Arquivos do Instituto Biológico
ISSN 1808-1657
https://doi.org/10.1590/1808-1657000032026

ESSAY
Plant Health

High-throughput sequencing in phytopathology: genomics-driven diagnostics and host-pathogen interactions

Laura Rossetto Pereira[1] https://orcid.org/0000-0001-5075-2356
Matheus Potsclam Barro[1,2] https://orcid.org/0000-0001-9365-7658
Ricardo Harakava[1] https://orcid.org/0000-0003-1431-2665
Juliana Freitas-Astúa[1,3] https://orcid.org/0000-0002-0506-6880
Pedro Luis Ramos-González[1,*] https://orcid.org/0000-0002-9640-6587

1. Instituto Biológico – Laboratório de Biologia Molecular Aplicada – São Paulo (SP), Brazil.
2. Universidade de São Paulo – Escola Superior de Agricultura Luiz de Queiroz – Departamento de Fitopatologia e Nematologia – Piracicaba (SP), Brazil.
3. Embrapa Mandioca e Fruticultura – Cruz das Almas (BA), Brazil.

*Corresponding author: plrg1970@gmail.com

ABSTRACT

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 polymerase chain reaction and enzyme-linked immunosorbent assay, 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 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins 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

Received: Feb 20, 2026 Accepted: Mar 9, 2026
Associate Editor: Silvia Galleti
Peer Review History: Double-blind Peer Review.

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 purification or isolation of the pathogen 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 – HTS 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 HTS methodologies (Nizamani et al., 2023). By generating large-scale DNA or RNA sequence data from numerous samples, HTS overcomes the limitations of methods that rely on prior assumptions about the causal agent and enables 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.

HTS TECHNOLOGIES: AN OVERVIEW

Also referred to as next-generation sequencing, 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 the last decade, propitiously, the cost per read base has been continually dropping as new technologies and platforms have entered the market, and the bioinformatic tools are more accessible on public servers around the world, demanding fewer computer facilities in small laboratories (Benz; Mitra, 2023; Lee, 2023).

Currently, second- and third-generation sequencing technologies are the predominant platforms used for HTS. 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 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 second-generation techniques, no pre-amplification is required, and data are obtained by processing one molecule at a time (single-molecule, real-time) (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 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 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 (Fox et al., 2025; Piombo et al., 2021; Singh 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 has not only 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 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 (Fox et al., 2025; Lee, 2023; Nizamani et al., 2023). 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 loop-mediated isothermal amplification 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.

AUTHORS' CONTRIBUTIONS

Conceptualization: Ramos-González, P. L.; Rossetto Pereira, L.; Potsclam Barro, M. **Investigation:** Rossetto Pereira, L.; Potsclam Barro, M.;Ramos-González, P. L. **Writing – original draft:** Rossetto Pererira, L; Potsclam Barro, M. **Writing – review and editing:** Ramos-González, P. L.; Rossetto Pereira, L.; Potsclam Barro, M.; Harakava, R.; Freitas-Astúa, J. **Funding acquisition:** Harakava, R.; Freitas-Astúa, J. **Supervision:** Ramos-González, P. L.; Freitas-Astúa, J. **Final approval:** Ramos-González, P. L.

AVAILABILITY OF DATA AND MATERIAL

Not applicable

FUNDING

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Finance Code 001 and 88887.993646/2024-00

Fundação de Amparo à Pesquisa do Estado de São Paulo
Grant No: 2019/25078-9

Conselho Nacional de Desenvolvimento Científico e Tecnológico
Grant No: 141517/2024-7

CONFLICTS OF INTEREST

Nothing to declare.

ETHICAL APPROVAL

Nothing to declare.

ACKNOWLEDGEMENTS

Not applicable.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

The authors declare that artificial intelligence tools were used in the preparation of this manuscript only for language editing and grammar correction. The authors take full responsibility for the content, interpretation, and conclusions of this work.

REFERENCES

ABUEG, L. A. L.; AFGAN, E.; ALLART, O.; AWAN, A. H.; BACON, W. A.; BAKER, D.; BASSETTI, M.; BATUT, B.; BERNT, M.; BLANKENBERG, D.; BOMBARELY, A.; BRETAUDEAU, A.; BROMHEAD, C. J.; BURKE, M. L.; CAPON, P. K.; ČECH, M.; CHAVERO-DÍEZ, M.; CHILTON, J. M.; COLLINS, T. J.; COPPENS, F.; CORAOR, N.; CUCCURU, G.; CUMBO, F.; DAVIS, J.; DE GEEST, P. F.; DE KONING, W.; DEMKO, M.; DESANTO, A.; BEGINES, J. M. D.; DOYLE, M. A.; DROESBEKE, B.; ERXLEBEN-EGGENHOFER, A.; FÖLL, M. C.; FORMENTI, G.; FOUILLOUX, A.; GANGAZHE, R.; GENTHON, T.; GOECKS, J.; GONZALEZ BELTRAN, A. N.; GOONASEKERA, N. A.; GOUÉ, N.; GRIFFIN, T. J.; GRÜNING, B. A.; GUERLER, A.; GUNDERSEN, S.; GUSTAFSSON, O. J. R.; HALL, C.; HARROP, T. W.; HECHT, H.; HEIDARI, A.; HEISNER, T.; HEYL, F.; HILTEMANN, S.; HOTZ, H. R.; HYDE, C. J.; JAGTAP, P. D.; JAKIELA, J.; JOHNSON, J. E.; JOSHI, J.; JOSSÉ, M.; JUM'AH, K.; KALAŠ, M.; KAMIENIECKA, K.; KAYIKCIOGLU, T.; KONKOL, M.; KOSTRYKIN, L.; KUCHER, N.; KUMAR, A.; KUNTZ, M.; LARIVIERE, D.; LAZARUS, R.; LE BRAS, Y.; LE CORGUILLÉ, G.; LEE, J.; LEO, S.; LIBORIO, L.; LIBOUBAN, R.; LÓPEZ TABERNERO, D.; LOPEZ-DELISLE, L.; LOS, L. S.; MAHMOUD, A.; MAKUNIN, I.; MARIN, P.; MEHTA, S.; MOK, W.; MORENO, P. A.; MORIER-GENOUD, F.; MOSHER, S.; MÜLLER, T.; NASR, E.; NEKRUTENKO, A.; NELSON, T. M.; OBA, A. J.; OSTROVSKY, A.; POLUNINA, P. V.; POTERLOWICZ, K.; PRICE, E. J.; PRICE, G. R.; RASCHE, H.; RAUBENOLT, B.; ROYAUX, C.; SARGENT, L.; SAVAGE, M. T.; SAVCHENKO, V.; SAVCHENKO, D.; SCHATZ, M. C.; SEGUINEAU, P.; SERRANO-SOLANO, B.; SORANZO, N.; SRIKAKULAM, S. K.; SUDERMAN, K.; SYME, A. E.; TANGARO, M. A.; TEDDS, J. A.; TEKMAN, M.; THANG, W. C.; THANKI, A. S.; UHL, M.; VAN DEN BEEK, M.; VARSHNEY, D.; VESSIO, J.; VIDEM, P.; VON KUSTER, G.; WATSON, G. R.; WHITAKER-ALLEN, N.; WINTER, U.; WOLSTENCROFT, M.; ZAMBELLI, F.; ZIEREP, P.; ZOABI, R. The Galaxy platform for accessible, reproducible, and collaborative data analyses: 2024 update. *Nucleic Acids Research*, v. 52, n. W1, p. W83-W94, 2024. https://doi.org/10.1093/nar/gkae410

AGLIETTI, C.; BENIGNO, A.; CACCIOLA, S. O.; MORICCA, S. LAMP reaction in plant disease surveillance: applications, challenges, and future perspectives. *Life*, v. 14, n. 12, p. 1549, 2024. https://doi.org/10.3390/life14121549

AHMAD, A.; SARASWAT, D.; EL GAMAL, A. A survey on using deep learning techniques for plant disease diagnosis and recommendations for development of appropriate tools. *Smart Agricultural Technology*, v. 3, p. 100083, 2024. https://doi.org/10.1016/j.atech.2022.100083

ANAYA-LÓPEZ, J. L.; CHIQUITO-ALMANZA, E.; ACOSTA-GALLEGOS, J. A.; TOSQUY-VALLE, O. H.; MONTERO-TAVERA, V.; GONZÁLEZ-PÉREZ, E. Diagnosis and characterization of plant viruses in Mexico using HTS: an approach to guide virus management and marker-assisted selection. *Discover Plants*, v. 2, n. 1, p. 246, 2025. https://doi.org/10.1007/s44372-025-00332-y

ARORA, I.; TOLLEFSBOL, T. O. Computational methods and next-generation sequencing approaches to analyze epigenetics data: profiling of methods and applications. *Methods*, v. 187, p. 92-103, 2021. https://doi.org/10.1016/j.ymeth.2020.09.008

BANIK, S. K.; NATH, R.; SHARMA, S.; KIRTANIYA, S.; NATH, D.; PATRA, J. K.; CHOUDHURY, M. D.; DAS TALUKDAR, A. RNAi as a potential tool for control and management of plant disease: an updated overview. In: CHEN, J.-T.; KHAN, M.; PARVEEN, A.; PATRA, J. K. (ed.). *Molecular and biotechnological tools for plant disease management*. Singapore: Springer Nature Singapore, 2025. p. 349-375.

BENZ, S.; MITRA, S. From genomics to metagenomics in the era of recent sequencing technologies. *Methods in Molecular Biology*, v. 2649, p. 1-20. https://doi.org/10.1007/978-1-0716-3072-3_1

BHAT, K. A.; MIR, R. A.; FAROOQ, A.; MANZOOR, M.; HAMI, A.; ALLIE, K. A.; WANI, S. M.; KHAN, M. N.; SAYYED, R. Z.; POCZAI, P.; ALMALKI, W. H.; ZARGAR, S. M.; SHAH, A. A. Advances in nematode identification: a journey from fundamentals to evolutionary aspects. *Diversity*, v. 14, n. 7, p. 536, 2023. https://doi.org/10.3390/d14070536

DANG, T.; WANG, H.; ESPINDOLA, A. S.; HABIGER, J.; VIDALAKIS, G.; CARDWELL, K. Development and statistical validation of e-probe diagnostic nucleic acid analysis (EDNA) assays for the detection of citrus pathogens from raw high-throughput sequencing data. *PhytoFrontiers*, v. 3, n. 1, p. 113-123, 2023. https://doi.org/10.1094/PHYTOFR-05-22-0047-FI

FIDAN, H.; CALIS, O.; ARI, E.; ATASAYAR, A.; SARIKAYA, P.; TEK, M. I.; IZMIRLI, A.; OZ, Y.; FIRAT, G. Knockout of elF4E using CRISPR/Cas9 for large-scale production of resistant cucumber cultivar against WMV, ZYMV, and PRSV. *Frontiers in Plant Science*, v. 14, p. 1143813, 2023. https://doi.org/10.3389/fpls.2023.1143813

FOWKES, A. R.; DUFFY, S.; MCGREIG, S.; VAZQUEZ-IGLESIAS, I.; ALRAISS, K.; BARRETT, B.; CONYERS, C.; WEBSTER, G.; WARD, R.; CHISNALL, K.; MYNETT, M.; ADAMS, I. P.; MACARTHUR, R.; HOWARD, B.; FOX, A. Comparison of sequencing methods for

obtaining a whole genome of pea necrotic yellow dwarf virus from U.K. peas. *PhytoFrontiers*, v. 3, n. 4, p. 795-801, 2023. https://doi.org/10.1094/PHYTOFR-01-23-0003-R

FOX, A.; BOTERMANS, M.; ZIEBELL, H.; FOWKES, A. R.; PARETA, N. F.; MASSART, S.; RODONI, B.; CHOOI, K. M.; KREUZE, J.; LAVA KUMAR, P.; CUELLAR, W. J.; CARVAJAL-YEPES, M.; MACDIARMID, R. M. Implications of high throughput sequencing of plant viruses in biosecurity – a decade of progress? *Peer Community Journal*, v. 5, p. e71, 2025. https://doi.org/10.24072/pcjournal.572

GAI, Y.; WANG, H. Plant disease: a growing threat to global food security. *Agronomy*, v. 14, n. 8, p. 1615, 2024. https://doi.org/10.3390/agronomy14081615

GAO, L.; XU, W.; XIN, T.; SONG, J. Application of third-generation sequencing to herbal genomics. *Frontiers in Plant Science*, v. 14, p. 1124536, 2023. https://doi.org/10.3389/fpls.2023.1124536

GONZÁLEZ-PÉREZ, E.; CHIQUITO-ALMANZA, E.; VILLALOBOS-REYES, S.; CANUL-KU, J.; ANAYA-LÓPEZ, J. L. Diagnosis and characterization of plant viruses using HTS to support virus management and tomato breeding. *Viruses*, v. 16, n. 6, p. 888, 2024. https://doi.org/10.3390/v16060888

HAMMOND, J.; HUANG, Q.; JORDAN, R.; MEEKES, E.; FOX, A.; VAZQUEZ-IGLESIAS, I.; VAIRA, A. M.; COPETTA, A.; DELMIGLIO, C. International trade and local effects of viral and bacterial diseases in ornamental plants. *Annual Review of Phytopathology*, v. 61, p. 73-95, 2023. https://doi.org/10.1146/annurev-phyto-021621-114618

JAUHARI, S.; AGRAWAL, K. K. A Comprehensive review on various plant diseases and impact on crop yield and quality. *Journal of Information Systems Engineering and Management*, v. 2025, n. 38s, p. 2468-4376, 2024. https://doi.org/10.52783/jisem.v10i38s.6866

KHAKIMOV, A.; SALAKHUTDINOV, I.; OMOLIKOV, A.; UTAGANOV, S. Traditional and current-prospective methods of agricultural plant diseases detection: A review. *IOP Conference Series: Earth and Environmental Science*, v. 951, n. 1, p. 012002, 2022. https://doi.org/10.1088/1755-1315/951/1/012002

KNOBLICH, M.; GURSINSKY, T.; GAGO-ZACHERT, S.; WEINHOLDT, C.; GRAU, J.; BEHRENS, S. E. A new level of RNA-based plant protection: dsRNAs designed from functionally characterized siRNAs highly effective against Cucumber mosaic virus. *Nucleic Acids Research*, v. 53, n. 5, p. 136, 2025. https://doi.org/10.1093/nar/gkaf136

KREUZE, J. F.; CUELLAR, W. J.; LAVA KUMAR, P.; BODDUPALLI, P.; OMONDI, A. B. New technologies provide innovative opportunities to enhance understanding of major virus diseases threatening global food security. *Phytopathology*, v. 113, n. 9, p. 1622-1629, 2023. https://doi.org/10.1094/PHYTO-12-22-0457-V

LEBAS, B.; ADAMS, I.; AL RWAHNIH, M.; BAEYEN, S.; BILODEAU, G. J.; BLOUIN, A. G.; BOONHAM, N.; CANDRESSE, T.; CHANDELIER, A.; DE JONGHE, K.; FOX, A.; GAAFAR, Y. Z. A.; GENTIT, P.; HAEGEMAN, A.; HO, W.; HURTADO-GONZALES, O.; JONKERS, W.; KREUZE, J.; KUTJNAK, D.; LANDA, B.; LIU, M.; MACLOT, F.; MALAPI-WIGHT, M.; MAREE, H. J.; MARTONI, F.; MEHLE, N.; MINAFRA, A.; MOLLOV, D.; MOREIRA, A.; NAKHLA, M.; PETTER, F.; PIPER, A. M.; PONCHART, J.; RAE, R.; REMENANT, B.; RIVERA, Y.; RODONI, B.; ROENHORST, J. W.; ROLLIN, J.; SALDARELLI, P.; SANTALA, J.; SOUZA-RICHARDS, R.; SPADARO, D.; STUDHOLME, D. J.; SULTMANIS, S.; VAN DER VLUGT, R.; TAMISIER, L.; TRONTIN, C.; VAZQUEZ-IGLESIAS, I.; VICENTE, C. S. L.; VOSSENBERG, B. T. L. H.; WETZEL, T.; ZIEBELL, H.; MASSART, S. Facilitating the adoption of high-throughput sequencing technologies as a plant pest diagnostic test in laboratories: a step-by-step description. *EPPO Bulletin*, v. 52, n. 2, p. 394-418, 2022. https://doi.org/10.1111/epp.12863

LEE, J.-Y. The principles and applications of high-throughput sequencing technologies. *Development & Reproduction*, v. 27, n. 1, p. 9-24, 2023. https://doi.org/10.12717/DR.2023.27.1.9

LELWALA, R. V.; LEBLANC, Z.; GAUTHIER, M. E. A.; ELLIOTT, C. E.; CONSTABLE, F. E.; MURPHY, G.; TYLE, C.; DINSDALE, A.; WHATTAM, M.; PATTEMORE, J.; BARRERO, R. A. Implementation of GA-VirReport, a Web-Based Bioinformatics Toolkit for Post-Entry Quarantine Screening of Virus and Viroids in Plants. *Viruses*, v. 14, n. 7, p. 1480, 2022. https://doi.org/10.3390/v14071480

LI, F.; LI, X.; ZHAO, S.; PAN, F.; LI, Z.; HAO, Y.; HE, J.; WANG, A.; KORMELINK, R.; ZHOU, X.; RNA, A. Antiviral RNA interference in plants: increasing complexity and intertwining with other biological processes. *Plant Communications*, p. 101490, 2025. https://doi.org/10.1016/j.xplc.2025.101490

MAINA, S.; DONOVAN, N. J.; PLETT, K.; BOGEMA, D.; RODONI, B. C. High-throughput sequencing for plant virology diagnostics and its potential in plant health certification. *Frontiers in Horticulture*, v. 3, p. 1388028, 2024. https://doi.org/10.3389/fhort.2024.1388028

MOHAMED, A.; JIN, Z.; OSMAN, T.; SHI, N.; TÖR, M.; JACKSON, S.; HONG, Y. Hotspot siRNA confers plant resistance against viral infection. *Biology*, v. 11, n. 5, p. 714, 2022. https://doi.org/10.3390/biology11050714

NIZAMANI, M. M.; ZHANG, Q.; MUHAE-UD-DIN, G.; WANG, Y. High-throughput sequencing in plant disease management: a comprehensive review of benefits, challenges, and future perspectives. *Phytopathology*, v. 5, n. 1, p. 44, 2023. https://doi.org/10.1186/s42483-023-00199-5

OGBUJI, N. G.; AGOGBUA, J. U. Genomics in plant pathogen identification and control. *Frontiers in Plant Science*, v. 16, p. 1661432, 2025. https://doi.org/10.3389/fpls.2025.1661432

PACHECO-DORANTES, C.; TOVAR-PEDRAZA, J. M.; OCHOA-MARTÍNEZ, D. L.; GONZÁLEZ-GARZA, R.; DIAZ-LARA, A. Unleashing the potential of high-throughput sequencing for plant virus and viroid detection in Mexico. *Frontiers in Microbiology*, v. 16, p. 1603010, 2025. https://doi.org/10.3389/fmicb.2025.1603010

PEREIRA, L. R.; RODRIGUES, M. C.; CHABI-JESUS, C.; RAMOS-GONZÁLEZ, P. L.; BARBOSA, C. J.; SANTOS, M. G.; COSTA, H.; MARO, L. C.; TASSI, A. D.; KITAJIMA, E. W.; HARAKAVA, R.; FREITAS-ASTÚA, J. Historical and Contemporary evidence confirms a Higrevirus as the causal agent of citrus zonate chlorosis in Brazil. *Viruses*, v. 17, n. 11, p. 1428, 2025. https://doi.org/10.3390/v17111428

PIOMBO, E.; ABDELFATTAH, A.; DROBY, S.; WISNIEWSKI, M.; SPADARO, D.; SCHENA, L. Metagenomics approaches for the detection and surveillance of emerging and recurrent plant pathogens. *Microorganisms*, v. 9, n. 1, p. 188, 2021. https://doi.org/10.3390/microorganisms9010188

RISTAINO, J. B.; ANDERSON, P. K.; BEBBER, D. P.; BRAUMAN, K. A.; CUNNIFFE, N. J.; FEDOROFF, N. V; FINEGOLD, C.; GARRETT, K. A.; GILLIGAN, C. A.; JONES K, C. M.; MARTIN, M. D.; MACDONALD, G. K.; NEENAN, P.; RECORDS, A.; SCHMALE, D. G.; TATEOSIAN, L.; WEI, Q. The persistent threat of emerging plant disease pandemics to global food security. *Applied Environmental Microbiology*, v. 89, n. 23, 2021. https://doi.org/10.1073/pnas.2022239118

RIVAREZ, M. P. S.; FAURE, C.; SVANELLA-DUMAS, L.; PECMAN, A.; TUŠEK-ŽNIDARIC, M.; SCHÖNEGGER, D.; DE JONGHE, K.; BLOUIN, A.; RASMUSSEN, D. A.; MASSART, S.; RAVNIKAR, M.; KUTNJAK, D.; MARAIS, A.; CANDRESSE, T. Diversity and pathobiology of an ilarvirus unexpectedly detected in diverse plants and global sequencing data. *Phytopathology*, v. 113, n. 9, p. 1729-1744, 2023. https://doi.org/10.1094/PHYTO-12-22-0465-V

RWAHNIH, M. Al; KLAASSEN, V.; ERICKSON, T.; ALABI, O. J.; STEVENS, K.; HWANG, M. S.; PORT, L. A new era in federal quarantine and state certification diagnostics at clean plant centers in the United States. *Plant Disease*, v. 109, n. 7, p. 1392-1403, 2025. https://doi.org/10.1094/PDIS-10-24-2104-FE

DNA sequencing with chain-terminating inhibitors. P*roceedings of the National Academy of Sciences of the United States of America*, v. 74, n. 12, p. 5463-5467, 1977. https://doi.org/10.1073/pnas.74.12.5463

SARMAH, D.; DHANABALAN, S. P.; JOHNSON, I.; SEKAR, C.; MARY, X. A.; KARTHIKEYAN, M. Diagnostic tools for detection of pathogens and diseases in plantation crops. *Journal of Microbiological Methods*, v. 239, n. 4, p. 107294, 2025. https://doi.org/10.1016/j.mimet.2025.107294

SINGH, B. K.; DELGADO-BAQUERIZO, M.; EGIDI, E.; GUIRADO, E.; LEACH, J. E.; LIU, H.; TRIVEDI, P. Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, v. 21, n. 10, p. 640–656, 2023. https://doi.org/10.1038/s41579-023-00900-7

SINGH, P.; BHARGAVA, P.; BHASKAR, S. S.; NARWARE, J.; NANDNI, S.; PITAMBARA; DEV, D. High-Throughput sequencing: A tool to curb banana diseases of quarantine importance. *European Journal of Plant Pathology*, v. 173, n. 2, p. 545-560, 2025. https://doi.org/10.1007/s10658-025-03075-4

SOUZA, J. O.; MELGAREJO, T. A.; MACEDO, M. A.; BARBOSA, T.; LIMA, R. N.; EDUARDO SILVEIRA SILVA, C.; RODRIGUES, C.; AL RWAHNIH, M.; ROBERTO SPOTTI LOPES, J.; INOUE-NAGATA, A. K.; GILBERTSON, R. L. How a chance finding and high-throughput sequencing helped unmask the probable causal agent of Brazilian curly top, a plant disease that disappeared over 70 years ago. *Journal of General Virology*, v. 107, n. 2, 2026. https://doi.org/10.1099/jgv.0.002188

STOBBE, A. H.; DANIELS, J.; ESPINDOLA, A. S.; VERMA, R.; MELCHER, U.; OCHOA-CORONA, F.; GARZON, C.; FLETCHER, J.; SCHNEIDER, W. E-probe diagnostic nucleic acid analysis (EDNA): a theoretical approach for handling of next generation sequencing data for diagnostics. *Journal of Microbiological Methods*, v. 94, n. 3, p. 356-366, 2013. https://doi.org/10.1016/j.mimet.2013.07.002

UNITED NATIONS. *Transforming our world: The 2030 Agenda for Sustainable Development*. New York: [s.n.], 2015. Available at: https://sdgs.un.org/2030agenda Acesso em: 17 fev. 2026.

VERMA, S.; PRIYANKA; AKHTER, Y.; SAXENA, S. A novel protocol to identify the siRNA hotspots for creating RNAi-based Begomovirus resistance. *Briefings in Functional Genomics*, v. 22, n. 1, p. 49-60, 2023. https://doi.org/10.1093/bfgp/elac042

VISWANATH, K. K.; HAMID, A.; ATEKA, E.; PAPPU, H. R. CRISPR/Cas, multiomics, and RNA interference in virus disease management. *Phytopathology*, v. 113, n. 9, p. 1661-1676, 2023. https://doi.org/10.1094/PHYTO-01-23-0002-V