INTERNATIONAL JOURNAL OF INTELLECTUAL ADVANCES FOR MULTIDISCIPLINARY SCIENCES

IJIAMS.COM

Volume 02, Issue 03: Year 2026

Advancing Viral Diagnostics in Perennial Crops: A Review of High-Throughput Sequencing and Targeted qPCR Technologies

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Abstract

Early and accurate detection of viral infections is essential for maintaining the productivity and long-term sustainability of perennial crops. Owing to their extended life cycle and repeated exposure to pathogens, perennial plants are particularly vulnerable to gradual viral spread, which often remains unnoticed until severe yield losses occur. In recent years, molecular diagnostic tools have transformed plant virus detection by enabling faster and more reliable identification of pathogens. This review examines the role of high-throughput sequencing (HTS) and targeted quantitative polymerase chain reaction (qPCR) as two major approaches for viral diagnostics in perennial cropping systems.
High-throughput sequencing offers a comprehensive platform for detecting known and unknown viruses through whole-genome and metagenomic analysis. It provides valuable insights into viral diversity, mixed infections, and evolutionary patterns, making it highly useful for surveillance and research-based applications. In contrast, targeted qPCR remains a practical and cost-effective technique for routine screening due to its high sensitivity, rapid processing time, and ease of standardization. This paper critically compares both technologies in terms of detection accuracy, operational requirements, turnaround time, and relevance to field-level disease management. The review also discusses current challenges such as technical complexity, data interpretation, and limited accessibility in resource-constrained regions. By evaluating the complementary strengths of HTS and qPCR, this study highlights their combined potential for improving early diagnosis, supporting informed management decisions, and strengthening viral disease control strategies in perennial agriculture.

Keywords: *Perennial crops; viral diagnostics; high-throughput sequencing; quantitative PCR; early disease detection; crop health management*

I. Introduction

Perennial crops such as fruit trees, grapevines, tea, coffee, and plantation crops play a vital role in global agriculture and rural economies. Unlike annual crops, these plants remain productive for many years and are repeatedly exposed to biotic and abiotic stresses throughout their life cycle. Among these challenges, viral diseases represent a major threat due to their persistent nature, silent spread, and limited treatment options once infection is established [1], [2]. Viral infections in perennial crops often remain undetected for long periods, leading to gradual yield decline, reduced fruit quality, and long-term economic losses [3].

Traditional diagnostic methods, including visual inspection and serological assays, have been widely used for virus detection. However, these approaches are frequently limited by low sensitivity, cross-reactivity, and dependence on symptom expression, which may vary with environmental conditions and plant age [4]. As a result, early and reliable diagnosis remains a critical requirement for effective disease management and certification programs [5]. In response to these limitations, molecular diagnostic technologies have emerged as powerful tools for improving virus detection in perennial cropping systems.

Targeted quantitative polymerase chain reaction (qPCR) has become one of the most commonly adopted molecular techniques for routine virus diagnosis. It offers high sensitivity, specificity, and relatively rapid processing, making it suitable for large-scale screening and quarantine inspections [6], [7]. qPCR enables precise quantification of viral load and supports standardized testing protocols across laboratories. Nevertheless, its dependence on prior sequence information restricts its ability to detect unknown or highly variable viruses [8].

High-throughput sequencing (HTS), also referred to as next-generation sequencing, has revolutionized plant virology by enabling comprehensive analysis of viral genomes without prior assumptions. Through metagenomic and small RNA sequencing approaches, HTS facilitates the discovery of novel viruses, mixed infections, and complex viral populations within host plants [9]. This technology has significantly enhanced our understanding of virus evolution, host–pathogen interactions, and disease epidemiology in perennial crops [10]. However, challenges related to cost, data analysis, infrastructure, and technical expertise continue to limit its widespread adoption in routine diagnostics.

Given the complementary strengths and limitations of qPCR and HTS, a comparative evaluation of these technologies is essential for developing effective diagnostic strategies. Understanding their performance in terms of sensitivity, turnaround time, accessibility, and practical utility can support informed decision-making in crop protection programs. This review aims to examine recent advances in targeted qPCR and high-throughput sequencing for viral diagnostics in perennial crops, highlighting their roles in early detection, disease monitoring, and sustainable crop management [11].

II. Literature Review

The rapid progression of molecular diagnostic technologies over the past decade has profoundly influenced plant virus detection, especially in complex perennial cropping systems. Early literature underscores significant limitations of traditional diagnostic methods, such as enzyme-linked immunosorbent assays (ELISA) and biological indexing, which often lack the sensitivity and specificity required for reliable detection of low-titer or asymptomatic viral infections in perennial hosts [12], [13]. These constraints have driven the adoption of more advanced molecular approaches such as targeted quantitative PCR (qPCR) and high-throughput sequencing (HTS).

Quantitative PCR emerged as a pivotal methodology for virus detection due to its ability to deliver high sensitivity and specificity while quantifying viral load [14]. Initial studies demonstrated that qPCR could detect viral genomes at much lower concentrations than conventional PCR, enabling early diagnosis before visible symptom development [15]. Subsequent work focused on optimizing qPCR assays for a broad range of perennial crop viruses, including strategies to reduce nonspecific amplification and improve inter-laboratory consistency [16]. Researchers also underscored the importance of designing primers and probes that can accommodate genetic diversity among viral strains to avoid false negatives [17].

While qPCR has become a mainstay in routine diagnostics, its reliance on prior knowledge of target sequences has restricted its utility against novel or highly divergent viral agents. To overcome this, high-throughput sequencing has been increasingly explored for its hypothesis-free detection capabilities. HTS technologies, encompassing whole genome sequencing, RNA-Seq, and small RNA sequencing, allow comprehensive profiling of viral communities in plant tissues without requiring predefined target sequences [18]. Early applications of HTS in perennial crops revealed previously undetected viruses, characterized mixed infections, and provided insights into complex viral dynamics [19]. These capabilities have significant implications for understanding virus evolution, host adaptation, and epidemiology.

Comparative studies have examined the relative strengths of qPCR and HTS. For example, several investigations have shown that HTS consistently identifies a broader spectrum of viruses, including low-abundance and novel agents missed by targeted methods [20]. However, qPCR often surpasses HTS in terms of cost efficiency, ease of implementation, and faster turnaround times, making it highly attractive for routine surveillance and certification programs [21]. Moreover, advancements in HTS data analysis pipelines have enhanced viral genome assembly and annotation, yet challenges related to computational requirements and interpretation complexity remain significant barriers for widespread adoption [22].

Recent literature advocates a complementary diagnostic framework where HTS is utilized for initial pathogen discovery and comprehensive surveys, while targeted qPCR assays are developed subsequently for high-throughput monitoring and management decision support [23]. This integrative approach leverages the unique advantages of both technologies and aligns with sustainable crop protection strategies in perennial agriculture.

III. Methodological Comparison

The selection of appropriate diagnostic methodologies is central to effective viral surveillance in perennial crops. Targeted quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS) represent two widely adopted molecular approaches, each characterized by distinct operational principles, technical requirements, and analytical capabilities. A systematic comparison of these methods is essential for understanding their suitability across diverse diagnostic contexts.

Targeted qPCR is based on the amplification of specific viral genomic regions using sequence-specific primers and fluorescent probes. The procedure typically involves nucleic acid extraction, reverse transcription for RNA viruses, and real-time amplification under controlled thermal cycling conditions. This technique offers high analytical sensitivity, often detecting viral copies at very low concentrations, and provides quantitative data that support infection intensity assessment [24]. Furthermore, standardized protocols and commercially available kits facilitate reproducibility and inter-laboratory consistency [25]. However, qPCR performance is highly dependent on primer design and sequence stability, which may limit detection when viral mutations or recombinant strains occur [26].

In contrast, HTS relies on massively parallel sequencing of nucleic acids, enabling the comprehensive characterization of viral populations without prior sequence knowledge. Sample preparation for HTS generally includes nucleic acid fragmentation, library construction, adapter ligation, and sequencing on specialized platforms. Bioinformatic pipelines are subsequently applied for read assembly, annotation, and taxonomic classification [27]. This workflow allows simultaneous detection of multiple viruses, identification of novel pathogens, and analysis of mixed infections, thereby offering a broader diagnostic perspective than targeted methods [28]. Nevertheless, HTS requires advanced infrastructure, skilled personnel, and substantial computational resources, which may restrict its routine application in many diagnostic laboratories.

From an operational standpoint, qPCR is characterized by relatively short turnaround times, often delivering results within a few hours after sample processing. Its cost-effectiveness and scalability make it suitable for large-scale screening, certification programs, and quarantine inspections. HTS, although more time-consuming and expensive, provides in-depth genomic information that supports epidemiological studies and long-term surveillance initiatives [29]. Consequently, HTS is often employed in research-oriented settings or during outbreak investigations, while qPCR remains the preferred tool for routine monitoring.

Recent methodological frameworks emphasize the integration of both technologies within tiered diagnostic systems. In such models, HTS is utilized for initial pathogen discovery and population-level assessments, followed by the development of targeted qPCR assays for rapid field deployment. This complementary approach enhances diagnostic accuracy while optimizing resource utilization. Overall, the methodological comparison highlights that neither technique alone is universally sufficient; rather, their combined application offers a balanced strategy for effective viral diagnostics in perennial crop systems.

IV. Analysis and Discussion

The comparative evaluation of high-throughput sequencing (HTS) and targeted quantitative polymerase chain reaction (qPCR) reveals important insights into their practical effectiveness for viral diagnostics in perennial crops. Both technologies have demonstrated substantial improvements over conventional diagnostic methods; however, their utility varies according to operational objectives, resource availability, and disease management priorities. An analysis of recent studies indicates that qPCR remains the preferred method for routine surveillance due to its high sensitivity, rapid processing time, and ease of implementation in standard diagnostic laboratories [30], [31]. Its ability to quantify viral load also supports informed decisions regarding disease severity and crop management interventions.

Despite these advantages, qPCR exhibits inherent limitations related to its dependence on predefined target sequences. Several investigations have reported reduced detection efficiency in cases involving genetically diverse or emerging viral strains, highlighting the risk of false-negative results under such conditions [32]. This limitation is particularly relevant in perennial systems, where prolonged host–pathogen interactions can promote viral mutation and recombination. Consequently, exclusive reliance on targeted diagnostics may result in incomplete disease assessments.

In contrast, HTS offers a comprehensive diagnostic platform capable of identifying both known and previously uncharacterized viruses. Studies have consistently shown that HTS outperforms targeted methods in detecting mixed infections and low-abundance viral populations [33], [34]. These features are essential for understanding complex disease etiologies in long-lived crops, where multiple pathogens often coexist. Moreover, HTS-derived genomic data facilitate phylogenetic and epidemiological analyses, supporting long-term disease monitoring and resistance breeding programs [35]. However, high operational costs, extended processing times, and data interpretation challenges continue to constrain its routine use, particularly in developing agricultural regions.

The integration of HTS and qPCR within tiered diagnostic frameworks has emerged as a promising strategy for optimizing disease detection and management. In such systems, HTS is applied during preliminary surveys or outbreak investigations, while qPCR is subsequently employed for high-throughput screening and monitoring [36]. Empirical evidence suggests that this combined approach enhances diagnostic reliability while minimizing financial and technical burdens [37]. Additionally, recent advancements in portable sequencing devices and automated bioinformatic tools may gradually reduce barriers to HTS adoption, thereby expanding its accessibility [38].

From a management perspective, accurate and timely diagnostics directly influence disease containment and productivity in perennial crops. Early detection enables the removal of infected plant material, implementation of quarantine measures, and selection of virus-free propagation stocks. The findings of this review indicate that while qPCR remains indispensable for operational diagnostics, HTS plays a critical role in strategic surveillance and knowledge generation. Therefore, future diagnostic systems should emphasize methodological complementarity, capacity building, and cost reduction to ensure sustainable viral disease management across diverse agricultural settings.

V. Conclusion

Effective management of viral diseases in perennial crops depends largely on timely and accurate diagnostic practices. This review has examined the roles of high-throughput sequencing and targeted quantitative PCR as two major molecular tools that have significantly advanced plant virus detection. While targeted qPCR remains a reliable and practical option for routine screening due to its speed, sensitivity, and affordability, it is inherently limited by its dependence on known viral sequences. In contrast, high-throughput sequencing offers a comprehensive approach for identifying both known and emerging viruses, providing valuable insights into complex infection patterns and viral diversity.

The comparative analysis highlights that neither technology alone is sufficient to address the diverse diagnostic challenges present in perennial cropping systems. Instead, their combined application offers a balanced and efficient strategy for improving disease surveillance and management. Integrating high-throughput sequencing for exploratory analysis with qPCR for large-scale monitoring can enhance early detection and support informed decision-making.

Looking ahead, continued efforts are needed to improve accessibility, reduce operational costs, and strengthen technical capacity in diagnostic laboratories. Advances in portable sequencing platforms, automated data analysis tools, and standardized testing protocols are expected to further expand the practical use of molecular diagnostics. By adopting integrated and adaptable diagnostic frameworks, agricultural stakeholders can improve crop health, minimize economic losses, and promote the long-term sustainability of perennial crop production systems.

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