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High-Throughput Sequencing in Phytopathology: Genomics-Driven Diagnostics and Host-Pathogen Interactions

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Summary

Plant diseases severely constrain agricultural productivity, exacerbating food insecurity, economic instability, and environmental degradation. Global trade and climate change further intensify pathogen spread, emergence, and host shifts. While traditional diagnostics and targeted assays, such as PCR and ELISA, improve specificity, they depend on prior knowledge and are limited in detecting novel or mixed infections. High-throughput sequencing (HTS) has emerged as a transformative, unbiased platform that allows comprehensive detection of known and unknown pathogens through metagenomics and transcriptomics. By generating large-scale genomic data, HTS supports pathogen discovery, epidemiological surveillance, quarantine systems, and genome-informed disease management. It underpins advanced strategies, including CRISPR-Cas editing and RNA interference, and accelerates the breeding of resistance. Despite challenges—such as bioinformatics standardization, cost, and data interpretation—HTS, when integrated with classical diagnostics and biological validation, represents a foundational technology for sustainable, proactive plant health management and global phytosanitary resilience.

Keywords: Next-generation sequencing, Plant pathogen detection, Plant health surveillance

Introduction

Plant diseases are a significant constraint on agricultural productivity, resulting in yield losses and reduced crop quality (Gai; Wang, 2024). Crop failures have detrimental socio-economic and environmental consequences, including food insecurity, price volatility, and increased import dependency in affected regions. Concomitantly, attempts to combat plant diseases may drive excessive pesticide use, potentially degrading soil health, impacting non-target organisms, and promoting pathogen resistance (Ahmad; Saraswat; El Gamal, 2023; Gai; Wang, 2024; Jauhari; Agrawal, 2024). Still, the economic burden and food insecurity imposed by plant disease can be further exacerbated by intense global trade within and among economic blocs, as well as by climate change. Individually or synergistically, these drivers facilitate the introduction of pathogens into new areas, the occurrence of spillover events, the emergence of new pathogen strains, and shifts in host-pathogen interactions (Hammond et al., 2023; Singh et al., 2023).

Effective plant disease management requires early detection and precise pathogen identification to guide targeted intervention and reduce losses (Khakimov et al., 2022; Ristaino et al., 2021). Advances in pathogen characterization and in the understanding of host-pathogen interactions are increasingly recognized as essential components for strengthening agricultural resilience and supporting the Sustainable Development Goals, particularly Goal 2 (Zero Hunger and Sustainable Agriculture) (United Nations, 2015). In this context, methodologies based on High-Throughput Sequencing (HTS) have emerged as rapid and reliable tools for unbiased pathogen detection, allowing comprehensive identification of known and novel plant-associated microorganisms without prior assumptions about their identities.

In this state-of-the-art overview, we critically evaluate the role of HTS as a transformative platform in plant pathology. We synthesize current advances in HTS-based diagnostics, surveillance, and genome-informed disease management, and discuss the challenges that must be addressed to integrate these technologies into sustainable plant health systems.

Pre-genomic identification of plant diseases

Early diagnosis of diseases is critical for timely intervention and effective management. Traditionally, plant disease diagnostics have relied on the characterization of host symptoms, indexing using indicator plants, morphological observations, and culture-based methods. These approaches often involve partial or complete pathogen purification or isolation in selective media, followed by visual identification using light or electron microscopy. Although foundational to phytopathology, these methods are frequently time-consuming, require specialized expertise and costly equipment, and may lack sensitivity and specificity.

The development of targeted molecular and serological assays, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), marked a major advancement by enabling highly specific detection of a known pathogen through nucleic acid amplification or antigen recognition. While those tools significantly improved diagnostic accuracy and speed, their effectiveness depends on prior knowledge of the etiological agent(s), limiting their utility in cases involving unknown, genetically similar, or mixed infections. For instance, the molecular identification of nematode species remains challenging due to high genetic similarities and the absence of universal markers capable of distinguishing species (Bhat et al., 2022). Thus, although the advent of PCR and ELISA represented a major advance over traditional techniques, they do not fully address the growing need for comprehensive and discovery-driven approaches in modern phytopathology diagnostics.

Genomics Era – high-throughput sequencing as a new toolkit in phytopathology

The limitations of traditional detection methods have accelerated the transition from targeted diagnostics, e.g., PCR and ELISA, to high-throughput sequencing (HTS) methodologies (Nizamani et al., 2023). By generating large-scale DNA or RNA sequence data from a plethora of samples, HTS overcomes the limitations of methods that depend on prior assumptions about the causal agent and enables the simultaneous analysis of complex or mixed infections. Coupled with traditional methods and advances in bioinformatics, HTS has expanded the scope of plant pathology beyond individual pathogen detection toward integrated surveillance, epidemiological tracking, and deeper studies of pathogens and their interactions with hosts, vectors, and the environment (Ristaino et al., 2021). Collectively, HTS approaches provide mechanistic insights and surveillance capacity that support the design of more precise and sustainable plant disease management strategies.

High-throughput sequencing technologies: an overview

Also referred to as next-generation sequencing (NGS), a marketing-heavy term coined around 2005 to describe the shift from DNA Sanger sequencing (Sanger; Nicklen; Coulson, 1977), HTS allows the simultaneous sequencing of millions of short DNA molecules, previously fragmented from the sample. HTS resulting sequences, known as “reads”, are appropriately assembled using bioinformatics tools. Over a decade, propitiously, the cost per read base has been continually dropping as new technologies and brands have arrived in the market, and the bioinformatic tools are more accessible on public servers around the world, demanding less computer facilities in small laboratories (Benz; Mitra, 2023; Lee, 2023).

Currently, second- and third-generation sequencing technologies are the predominant platforms used for high-throughput sequencing. They mainly differ in the technological architecture, which produces short or long reads, respectively. Second-generation

sequencing techniques use the sequence-by-synthesis (SBS) approach and produce short reads. Fragmented DNA molecules are amplified and allowed to form “clusters” on the surface of a sequencing flow cell, which are used as templates to synthesize complementary DNA. The addition of each new nucleotide during the synthesis is detected. In the third-generation sequencing, no amplification step is required, and different technologies are available. In one of them, the nucleic acid molecules are directly sequenced, generating long reads. DNA or RNA molecules are pulled through a pore-forming protein (nanopore), and variations in an existing electric potential are recorded. Each time a nucleotide passes through the pore, base-specific changes in the electric current are detected. Third-generation sequencing also includes a second platform using SBS, but unlike the second-generation techniques, no pre-amplification is required, and data is obtained by processing one molecule at a time (single-molecule, real-time; SMRT) (Gao et al., 2023).

Technologies from both generations can generate millions to billions of reads, demanding data analysis steps that involve bioinformatics pipelines for read quality filtering and assembly, and comparative genomics to ensure accurate biological interpretation (Arora; Tollefsbol, 2021). Revealed genomic data are at the foundation of diverse applications across phytopathology, establishing the critical link between sequence output and practical insights, from pathogen discovery to tracking outbreaks and resistance breaking.

Unbiased detection of known and novel pathogens

The genomes of more diverse plant pathogens can be revealed by sequencing total DNA (metagenomic) or RNA (transcriptomics) from a sample, capturing their entire genetic landscape. Metagenomics allows for the characterization of plant and pathogen genomes, enabling the identification of disease-associated traits and mutations, and the discovery of microorganisms directly from field samples (Pacheco-Dorantes et al., 2025; Rivarez et al., 2023). Transcriptomics, in turn, can be particularly valuable for detecting RNA viruses, analyzing host gene expression profiles, and detecting replicating viral genomes. Beyond mere detection, RNA sequencing (RNA-seq) can provide functional insight by quantifying differential gene expression, uncovering alternatively spliced isoforms of a gene, and comparing transcriptomic responses across healthy and diseased plants (Lee, 2023).

The interpretation of HTS datasets relies on bioinformatics pipelines that compare unknown sequences to reference databases, enabling the taxonomic assignment of novel viruses, viroids, bacteria, and fungi. The routine application of HTS in plant health surveillance, for example, includes the development of short, curated electronic probes (e-probes) (Stobbe et al., 2013). Pathogen-specific e-probes have been successfully implemented for the detection of known viruses, bacteria, viroids, and phytoplasmas affecting citrus (Dang et al., 2023; Stobbe et al., 2013).

The exceptional performance of HTS in pathogen discovery is likely best illustrated by its ability to resolve the etiology of historically elusive diseases. In Brazil, curly top disease of beets (CTD) and citrus zonate chlorosis (ZC) were first recorded in the 1900s and 1930's, respectively, but their causal agents remained unidentified for decades, partly due to the sporadic appearance of symptoms. Recently, when symptomatic plants re-emerged, HTS analyses enabled the association of ZC with hibiscus green spot virus 2 (*Higrevirus waimanalo*, family *Kitaviridae*) and CTD with a novel virus of the genus *Topilevirus* (family *Geminiviridae*) (Pereira et al., 2025; Souza et al., 2026). Those studies also identified the vectors of the two viruses, thereby closing long-standing diagnostic gaps.

Integrating HTS into Plant Health Surveillance and Quarantine Systems

HTS can strengthen both the implementation of quarantine and biosecurity regulations and the active surveillance of pathogen populations across agricultural and natural ecosystems (Piombo et al., 2021; Singh et al., 2025; Fox et al., 2025). Plant quarantine depends on the accurate detection of regulated pathogens to prevent their introduction or limit their spread. HTS provides a culture- and symptom-independent approach capable of a sensitive screening of imported germplasm and planting material (Rwahnih et al., 2025). However, the routine application of HTS in diagnostic laboratories must include the operation of user-friendly bioinformatics toolkits designed for non-specialists, which is often the most labor-intensive step in the process. Progress toward practical solutions has included the use of pathogen-specific e-probes, as well as broader platforms such as GA-VirReport, which automate the detection of regulated viruses from raw sequencing data (Dang et al., 2023; Lebas et al., 2022; Lelwala et al., 2022). These and other bioinformatic tools are available through Galaxy (<https://usegalaxy.eu/>; <https://usegalaxy.org/>), an open-source, web-based platform that provides public computational infrastructure for accessible and reproducible HTS data analysis without requiring local infrastructure (Abueg et al., 2024). Beyond regulatory compliance, HTS provides high-resolution data for tracing pathogen movement, monitoring population dynamics, and anticipating emerging threats. Whole-genome sequencing of field samples allows the reconstruction of invasion routes, identification of new strains or variants, and the tracking of fungicide or antibiotic resistance alleles directly from environmental samples (Nizamani et al., 2023). On the other hand, metagenomic screening of arthropod vectors, water, or soil can function as an early warning system, revealing potentially invasive pathogens before major outbreaks occur. Surveillance programs are increasingly integrating HTS with predictive modelling. In the United Kingdom, for example, large-scale HTS-based surveillance of pea crops not only has detected expected viruses but also revealed the presence of unexpected pathogens (Fowkes et al., 2023). Such approaches are particularly critical in the context of climate change, which is reshaping the geographical distribution of both pathogens and vectors. By combining genomic data with environmental and spatial models, HTS-informed surveillance can support proactive rather than reactive responses to virus emergence (Kreuze et al., 2023).

Disease management

The integration of HTS into resistance breeding and disease management marks a shift from phenotype-driven selection toward genome-informed precision intervention. HTS provides the foundational genomic intelligence required to design, deploy, and monitor durable resistance strategies, serving as the essential discovery engine upon which modern biotechnological tools depend (Anaya-López et al., 2025; Ogbuji; Agogbua, 2025; Viswanath et al., 2023). Both CRISPR-Cas genome editing and RNA interference require detailed knowledge of the target sequences that can be obtained through HTS. Armed with this HTS-derived knowledge, the CRISPR-Cas system permits the precise modification of either viral genomes or host susceptibility factors. Direct targeting of viral DNA or RNA can attenuate replication and systemic movement, while editing host susceptibility genes disrupts the compatibility mechanisms that pathogens depend upon - an approach that often yields durable and broad-spectrum resistance (Fidan et al., 2023). RNA interference, in turn, harnesses a conserved antiviral pathway induced by double-stranded RNA, which is processed into small interfering RNAs that guide the degradation of complementary transcripts (Li et al., 2025). This sequence-specific silencing strategy has shown promise against pathogens for which conventional resistance genes are unavailable or have been overcome (Banik et al., 2025). Furthermore, profiling viral small

interfering RNAs generated during natural infection can reveal genomic "hotspots", those most frequently processed by the plant silencing machinery, providing a guide for the rational design of effective dsRNAs (Knoblich et al., 2025; Mohamed et al., 2022; Verma et al., 2023). In both cases, HTS-derived transcriptomic data guides intervention design by revealing which genetic pathways are mobilized during infection, informing strategic decisions - whether to enhance resistance gene function, disable susceptibility factors, or select optimal viral targets for silencing.

Despite its transformative potential, genome-driven disease management continues to face technical and practical hurdles. These include off-target effects in CRISPR editing, the environmental instability of topical dsRNA formulations, evolving regulatory frameworks for gene-edited crops, and the need for scalable, cost-effective dsRNA production. As an alternative approach, HTS can also accelerate traditional breeding by rapidly identifying resistance-associated markers and candidate genes, while simultaneously supporting the design pipelines of next-generation biotechnological tools (Anaya-López et al., 2025; González-Pérez et al., 2024). The rational, knowledge-driven development of durable and sustainable disease resistance is no longer conceivable without the genomic and transcriptomic foundation provided by HTS.

Challenges and Future Prospects

Although HTS represents a major technological advance, its widespread adoption in plant pathology continues to face significant obstacles. These include the absence of standardized and validated bioinformatics pipelines, the difficulty of distinguishing true pathogens from harmless endophytes or saprobes in complex metagenomic datasets, and the challenge of assigning biological relevance to detected sequences (Lee, 2023; Nizamani et al., 2023; Fox et al., 2025). Importantly, HTS alone cannot determine whether a detected microorganism is the causal agent of disease or merely part of the background microbiota. Therefore, sequencing-based detection must be integrated with biological assays, pathogenicity tests, host interaction studies, and classical diagnostic approaches to establish causal relationships and properly characterize novel or unexpected microorganisms. Moreover, the cost and technical expertise required for HTS remain prohibitive for many routine diagnostic laboratories, necessitating clear criteria for its use over conventional methods. The adoption of a modern diagnostic framework will require HTS as a first-line tool to address complex diseases of unknown etiology and to provide broad-spectrum surveillance. This strategy can be complemented by faster, cheaper targeted assays such as LAMP and qPCR for routine confirmation and monitoring (Aglietti et al., 2024; Sarmah et al., 2025). In this context, HTS would function as an early warning system within national laboratories, integrating genomic information into real-time phytosanitary networks and allowing the global tracking of emerging pathogens, the safe exchange of germplasm, and coordinated responses to transboundary disease threats (Nizamani et al., 2023). While concerns regarding labor-intensive protocols, data interpretation, and equitable access to technology persist, these limitations can be mitigated through capacity building, user-friendly bioinformatics toolkits, and sustained investment in global partnerships (Maina et al., 2024). Ultimately, HTS represents a foundational technology for a more sustainable and proactive paradigm in plant health – one that integrates genomic insights into every layer of disease management, from resistance breeding to ecosystem surveillance.

Declarations

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Ethical approval

Not applicable.

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