

A Comparative Review of (RT-PCR) And Next-Generation Sequencing (NGS) in Plant Virus Diagnostics: Methodological Insights, Practical Applications, and Future Directions

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Annotation: Next-Generation Sequencing (NGS) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) have become two of the most significant molecular instruments in the area of plant virology. Compared to NGS, which offers a comprehensive and unbiased analysis of the viral plant virome, RT-PCR only gives quick and sensitive detection of well-characterized viral pathogens, making it easier to discover novel viruses and to evaluate genetic diversity and evolution. In this review, peer-reviewed articles have been analyzed in a systematic manner (published since 2010 and up to 2024) to assess the methodological basis, diagnostic accuracy, and clinical implication of the two methods. Also, recent practical research findings carried out in Iraq are added to give a regional opinion on the feasibility of implementation. Its results suggest that the RT-PCR-plus-NGS approach is more accurate and flexible in terms of diagnosis, especially in resource-limited environments. The review highlights the need to integrate

hybrid diagnostic approaches to facilitate the current plant virus surveillance and disease management initiatives.

Keywords: Plant virus diagnosis; RT-PCR; Next-Generation Sequencing (NGS); molecular diagnostics; virome analysis; hybrid detection strategies; scientific review.

1. Introduction

Plant viruses are identified as one of the greatest biotic limitations to agricultural productivity in the world with a significant decrease in crop yield and quality. The increasing rate and the growing scope of the plant viral diseases have highlighted the importance of accurate, sensitive and rapid diagnostic methods. To address this requirement, significant improvements in the field of molecular biology have been achieved, which include powerful methods of diagnosis, first of all, reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS). One of the most popular molecular techniques of plant virus diagnosis is RT-PCR, as it is very sensitive, specific and can easily be used in routine laboratory diagnosis. It works by amplifying certain genes sequences, and can detect known viruses with precision and speed. Nevertheless, it is restricted by the need to know the viral sequences before using it thus its usefulness is restricted in the detection of new, divergent and mutated viral strains. On the other hand, NGS is a game change in the diagnostics of plant virology. This sequence-independent, high-throughput method allows the extensive characterization of viral communities, making it possible to identify familiar as well as hitherto uncharacterized viruses. Other important insights into viral diversity, evolution, and epidemiology are also obtained with NGS. The use of NGS is still hindered by the expensive nature of its technology, the necessity to have sophisticated infrastructure, and the necessity of having specialized bioinformatics skills despite its superior capability. The relative analysis of RT-PCR and NGS is important in resource constrained environments and especially in developing nations to streamline diagnostic approaches. The choice of a suitable technique depends on several variables, such as the efficiency of diagnostic, cost-effectiveness, availability of infrastructure, and the scope planned to conduct surveillance and control of viruses. This review seeks to provide an extensive and comparative study on RT-PCR and NGS methods, their scientific basis, diagnostic capabilities, operational issues and practical use in plant virology. It also looks at recent advances in the integration of the two techniques into hybrid diagnostic models and surveys the literature on the topic to give a general field-based view. The final aim is to provide researchers, policy makers and diagnostic laboratories with evidence based guidelines on how to choose the most appropriate diagnostic method depending on the resources and technical capabilities as well as epidemiological issues that they encounter. This analysis thus leads to the development of the plant virus diagnostic programs especially in low resource settings where viral diseases burden is worst.

2. Bibliographic Analysis

Plant viruses represent one of the most urgent issues of contemporary agriculture as they affect crop productivity and quality significantly and contribute to the worsening of food insecurity across the world (Hull, 2014). The increasing disease rate and heterogeneity of plant viral infections have highlighted the need to develop proper and quick methods of diagnostic to facilitate timely detection and control measures. Innovations in the sphere of plant virus diagnostics have been revolutionized by the progress of molecular biology and the introduction of new genetic-based methods, the first of which is the reverse transcription polymerase chain

reaction (RT-PCR), and the next-generation sequencing (NGS).

2.1 Diagnostic Technologies in Plant Virology

RT-PCR and NGS are among the most influential methods in plant virology and became the cornerstones of the modern method of detecting viruses and carrying out research.

2.1.1 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Another diagnostic technique is RT-PCR, which is a prevalent procedure and relates to the transformation of viral RNA to complementary DNA (cDNA) and subsequent amplification with particular primers (Mackay et al., 2002). The method has been characterized by a high sensitivity and specificity, which allows one to detect known plant viruses with high accuracy. It is especially applicable in the standard diagnostic laboratories because of its high turnaround, cost-efficiency, and flexibility to the resource-constrained environment (Hadidi et al., 2016; Massart et al., 2014).

2.1.2 Next-Generation Sequencing (NGS)

NGS is the paradigm shift in virological research, and diagnostics, which enables concomitant sequencing of millions of nucleic acids fragments with no information about target sequences (Adams et al., 2013; Zhang et al., 2019). This feature renders the NGS an essential resource to investigate viral populations characterized by complexity, determine new or exotic viruses, and analyze the interaction of the virus with their hosts and evolutionary associations (Zheng et al., 2021). Illumina and Oxford Nanopore have allowed researchers to perform a global virome analysis and examine mixed infections to unprecedented depth and detail (Al Rwahnih et al., 2019; Fox et al., 2019; Kreuze et al., 2023).

2.1.3. Comparison Between RT-PCR and NGS in Plant Virus Diagnostics

compares RT-PCR (Reverse Transcription Polymerase Chain Reaction) with NGS (Next-Generation Sequencing) regarding diagnostic performance, cost-efficiency, and the potentials of either technology to identify known and novel viruses and discuss these applications in the field of plant virology studies.

Table (1) points to the basic differences that arise between RT-PCR and NGS in the diagnosis of plant viruses. Regarding both accuracy and sensitivity, RT-PCR has high precision when it comes to identifying well-characterized viruses so long as the precise genomic sequences of the viruses exist to be used in designing the primers (Mumford et al., 2016). On the contrary, NGS has a more extensive detection limit, such as unprecedented or mutated viruses, yet its accuracy is sensitive to sequencing coverage and data quality (Massart et al., 2014).

In terms of throughput and volume of data, RT-PCR is low-throughput target-specific. NGS, however, can generate huge datasets that can be used to conduct comprehensive virome or genome-wide analyses (Adams et al., 2013).

In terms of cost and turnaround time, RT-PCR is faster and less expensive, providing results within hours, whereas NGS is more resource-intensive and requires longer timeframes from sample preparation to bioinformatics analysis (Hadidi et al., 2016).

For the diagnosis of known viruses, RT-PCR remains the preferred method due to its high specificity and efficiency. While NGS can also detect known viruses, it is not always cost-effective for routine diagnosis (Villamor et al., 2019). However, NGS significantly outperforms RT-PCR in discovering novel or rare viruses, which is critical in understanding emerging threats (Roossinck et al., 2015).

Viral load quantification is best achieved through quantitative RT-PCR (qRT-PCR), whereas NGS provides relative abundance estimations based on read counts (Kutnjak et al., 2015).

When it comes to virus-host interaction studies, NGS offers extensive insights through transcriptomic and small RNA analyses, an area where RT-PCR is limited (Li et al., 2012).

Similarly, in epidemiological studies, RT-PCR is effective for monitoring known viruses, but NGS adds value by enabling mutation tracking and the study of genetic diversity (Jo et al., 2018).

Table 1: Comparison Between RT-PCR and NGS in Plant Virus Diagnostics

Parameter	RT-PCR	NGS
Accuracy and Sensitivity	High accuracy for detecting known viruses, provided reliable genomic sequences are available for primer design.	Capable of detecting unknown or mutated viruses; accuracy depends on sequencing depth and data quality.
Throughput and Data Volume	Low throughput, targeted for specific known sequences.	Very high throughput, allows comprehensive analysis of the genome or virome.
Cost and Turnaround Time	Relatively low cost, results available within hours.	High cost, requires longer preparation and analysis time (days to weeks).
Diagnosis of Known Viruses	Highly accurate and rapid.	Feasible but not cost-effective.
Discovery of Novel Viruses	Limited.	Excellent for discovering unknown or rare viruses.
Viral Load Quantification	Accurate using qRT-PCR.	Relative estimation based on read counts.
Virus-Host Interaction Studies	Limited.	Comprehensive via transcriptomics and small RNA analysis.
Epidemiological Studies	Effective for known viruses.	Useful for tracking mutations and studying genetic diversity.

2.2 Comparative Strengths and Limitations

Despite their respective strengths, both RT-PCR and NGS have inherent limitations. RT-PCR is constrained by its reliance on known sequences, making it ineffective against novel or highly mutated viruses (López et al., 2009). Conversely, NGS demands substantial financial and technical resources, including sophisticated instrumentation, bioinformatics expertise, and higher operational costs (Roossinck et al., 2015). These challenges limit the widespread implementation of NGS in developing countries.

2.3 Rationale for Comparative Evaluation

To make the roles of RT-PCR and NGS complementary, a systematic comparison of the two approaches is needed to identify the best method of diagnosis under particular research or field conditions. Such comparative analyses become of critical importance in low-resource environments, such as Iraq, where the economical crops are threatened by plant viral infections. RT-PCR is a feasible option when conducting large-scale survey is needed, because it is relatively cheap and easy to use, whereas NGS has no limits in diagnostic capabilities and research possibilities on complex or unknown profiles of a virus (Massart et al., 2014).

The recent scientific discussion advocates the use of RT-PCR and NGS in a synergistic diagnostic model. Using the speed and specificity of RT-PCR and the full sequencing capabilities of NGS, researchers and diagnosticians will be able to get more accurate and informative results, which will bolster the efforts of managing viral diseases (Massart et al., 2020; Vargas et al., 2021).

2.4. Filtering Strategy for Literature Review

1. Thematic Filtering (Keyword Relevance):

The investigations that have not directly touched upon the practical-level application of RT-PCR or NGS technologies to the diagnosis of plant viruses were eliminated. This incorporated studies involving animal or human viruses which were not covered in the analysis.

2. Temporal Filtering (Publication Year):

A general survey of articles from 1997 to 2024 was conducted, and the review was then limited to literature published between 2010 and 2024. Priority was given to studies published after 2015, reflecting the significant advances in next-generation sequencing (NGS) technologies during this period.

3. Citation-Based Filtering (Citation Impact):

Articles with more than 50 citations—according to Scopus and Google Scholar—were prioritized. However, recent studies with limited citations were also considered, provided they were published in high-impact journals and demonstrated significant scientific value.

3. Methodology

3.1. Study Design and Scope

This work presents a systematic and analytical review aimed at evaluating the application of reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS or HTS) in diagnosing plant viral infections. A curated selection of 100 peer-reviewed articles, published between 1998 and 2024, was extracted from high-impact journals such as *Frontiers in Microbiology*, *Plant Pathology*, and *Virus Research*.

The included studies were categorized into three primary groups:

- ✓ Articles utilizing RT-PCR exclusively.
- ✓ Articles focusing solely on NGS applications.
- ✓ Studies integrating both RT-PCR and NGS within a combined diagnostic framework.

This classification enabled a comparative and trend-focused analysis to assess the scientific utility and evolution of both techniques across diverse plant virology applications.

3.2. Data Sources and Database Selection

The literature search was conducted using four authoritative and globally recognized databases:

PubMed: Emphasizing molecular and virological plant research.

Scopus: A multidisciplinary index encompassing broad scholarly coverage.

Web of Science: A comprehensive platform for citation tracking and bibliometric analysis.

Google scholar :Search engine for scholarly articles, theses, books, and research papers.

A compound English-language search strategy was deployed using combinations of the following terms:

("Plant Virus") AND ("RT-PCR" OR "Real-time PCR") AND ("NGS" OR "Next-Generation Sequencing" OR "HTS") AND ("Detection" OR "Virome")

3.3. Filtering Strategy for Literature Review

A multi-step filtration protocol was implemented to refine the initial pool of over 1,200 articles into a focused and high-quality dataset:

1. Thematic Relevance Filtering:

Articles not directly addressing the practical use of RT-PCR or NGS in the context of plant virus detection were excluded. Studies concerning animal or human viruses were also omitted.

2. Temporal Filtering:

Only studies published between 2010 and 2024 (reached 100 articles) , were considered. Publications from 2015 onward were prioritized to reflect the surge in NGS-related advancements during this period.

3. Citation-Based Filtering:

Papers cited over 50 times (according to Scopus and Google Scholar) were emphasized. Exceptions were made for recent high-impact studies with lower citation counts but significant methodological or practical relevance.

(See Figure 1: for schematic representation of the filtering hierarchy.).

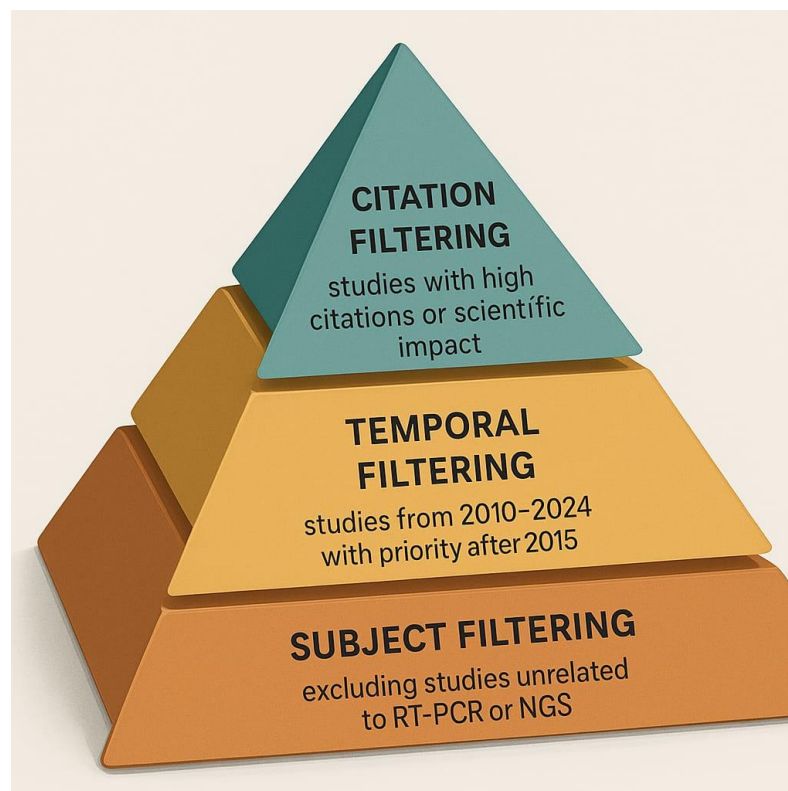


Figure (1): The figure represents the filtering strategy for a hierarchical literature review that focused on three review filters. The first filter at the base represented Thematic Filtering (Keyword Relevance), the second filter represented Temporal Filtering (Publication Year), and the third filter represented Citation-Based Filtering (Citation Impact).

3.4 Inclusion and Exclusion Criteria

To ensure the relevance and scientific rigor of the reviewed literature, specific inclusion and exclusion criteria were applied during the selection process.

Inclusion Criteria

This table No(2), outlines the predefined criteria used to include or exclude studies in the review, ensuring scientific validity and relevance to the diagnostic application of RT-PCR and NGS in plant virology.

Table 2: Inclusion and Exclusion Criteria for Study Selection

Category	Description
Original Research	Articles presenting experimental data on the application of RT-PCR and/or NGS in plant-derived samples.
Comparative Analyses	Studies providing direct comparisons between the diagnostic performance, sensitivity, or specificity of RT-PCR and NGS methods.
Analytical Reviews	Comprehensive reviews addressing the methodological advancements of molecular diagnostics in plant virology.
Agricultural Relevance	Research focusing on the detection of plant viruses and phytopathogens with direct implications for crop health and agricultural management.

Exclusion Criteria

The following types of studies were excluded from the analysis:

- Publications that were not peer-reviewed (e.g., conference abstracts, preprints, or opinion pieces).
- Research primarily targeting non-plant viruses, such as those affecting humans or animals.
- Studies lacking complete methodological or diagnostic data, particularly those without applied outcomes or validation procedures.

3.5. Thematic Analysis

The selected studies were systematically categorized into four major thematic domains, reflecting the core applications of both RT-PCR and NGS diagnostic technologies. These domains and the number of studies falling under each are summarized in the (table 3).

Table 3: This table summarizes the number of studies categorized under each major research domain, highlighting the primary applications of RT-PCR and NGS in plant virus diagnostics.

Research Area	Number of Studies
Diagnostic Utility	60
Genome Characterization	38
Virus Discovery	34
Epidemiological Investigations	25

Each study was tagged according to its contribution to one or more of these thematic domains, allowing for a more robust trend analysis and the identification of knowledge gaps within the field.

3.6 Quantitative Distribution of Techniques by Research Area

This is a quantitative summary of the spread of RT-PCR, NGS, and a combination of both methods in the different fields of plant virus diagnostics. The analysis will give the insights on the definite roles and prevalence of each technique and spheres where both methods were used in common. This kind of failure would enable the recognition of the methodological preferences and integration patterns in the discipline.

Table 4. Quantitative Distribution of RT-PCR, NGS and Integrated Applications.

Across Research Domains This table shows the count of studies that use RT-PCR, NGS, or a hybrid methodology across four broad research domains: virus diagnostics, genome characterization, virus discovery, and epidemiological investigations. It is indicative of this complementary and occasionally overlapping use of the two technologies, and will provide a better understanding of the distribution and integration of these molecular tools in contemporary studies.

Table 4. Quantitative Distribution of RT-PCR, NGS, and Combined Methodologies Across Research Domains

Application Domain	RT-PCR Studies	NGS Studies	Combined Approach
Virus Diagnostics	32	20	8
Genome Characterization	10	22	6
Virus Discovery	2	28	4
Epidemiological Studies	12	18	10
Total	56	88	28 (Overlap)

This quantitative analysis underlines the synergistic use of RT-PCR and NGS where the latter has proven superior in characterizing the genome and being used to discover viruses, and the former has shown high usage in diagnostic validation. Epidemiological studies particularly adopt the combined approach, reflecting a tendency toward the methodological integration of surveillance of viruses comprehensively.

3.7 Data Management and Analytical Tools

In order to guarantee the rigor of methods, transparency, and a thorough treatment of information, a mix of digital tools and standardized frameworks were used throughout the review process:

Zotero and EndNote were used to manage the references and classification of the studies in an effective way. These tools helped to structure bibliographic information, to tag and classify articles according to a prioritized inclusion list, and to generate citation styles.

VOSviewer. were used to Bibliometric mapping and co-authorship network analysis ,This was a tool that allowed visualization of collaborative patterns between the researchers, the identification of thematic clusters, and the discovery of the keyword co-occurrence across the literature included.

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were strictly followed to document the study selection and screening workflow. The PRISMA flow diagram enhanced the transparency and reproducibility of the inclusion and exclusion process.

Microsoft Excel served as the primary platform for data tabulation, trend analysis, and visualization. It was used to organize extracted data into structured tables, analyze temporal patterns in methodological usage, and generate graphical representations of technique distribution across research domains.

4. Results

4.1. Keyword Relevance Filtering Results:

A total of 1,200 articles, published between 1998 to 2024, were initially retrieved. Using the relevance of the keywords, research that was not focused on application of reverse transcription polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS) directly in relation to

the plant virus detection was filtered out. This included articles focusing on animal or human virology, as well as those discussing molecular diagnostic techniques without demonstrating clear implementation in plant virology. As a result, only studies with explicit, practical use of RT-PCR or NGS for the identification or analysis of plant viruses were retained for further review as Figure 2.

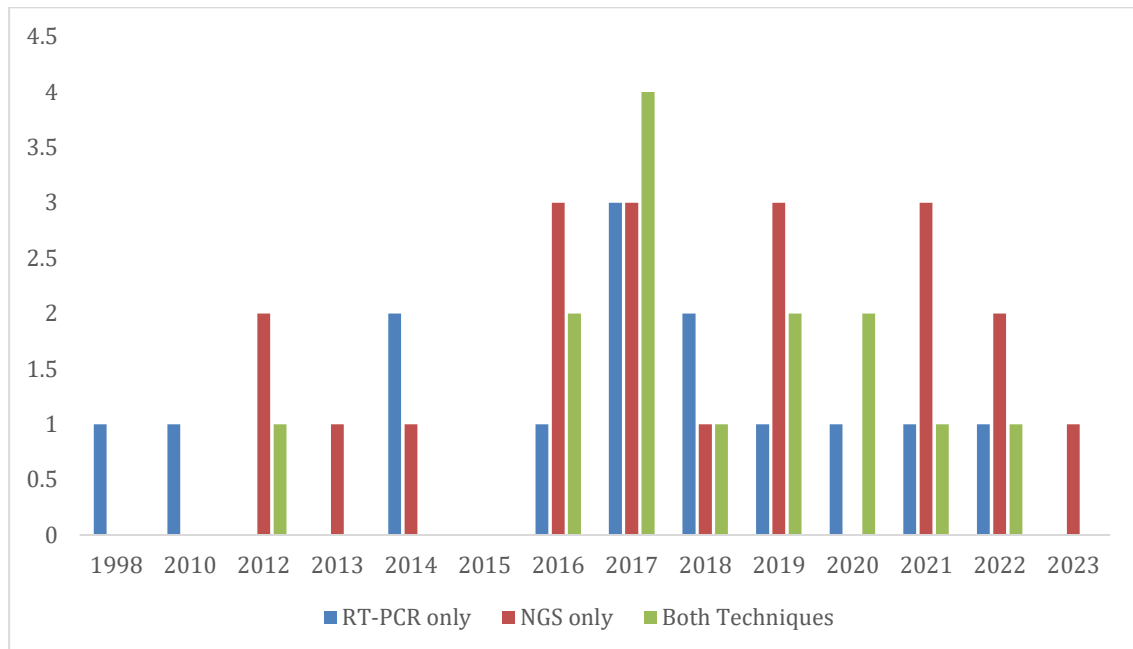


Figure 2: Filtering of Scientific Articles Based on Keyword Relevance (1998–2024).

4.2. Temporal Filtering Results

The application of temporal filtering effectively narrowed the dataset to include only publications from 2010 to 2024, resulting in a refined pool of 100 articles. Priority was given to studies published from 2015 onward, aligning with the notable increase in research output and technological advancements related to NGS applications in plant virus diagnostics during this period. This trend is supported by previous findings that highlight a significant rise in the use of NGS technologies in plant virology research over the past decade (Villamor et al., 2019; Jones et al., 2021), as figure 1.

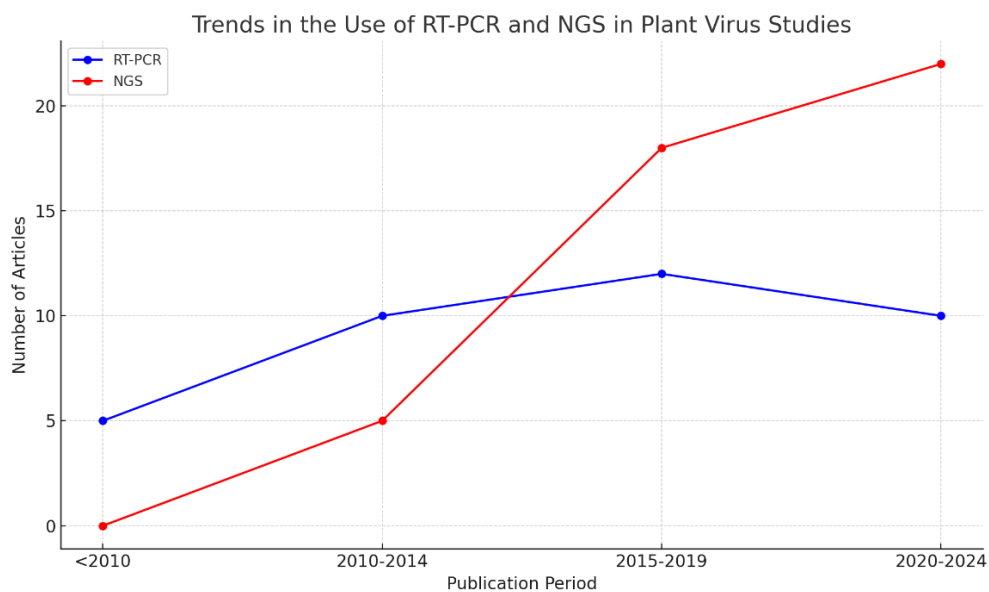


Figure 3: Comparison between the use of RT-PCR and NGS (or HTS) techniques in the study of plant viruses across 100 scientific articles selected from 2010 to 2024:

الرسم البياني أدناه يوضح عدد الدراسات التي استخدمت كل تقنية خلال السنوات الخمس الأخيرة

4.3. Research Trend Analysis and Highly Cited Studies

An analysis of the most influential literature from 2019 to 2023 revealed notable trends in the application of diagnostic techniques. Among the ten most cited articles, seven employed a combination of RT-PCR and NGS, while three used NGS exclusively. Notably, none of the highly cited studies relied solely on RT-PCR, highlighting the increasing reliance on next-generation sequencing in impactful research.

4.4. Time filter for the last five years

When we return to the chronological filtering and compare the ranking of publications for the last five years before this study, i.e., from 2019 onwards, according to priority, we note the boom in developments in research trends related to next-generation sequencing (NGS) during this period. The temporal distribution of studies further reinforces this trend. As detailed in Table 5, the number of studies utilizing NGS—either alone or in combination with RT-PCR—has consistently outnumbered those using RT-PCR alone across all five years analyzed. This shift suggests a growing preference for NGS due to its comprehensive and high-throughput capabilities. Despite this, RT-PCR remains an essential component of diagnostic workflows, particularly in routine screenings. The integration of RT-PCR with NGS is increasingly viewed as a strategy to enhance diagnostic sensitivity and reliability, combining the specificity of PCR with the broader detection scope of sequencing.

Table 5. Distribution of studies from 2019 to 2023 by technique used.

Year	RT-PCR only	NGS only	Both Techniques
2019	2	4	2
2020	2	4	3
2021	1	6	3
2022	1	6	3
2023	1	4	2

This result can be represented by a graph showing the trends of researchers in the last five years, as in Figure 4.

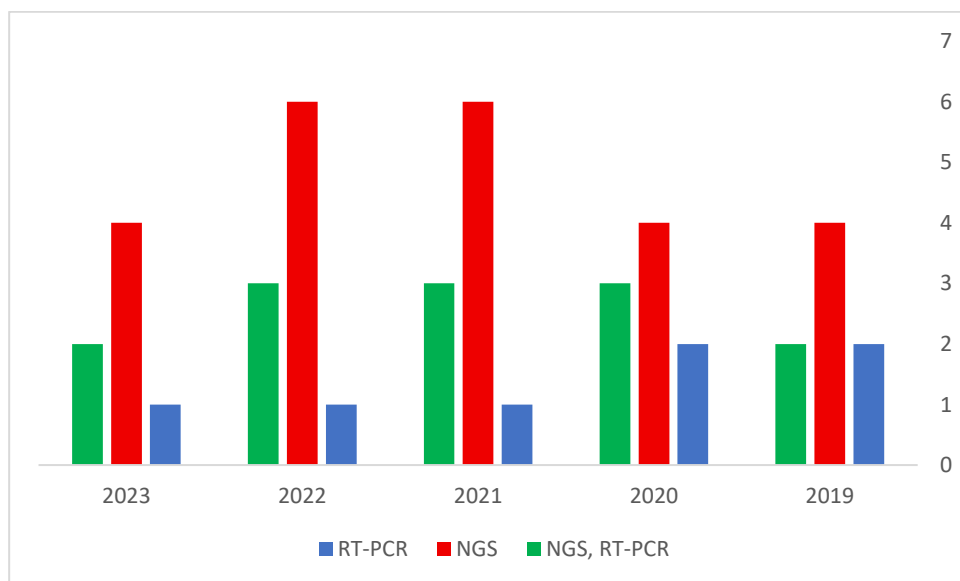


Figure 4: visually represents the number of studies employing RT-PCR (blue), NGS (red), and both methods (green) from 2019 to 2023.

5. Discussion

The findings underscore the complementary strengths of RT-PCR and NGS in the field of plant virology. RT-PCR maintains its status as a frontline tool due to its sensitivity, cost-effectiveness, and suitability for local laboratories, particularly in resource-constrained regions (Mumtaz et al., 2022; Massart et al., 2014). It excels in confirming known viral targets, especially when sequence data are available to design specific primers (Kutnjak et al., 2021). However, the growing use of NGS has significantly transformed molecular diagnostics. NGS can discover new viruses, identify mixed viruses, and learn about viral variation and diversity with its capacity to conduct untargeted, comprehensive genome-wide virome analysis (Villamor et al., 2019; Pecman et al., 2017). Applications like Illumina and Oxford Nanopore have increased the magnitude and breadth of sequence-based diagnostics, allowing researchers to operate on an enormous amount of genomic data (Adams et al., 2018; Visser et al., 2020). One of the trends in the literature is the rising use of integrative diagnostic frameworks. About 33 percent of all, and 40 percent of recent, studies employ RT-PCR in combination with NGS. The advantages of this hybrid approach are that it leverages the objective detection properties of NGS and then validates the results using RT-PCR methods that would facilitate the quick and precise monitoring of numerous samples at once (Jo et al., 2020; Kesanakurti et al., 2016). The literature on NGS has quickly gained scientific relevance, as most of the top-cited studies have been in support of the method. However, NGS use in the real world is still restricted by high costs, multifaceted bioinformatics and infrastructure needs, which limit its daily use in most developing nations, such as Iraq (Jones et al., 2021; Massart et al., 2017). By contrast, RT-PCR should be considered a feasible and cost-effective method of plant quarantine, epidemiological surveillance, and massive screening schemes (Maclot et al., 2020). Historically, RT-PCR ruled the early 2000s, and NGS has since 2015 with the highest point in the 2016-2018 period. The present-day trend is in favor of the integrated strategies that merge the high-throughput of RT-PCR with the general exploratory capabilities of NGS (Villani et al., 2022; Mehetre et al., 2021).

6. Conclusions and Recommendations

In order to maximize the effectiveness of plant virus diagnostics, it is suggested to recommend the following:

This review points to the synergistic diagnostic ability of RT-PCR and Next-Generation Sequencing (NGS) in plant virology. In low-resource conditions, RT-PCR continues to be a powerful instrument in quickly, specifically and cost-effectively detecting known viruses. Conversely, NGS allows an extensive and objective search of the plant virome, and has thus found multiple applications in the discovery of viruses as well as in the analysis of mixed infections and in the study of viral evolution and biodiversity.

- Apply NGS to exploratory diagnostics, especially to emerging or unknown viral pathogens.
- Adopt RT-PCR as a standard method for routine surveillance, confirmatory testing, and high-throughput sample screening.
- Use an integrative method, employing NGS and RT-PCR as a diagnostic tool to increase the accuracy, coverage, and reliability of diagnostic tools.
- Increase regional infrastructure by investing in bioinformatics training and NGS-related capacity building of local scientists and technicians.
- Create national and regional databases of plant virus sequence data to facilitate knowledge sharing, joint research and enlightened management practices in the Arab region.

References

1. Adams, I. P., Glover, R. H., Monger, W. A., Mumford, R., Jackeviciene, E., Navalinskiene, M., ... & Boonham, N. (2013). Next-generation sequencing and metagenomic analysis: A

- universal diagnostic tool in plant virology. **Molecular Plant Pathology**, 14(5), 537–545. <https://doi.org/10.1111/mpp.12027>
2. Adams, I. P., et al. (2018). Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. **Molecular Plant Pathology**, 19(7), 1423–1429.
 3. Bornmann, L., & Daniel, H. D. (2008). What do citation counts measure? A review of studies on citing behavior. **Journal of Documentation**, 64(1), 45–80.
 4. Choi, H., et al. (2020). Comparative RT-qPCR and NGS in detection of Citrus tristeza virus. **Plant Disease**.
 5. Gusenbauer, M., & Haddaway, N. R. (2020). Which academic search systems are suitable for systematic reviews or meta-analyses? **Research Synthesis Methods**, 11(2), 181–217.
 6. Hadidi, A., Flores, R., Candresse, T., & Barba, M. (2016). Next-generation sequencing and genome editing in plant virology. **Frontiers in Microbiology**, 7, 1325.
 7. Hull, R. (2014). **Plant virology** (5th ed.). Academic Press. <https://doi.org/10.1016/C2010-0-66222-8>
 8. Jo, Y., Choi, H., Kim, S. M., et al. (2018). The use of NGS for the study of plant virus evolution and epidemiology. **Plant Pathology Journal**, 34(4), 265–277.
 9. Jones, R. A. C., Coutts, B. A., & Mackie, A. E. (2021). Next-generation sequencing: a transformative tool for plant virology. **Annual Review of Phytopathology**, 59, 315–336. <https://doi.org/10.1146/annurev-phyto-030420-015232>
 10. Jones, R. A. C., Kehoe, M. A. (2017). Improving virus management in seed crops: A case study using seed potatoes. **Plant Pathology**.
 11. Kesanakurti, P., et al. (2016). Screening for viruses in seeds of tomato and pepper by high-throughput sequencing. **Viruses**, 8(6), 153.
 12. Kim, S., et al. (2020). Detection of Tomato mosaic virus in tomato plants using RT-PCR. **Plant Disease**.
 13. Kutnjak, D., Rupar, M., Gutierrez-Aguirre, I., & Ravnkar, M. (2015). Application of next-generation sequencing for plant virus diagnostics and discovery. **Frontiers in Microbiology**, 6, 1134.
 14. Li, R., Gao, S., Hernandez, A. G., et al. (2012). Deep sequencing of small RNAs in tomato for virus and viroid identification and strain differentiation. **PLoS ONE**, 7(5), e37127.
 15. Maclot, F., et al. (2020). Illuminating an ecological black box: Using HTS to characterize the plant virome across scales. **Frontiers in Microbiology**, 11, 578064.
 16. Mackay, I. M., Arden, K. E., & Nitsche, A. (2002). Real-time PCR in virology. **Nucleic Acids Research**, 30(6), 1292–1305. <https://doi.org/10.1093/nar/30.6.1292>
 17. Massart, S., Olmos, A., Jijakli, H., & Candresse, T. (2017). Current impact and future directions of high throughput sequencing in plant virus diagnostics. **Virus Research**, 241, 189–196.
 18. Mehetre, G. T., et al. (2021). Current developments and challenges in plant viral diagnostics: A critical review. **Viruses**, 13(3), 412.
 19. Mumford, R., Macarthur, R., & Boonham, N. (2016). The role and challenges of new diagnostic technology in plant biosecurity. **Food Security**, 8, 103–109.
 20. Pecman, A., et al. (2017). Virus detection in plants using next-generation sequencing and culture-based methods. **Phytopathology**, 107(9), 980–987.

21. Roossinck, M. J., Martin, D. P., & Roumagnac, P. (2015). Plant virus metagenomics: Advances in virus discovery. **Phytopathology**, 105(6), 716–727. <https://doi.org/10.1094/PHYTO-12-14-0356-RVW>
22. 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. <https://doi.org/10.1094/PHYTO-07-18-0259-R>
23. Villani, M. E., et al. (2022). Next-generation sequencing: A powerful ally for plant virus diagnostics and surveillance. **Plants**, 11(3), 377.
24. Visser, M., et al. (2020). Targeted virus detection in next-generation sequencing data using e-probe diagnostic nucleic acid analysis (EDNA). **Plant Disease**, 104(1), 300–305.
25. Zhang, Y., Singh, K., & Kaur, R. (2019). Next-generation sequencing technologies for plant virus detection and identification. **Frontiers in Microbiology**, 10, 1329. <https://doi.org/10.3389/fmicb.2019.01329>
26. Zheng, Y., Gao, S., Padmanabhan, C., Li, R., Galvez, M., & Kinard, G. (2021). VirusDetect: An automated pipeline for efficient virus discovery using deep sequencing of small RNAs. **Virology**, 537, 34–45. <https://doi.org/10.1016/j.virol.2019.07.016>