 359–369.

Roossinck, M. J., Martin, D. P., & Roumagnac, P. (2015). Plant virus metagenomics: Advances in virus discovery. *Phytopathology, 105*(6), 716–727.

Roux, S., Matthijnssens, J., & Dutilh, B. E. (2021). Metagenomics in virology. *Encyclopedia of Virology*, 133–140.

Sadvakasova, A. K., Kossalbayev, B. D., Zaletova, D., Bauenova, M. O., Huang, Z., Zharmukhamedov, S. K., et al. (2025). Combating phytopathogens by integration of metagenomics and phototrophic biotechnologies: Toward sustainable agricultural practices. *Critical Reviews in Plant Sciences, 44*(2), 70–87. https://doi.org/10.1080/07352689.2025.2494872

Sankaran, S., Mishra, A., Ehsani, R., & Davis, C. (2010). A review of advanced techniques for detecting plant diseases. *Computers and Electronics in Agriculture, 72*, 1–13.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution, 3*, 430–439.

Schoenfeld, T., Liles, M., Wommack, K. E., Polson, S. W., Godiska, R., & Mead, D. (2010). Functional viral metagenomics and the next generation of molecular tools. *Trends in Microbiology, 18*(1), 20–29.

Shakir, S., Zaidi, S. S., Hashemi, F. S. G., Nyirakanani, C., & Vanderschuren, H. (2023). Harnessing plant viruses in the metagenomics era: From the development of infectious clones to applications. *Trends in Plant Science, 28*(3), 297–311. https://doi.org/10.1016/j.tplants.2022.10.005

Simmonds, P., Adams, M. J., Benkő, M., Brister, J. R., Carstens, E. B., Davison, A. J., et al. (2017). Virus taxonomy in the age of metagenomics. *Nature Reviews Microbiology, 15*(3), 161–168.

Sommers, P., Chatterjee, A., Varsani, A., & Trubl, G. (2019). Integrating viral metagenomics into an ecological framework. *Annual Review of Virology, 8*, 113–158.

Tiedge, K., Destremps, J., Solano-Sanchez, J., et al. (2022). Foxtail mosaic virus-induced gene silencing (VIGS) in *Panicum virgatum* L. *Plant Methods, 18*, 71. https://doi.org/10.1186/s13007-022-00903-0

Tremblay, J., Schreiber, L., & Greer, C. (2022). High-resolution shotgun metagenomics: The more data, the better? *Briefings in Bioinformatics*, bbac443.

Vargas-Albores, F., Martinez-Cordova, R., Martinez-Porchas, M., Calderón, K., & Lago-Leston, A. (2018). Functional metagenomics: A tool to gain knowledge for agronomic and veterinary sciences. *Biotechnology and Genetic Engineering Reviews, 35*, 1–23. https://doi.org/10.1080/02648725.2018.1513230

Villamor, D. E. V., Ho, T., Al Rwahnih, M., Martin, R. R., & Tzanetakis, I. E. (2019). High throughput sequencing for plant virus detection and discovery. *Phytopathology, 109*(5), 716–725.

Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence classification using exact alignments. *Genome Biology, 15*, R46. https://doi.org/10.1186/gb-2014-15-3-r46

Xiao, Z., Xing, M., Xing, L., Zhiyuan, F., Limei, Y., Yangyong, Z., et al. (2020). An efficient virus-induced gene silencing (VIGS) system for functional genomics in Brassicas using a cabbage leaf curl virus (CaLCuV)-based vector. *Planta, 252.* https://doi.org/10.1007/s00425-020-03454-7

Yadav, R., Kohli, A., & Prakash, J. (2022). Metagenomics: Current research, application and computational analysis. *Journal of Medical Pharmaceutical and Allied Sciences, 11*, 4901–4908. https://doi.org/10.55522/jmpas.V11I3.2931

Zhao, L., Rosario, K., Breitbart, M., & Duffy, S. (2019). Eukaryotic circular Rep-encoding single-stranded DNA (CRESS DNA) viruses: Ubiquitous viruses with small genomes and a diverse host range. *Advances in Virus Research, 103*, 71–133.

Peer-review history:
The peer review history for this paper can be accessed here:
https://pr.sdiarticle5.com/review-history/147810