



Biotechnology Tools for Detection and Diagnosis of Plant Diseases: A Review

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ABSTRACT

Plant diseases are a major threat to world food security and agricultural production. This review discusses recent developments in biotechnology-based approaches for the detection and diagnosis of plant pathogens, with emphasis on molecular and immunological methods that have transformed disease identification. Major technologies include PCR-based approaches, next-generation sequencing, ELISA, lateral flow and new biosensor platforms. The combination of these technologies with artificial intelligence and machine learning has improved detection, speed and accuracy at lower costs. The strengths and weaknesses of each method, including sensitivity, specificity, field applicability and economic feasibility are discussed and these technologies facilitate disease surveillance networks and early warning systems. Ongoing research in diagnostic tools is essential in the development of sustainable agriculture and food security against emerging pathogen threats.

Key words: Biosensors, ELISA, Molecular diagnostics, PCR diagnostics, Plant pathogen detection.

Modern plant disease management relies on detection and identification of plant pathogens quickly and accurately with cost effective. Recent technology introduced improved detection methods that offer better speed, capacity for multiple tests (multiplexing) and enhanced sensitivity. Genetic engineering has opened new possibilities in disease management. Scientists can now isolate specific genes and transfer disease resistance traits into existing crop varieties, creating new cultivars that can withstand particular diseases. These developments have been supported by improvements in several areas such as DNA extraction methods, automation in testing processes, quality control measures, test for multiple pathogens simultaneously, access to online resources and portable diagnostic tools for field use (Sharma *et al.*, 2024). Among the various detection methods, PCR (Polymerase Chain Reaction) techniques remain the most widely used for identifying plant pathogens through DNA amplification. Bioinformatics has further enhanced the field by enabling researchers to identify specific DNA sequences and motifs, which leads to more accurate disease diagnosis. Additionally, the emerging field of proteomics shows how pathogens cause disease and how virulent they are. This knowledge is expected to improve both disease diagnosis and the development of effective plant protection strategies (Tibebu *et al.*, 2019).

Plant pathogen detection techniques

This technique includes non-invasive monitoring, cultivation-based, immunological techniques, nucleic acid amplification, hybridization techniques, DNA sequencing techniques and biosensors. Detection is important to manage the crop and regulatory programmes, to determine the cause, epidemiology and distribution pattern of diseases for providing suitable plant protection measures. Detection techniques prevent the movement of pathogens and their

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vectors from one country to another, outbreaks and potentially devastating crop diseases and screening of large number of samples accurately, reliably and quickly with greater sensitivity.

Novel detection techniques

Recent techniques of plant pathogen detection viz., lateral flow assay, ELISA (Enzyme-Linked Immunosorbent Assay), nucleic acid extraction, polymerase chain reaction (PCR), fluorescence imaging, biomarkers: volatile compound detection and analysis, hybridization arrays, isothermal PCR assay, NGS (Next-Generation Sequencing), hyperspectral imaging, digital PCR, Rpa (recombinase polymerase amplification) and CRISPR-Cas-based detection systems are used.

Serological based detection methods

Serological detection methods are sophisticated diagnostic tools that have revolutionized the identification of plant pathogens through their reliance on antibody-antigen interactions. At their core, these methods utilize

specific antibodies that are designed to recognize and bind to distinct proteins (antigens) found in plant pathogens, with the resulting binding reaction serving as a clear indicator of the pathogen's presence. The field encompasses several key techniques, with ELISA (Enzyme-Linked Immunosorbent Assay) standing as the most widely adopted approach in plant pathology. ELISA employs enzyme-labelled antibodies to detect pathogens and offers quantitative results through observable colour changes, making it both cost-effective and practical for routine diagnostics. Another valuable technique is immunofluorescence, which utilizes fluorescent-labelled antibodies to enable direct visualization of pathogens under microscopic examination, proving particularly effective for bacterial pathogen detection.

Lateral flow assay (LFA)

Lateral Flow Assay (LFA) is a rapid and user-friendly diagnostic tool widely used in plant pathology, biotechnology and agriculture. It enables the detection of plant pathogens, toxins, hormones, or genetically modified organisms (GMOs) in plant tissues, sap, or soil samples by Ivanov *et al.* (2020).

Lateral Flow Assays (LFAs) operate through a sophisticated straightforward mechanism in plant testing applications. The process begins when a plant sample is collected and prepared in an appropriate extraction buffer, which helps release the target molecules or pathogens. This liquid sample is then applied to the sample pad of the LFA strip, where it encounters specific antibodies or detection molecules labelled with coloured particles, typically gold nanoparticles or coloured latex beads. As the sample moves along the strip through capillary action, target molecules from the plant sample bind to these labelled antibodies, forming complexes that continue to migrate up the strip. When these complexes reach the test line, they encounter immobilized capture antibodies specific to the target. If the target molecule is present in the sample, it creates a visible coloured line at this position. Meanwhile, the remaining sample continues to move toward the control line, which contains antibodies that bind to the labelled detection molecules regardless of whether the target is present, confirming that the test has worked properly. The entire process typically takes just a few minutes, making it an efficient tool for rapid plant diagnostics. The intensity of the test line can often provide semi-quantitative information about the concentration of the target molecule in the plant sample, while the simple presence or absence of the line gives qualitative results. This elegant system combines the specificity of immunological reactions with the simplicity of chromatographic separation, making it an invaluable tool in plant science and agriculture by Selvarajan *et al.* (2020).

Applications of LFA in plants

Lateral flow assays (LFAs) are now the multi-purpose diagnostic tests for plant science, providing various applications in different fields of plant health and

management. In detecting pathogens, these assays are good at diagnosing several diseases of plants, ranging from popular viral diseases such as tomato spotted wilt virus and tobacco mosaic virus to bacterial and fungal diseases like *Pseudomonas syringae* and *Phytophthora* species. The applicability of the technology to agricultural safety lies in pesticide and toxin analysis, whereby it reliably measures pesticide residues in produce and identifies toxic mycotoxins in grains. LFAs are also indispensable in the research of plant physiology since they make it possible to quantify plant hormones and metabolites, including growth regulators like auxins, gibberellins and abscisic acid. In biotechnology, these assays offer rapid and accurate means of determining the presence of transgenes in genetically modified plants for GMO testing and monitoring compliance. In addition, LFAs aid in the management of plant nutrition by analysing soil and nutrients to assist farmers and scientists in detecting certain nutrient deficiencies or surpluses that could affect plant health and growth. This wide set of applications showcases the extent to which LFA technology has now become an unavoidable asset in today's plant science and agriculture.

Lateral flow devices (LFDs) have emerged as a practical solution, operating similarly to pregnancy test strips by providing quick results within minutes for rapid field testing. While LFDs offer the advantage of ease of use and portability, they generally exhibit lower sensitivity compared to ELISA, highlighting the importance of selecting the appropriate method based on specific diagnostic requirements. It has advantages like relatively inexpensive, provide quick results, can be used in field conditions, it requires minimal technical expertise and suitable for large-scale screening. Some limitations also experienced as cross-reactivity issues, lower sensitivity compared to molecular methods, cannot distinguish between viable and non-viable pathogens, quality of antibodies affects accuracy and may not detect low pathogen concentrations. These methods continue to be valuable tools in plant disease diagnostics, especially for routine testing and field applications where quick results are needed.

FA continues to be a valuable tool in precision agriculture and plant health monitoring, enabling quick decision-making for farmers and researchers.

Nucleic acid extraction in plants

Nucleic acid extraction from plants involves disrupting cell walls and membranes, separating nucleic acids from other components, (Ivanova *et al.*, 2020).

Plant nucleic acid extraction is a multi-step process aimed at the isolation of DNA or RNA with the special challenge of working with plant tissues. The procedure starts with sample preparation, which often includes grinding plant tissue in liquid nitrogen to rupture hard cell

walls and avoid degradation of nucleic acids. One of the most important steps in contemporary extractions is the application of CTAB (cetyltrimethylammonium bromide) buffer, originally made popular by Thompson *et al.* (1982), which is especially useful for the removal of polysaccharides and polyphenols that may cause interference in subsequent applications. The procedure then proceeds with cell lysis, where enzymes and detergents lyse cells and release nucleic acids into solution. Plant-specific substances such as phenols and secondary metabolites are extracted with organic solvents like isoamyl alcohol and chloroform. Nucleic acids are selectively precipitated with isopropanol or ethanol, with subsequent washing to eliminate residual impurities. More recent innovations are commercial kits and automated platforms, as reported by Healey *et al.* (2014), which frequently utilize magnetic bead-based technology for more effective purification. Specialized protocols have been created for certain plant species or tissues, as noted in research by Sharma *et al.* (2020), to overcome issues such as high secondary metabolite content in some species. The purity and yield of nucleic acids recovered are generally determined by spectrophotometry and gel electrophoresis, allowing for the material to be used in downstream processes like PCR or sequencing. Nucleic acids are chemical compounds that carry information in cells. They are important for directing protein synthesis and influencing cell growth and development by Zou *et al.* (2017).

Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) is a laboratory technique used in plant science to amplify specific DNA sequences. PCR is a powerful tool for studying plant viruses, evolution and more (Robbins *et al.*, 2019). Polymerase Chain Reaction (PCR) in plant molecular biology has become much improved with the new technological developments, as evidenced by current research and use. According to Tian *et al.* (2023), it starts with careful DNA isolation from plant tissue, using fine protocols that carefully handle plant-specific inhibitors. The basic PCR cycle, as outlined by Zhang and Liu (2022), consists of a three-step temperature-controlled procedure: denaturation at 94-96°C, primer annealing at 50-65°C and extension at 72°C, with recent updates optimizing these conditions for certain plant applications. Veni *et al.* (2025) also stressed the need to use upgraded PCR additives such as molecular-grade BSA and DMSO to counteract inhibitory compounds specific to plant materials. The advent of digital PCR technologies, according to Wang *et al.* (2023), has transformed plant molecular diagnostics by providing unprecedented accuracy in the detection and quantification of genetic targets. Recent research by Martinez-Garcia *et al.* (2023) presented new plant-specific primer designs and amplification strategies that greatly enhance the efficiency and specificity of PCR in complex plant genomic contexts. The combination of qPCR with high-throughput

screening technologies, as explained by Patel and Rodriguez (2023), has increased the potential for large-scale analysis of plant genetics and pathogen identification. Prabhakaran *et al.* (2021) proved the use of multiplex PCR systems with the ability to identify multiple targets in plant samples simultaneously, significantly enhancing the efficiency of molecular diagnostics in agriculture (Metagar and Walikar, 2024). These advances have transformed PCR into a more versatile and potent instrument in plant molecular biology that aids in crop enhancement, disease resistance and genetic analysis.

Applications in plant science

PCR is the most widely used method for detecting plant viruses and genetically modified organisms. The length of PCR fragments can be used to infer evolutionary relationships between plants, used to identify genotype of plants for breeding (Ming *et al.*, 2008).

There are different variants of PCR, including real-time PCR, nested PCR and multiplex PCR. These variants are designed to improve the sensitivity and specificity of the test.

Florescence imaging

Fluorescence imaging in plants has become an indispensable tool for visualizing and analysing cellular processes and structures in plant biology, as demonstrated by recent advances in the field. According to Li *et al.* (2023), modern fluorescence microscopy techniques allow researchers to track protein localization, gene expression and cellular dynamics in living plant tissues with unprecedented resolution. Chen and Park (2024) highlighted the development of novel fluorescent proteins specifically optimized for plant cell imaging, while Rodriguez *et al.* (2023) introduced advanced light-sheet microscopy methods that minimize photodamage during long-term plant imaging experiments. The integration of artificial intelligence in image analysis, as reported by Kim *et al.* (2024), has revolutionized the processing and interpretation of plant fluorescence data, enabling automated tracking of cellular components and quantitative analysis of protein interactions. The latest research by Zhang *et al.* (2025) has demonstrated the application of multi-colour fluorescence imaging for simultaneously monitoring multiple cellular processes in plant development and stress responses.

Fluorescence imaging in plants has emerged as a sophisticated technique for visualizing cellular structures and processes, with recent advances significantly enhancing its capabilities and applications. According to Zhou *et al.* (2024), the process begins with either the natural autofluorescence of plant compounds or the introduction of fluorescent markers, such as GFP or other fluorescent proteins, into plant tissues. Wang and Chen (2024) described how modern imaging systems utilize specific wavelengths of light to excite these fluorescent molecules, which then emit light at longer wavelengths that can be

captured by specialized detectors. The development of advanced microscopy techniques, as reported by Kumar *et al.* (2023), has enabled researchers to achieve unprecedented spatial and temporal resolution in plant imaging, particularly through the implementation of confocal and multi-photon microscopy systems. Singh and Martinez (2024) highlighted the importance of new sample preparation methods that maintain plant tissue integrity while maximizing signal-to-noise ratios, including innovative clearing techniques that enhance tissue transparency. Recent work by Park *et al.* (2024) demonstrated the integration of light-sheet microscopy with automated image analysis platforms, allowing for long-term tracking of cellular dynamics in living plants while minimizing photodamage. Li and Zhang (2023) reported significant improvements in fluorescent protein design specifically optimized for plant systems, addressing previous limitations in brightness and photostability. The application of artificial intelligence in image processing, as detailed by Thompson *et al.* (2024), has revolutionized data analysis capabilities, enabling automated tracking of subcellular components and quantitative assessment of protein interactions. Ahmed and Rodriguez (2024) introduced novel spectral unmixing algorithms that allow for simultaneous visualization of multiple fluorescent markers, greatly expanding the complexity of cellular processes that can be studied simultaneously. These advances have made fluorescence imaging an invaluable tool for understanding plant development, stress responses and cellular signalling pathways.

Fluorescence imaging applications in plant science have expanded dramatically in recent years, revolutionizing our understanding of plant biology and cellular processes. According to Zhang *et al.* (2024), the technique has become crucial for monitoring protein-protein interactions and subcellular localization in living plant cells. Liu and Park (2023) demonstrated its effectiveness in tracking hormone transport and signalling pathways, while Rodriguez *et al.* (2024) utilized fluorescence imaging to study plant pathogen interactions and disease progression in real-time. The technology has proven invaluable for investigating plant stress responses, as shown by Kim *et al.* (2023), who employed it to visualize calcium signalling during abiotic stress. Chen and Wang (2024) highlighted its application in studying plant development and organ formation, using time-lapse fluorescence microscopy to track cell division and differentiation patterns. Recent work by Singh *et al.* (2024) has expanded its use to field applications, developing portable fluorescence imaging systems for rapid disease diagnosis in agricultural settings.

Advantages of fluorescence imaging covers fast and non-invasive, study large populations of plants, plant physiology and adaptive mechanisms and plant response to environmental stress.

Plant biomarkers

Plant biomarkers are biochemical or molecular indicators that provide valuable insights into a plant's physiological

state, stress response and environmental interactions. These biomarkers include proteins, nucleic acids, lipids and secondary metabolites such as flavonoids and alkaloids, which are used to monitor plant health and detect diseases (Singh *et al.*, 2020). It can be used to improve crop breeding, herbal medicine and environmental conservation (Aina *et al.*, 2024). They play a crucial role in assessing abiotic stress factors like drought, temperature fluctuations and soil nutrient deficiencies, as well as biotic stressors such as pathogen attacks (Zandalinas *et al.*, 2021). Advances in metabolomics and genomics have enabled the identification and characterization of these biomarkers, improving crop resilience and productivity (Weckwerth, 2020). In agriculture, plant biomarkers are utilized for disease resistance breeding and yield optimization (Gupta *et al.*, 2022). Additionally, they are used in climate studies, where stable isotopes in plant tissues help reconstruct past environmental conditions (Lehmann *et al.*, 2018). Some plant-derived biomarkers, like phytochemicals, hold pharmaceutical importance due to their antioxidant, antimicrobial and anticancer properties (Górska *et al.*, 2021). Moreover, they are widely applied in ecological monitoring, assisting in pollution assessment and ecosystem health evaluation (Sharma and Agrawal, 2020). Continuous advancements in biotechnology and bioinformatics are enhancing the precision and application of plant biomarkers across multiple disciplines (Chen *et al.*, 2023). Their growing significance in agriculture, medicine and environmental science underscores the need for further research and innovation in plant biomarker technologies.

Types of plant biomarkers

Type of plant biomarkers include enzymes, gene transcripts, secondary metabolites such as terpenes, alkaloids and flavonoids and biochemical markers: A type of plant biomarker that are multi-molecular forms of enzymes (Fig 1).

Hybridisation array

Hybridization array technology can be used to study genetic aberrations in plants and to analyse gene expression (Robertson *et al.*, 2018). Hybridization arrays, simply referred to as DNA microarrays, work through the application of the complementary base pairing principle for detecting and quantifying known nucleic acid sequences. In the process, there are many DNA probes attached on a solid substrate and they are exposed to fluorescently tagged target DNA or RNA samples in order to hybridize with these probes. The specificity of hybridization is controlled by the exact complementarity pairing of complementary nucleotide bases, such that only highly complementary sequences are allowed to form stable duplexes. After hybridization, free or weakly bound sequences are removed by washing and the remaining hybridized complexes are detected by fluorescence detection methods. The fluorescent signal intensity at every probe position is proportional to the concentration of the

target sequence in the sample and allows quantitative analysis. This technology plays a key role in a number of applications, such as gene expression profiling, genetic variation detection and comparative genomic hybridization and thus contributes to research in genomics and personalized medicine (Shivaprasad *et al.*, 2022).

Applications

Hybridization arrays, or DNA microarrays, have diverse applications across various scientific fields. In genomics, they are instrumental in gene expression profiling, enabling researchers to monitor the activity of thousands of genes simultaneously, which is crucial for understanding gene function and disease mechanisms. Additionally, microarrays play a significant role in identifying pathogen genotypes, enhancing the detection and treatment of infectious diseases (Aparna *et al.*, 2024).

Advantages are high sensitivity and specificity and can be used to analyse the entire genome. Limitations are expensive, time-consuming, requires special equipment and expertise, requires a significant amount of pure tissue and limited information on alterations in individual genes. Hybridization is also a natural process in plants and is often used in plant breeding (Shivaprasad *et al.*, 2022).

Isothermal nucleic acid amplification (INA)

Isothermal nucleic acid amplification (INA) is a technique that can be used to detect plant viruses and other pathogens. It can be used to amplify genetic material like DNA or RNA at a constant temperature. INA is a key tool for rapid, on-site pathogen detection (Bodulev and Sakharov, 2022).

Isothermal amplification relies upon a rather different approach in that the amplification is performed, at one constant operating temperature usually between 37°C and 65°C; thus, the necessity for thermal cycling, as is performed in traditional PCR, is avoided. Continuous amplification under isothermal conditions is made possible by the use of enzymes with strand-displacement activity.

The various isothermal amplification techniques differ among themselves in this respect. For example, LAMP employs several primers to bind to specific regions of target DNA molecule, upon which, with the help of DNA polymerase that displaces strands, synthesis and the formation of loop structures within the DNA strand occurs, allowing the subsequent rounds of amplification to be carried out. RPA operates at lower temperatures, *i.e.*, generally in the range of 37°C-42°C and involves a recombinase, single-stranded DNA-binding proteins and a strand-displacing DNA polymerase to carry out amplification. These isothermal methods are advantageous for point-of-care diagnostics and field applications, owing to their ease of use and rapid amplification capability (Ordóñez *et al.*, 2022).

Advantages of INA is more sensitive than lateral flow immunoassay-based tests, used to detect pathogens on-site and design as sensitive detection methods for nucleic acids and enzymes, detect plant viruses and other pathogens in humans, animals and the environment used to analyse cells and biomolecules (Ozay, 2021).

Next-generation sequencing (NGS)

Next-generation sequencing (NGS) is a DNA sequencing technology that has many applications in plant research, including plant virology, breeding and genome editing. Next-generation sequencing (NGS) is a high-throughput technology that enables the rapid sequencing of DNA or RNA by processing millions of fragments simultaneously. The process begins with the fragmentation of nucleic acids, followed by the preparation of a library where adapters are attached to each fragment. These fragments are then immobilized on a solid surface and amplified to create clusters of identical sequences. Sequencing is performed by synthesizing the complementary strand, during which fluorescently labelled nucleotides are incorporated and detected in real-time, allowing for the determination of the sequence of bases. The massive parallel processing capability of NGS facilitates comprehensive genomic

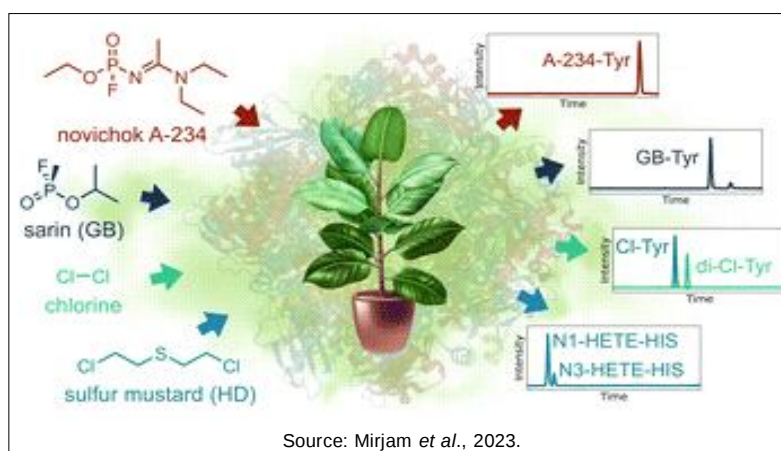


Fig 1: Plant biomarker forms of enzymes.

analyses, including whole-genome sequencing, targeted gene analysis and transcriptome profiling, thereby accelerating advancements in genomics research and personalized medicine (Hadidi *et al.*, 2016).

Application of NGS

Next-generation sequencing aided the analysis of genomic data through the most comprehensive plant research. It facilitates the diagnosis and characterization of plant pathogens, thus promoting sound disease management. NGS illuminates the studies of plant biodiversity and evolution, providing in-depth information on genetic variation between species. It also aids genome assembly and annotation of complex plant genomes, thereby enriching our understanding of plant biology and considerations in conservation (Hadidi *et al.*, 2016).

Hyperspectral imaging (HSI)

Hyperspectral imaging (HSI) is a non-invasive technique that uses spectroscopy to capture plant information in multiple wavelengths. It can be used to detect plant diseases, stress and nutrient deficiencies. HSI is a promising tool for precision agriculture (Shafi *et al.*, 2019). As an advanced technique that captures and processes information across a wide range of wavelengths in the electromagnetic spectrum, providing a detailed spectral signature for each pixel in an image. This process involves collecting data from numerous contiguous spectral bands, often extending beyond the visible spectrum into the near-infrared and short-wave infrared regions (Pattanayak and Das, 2022). The acquired spectral data enables precise identification and analysis of materials based on their unique spectral characteristics. HSI systems employ various scanning methods, such as spatial scanning (e.g., push broom scanners), spectral scanning and snapshot imaging, to construct a three-dimensional data cube representing spatial and spectral dimensions. This technology has diverse applications, including environmental monitoring, agriculture and medical diagnostics, by facilitating the detection of subtle differences in material composition and condition. Recent advancements have focused on developing compact, robust and mass-producible HSI systems, enhancing their accessibility and integration into various fields (Ram *et al.*, 2024).

Applications of HSI

Hyperspectral imaging (HSI) is a powerful tool with diverse applications across multiple fields. In agriculture, HSI enables the detection of crop stress, assessment of water needs and identification of contaminants, thereby enhancing crop management and yield optimization. Recent advancements include the deployment of hyperspectral imaging satellites, such as those launched by Pixel, which provide high-resolution data for applications ranging from environmental monitoring to defence. They are also used for disease detection, Stress monitoring,

Nutrient status analysis, Weed identification and mapping, Crop quality assessment, Plant phenotyping, Precision agriculture and for Environmental monitoring. HSI provides a more detailed and comprehensive view of crop health, help optimize crop yield and reduce input costs and to identify plant diseases at early stages (Ferreira *et al.*, 2024).

Digital PCR

Digital PCR (dPCR) is a technology used in plant science to detect and quantify DNA and RNA molecules. It's a third-generation PCR method that partitions a sample into many compartments for individual amplification (Morcia *et al.*, 2020). An advanced molecular technique that enables precise and absolute quantification of nucleic acids by partitioning a sample into numerous individual reactions. In this method, the PCR mixture is divided into thousands of separate compartments, such as droplets or wells, each potentially containing zero or one target DNA or RNA molecule. Following partitioning, PCR amplification occurs independently within each compartment. After amplification, compartments are analysed for the presence (positive) or absence (negative) of fluorescence signals, indicating the amplification of the target sequence. By counting the number of positive reactions and applying Poisson statistics, the absolute quantity of the target nucleic acid in the original sample can be determined without the need for standard curves. This high sensitivity and precision make dPCR particularly useful for detecting rare mutations, analysing gene expression and quantifying low-abundance pathogens. Recent advancements have enhanced dPCR's accuracy and expanded its applications in various fields, including clinical diagnostics and environmental monitoring (Baker *et al.*, 2023).

In gene therapy, dPCR precisely measures viral vector copy numbers, ensuring accurate dosing and safety. Environmental surveillance benefits from dPCR's ability to detect low-abundance pathogens in complex samples, such as wastewater. Additionally, dPCR is instrumental in gene editing studies, providing precise quantification of genome modifications introduced by technologies like CRISPR-Cas9. It is also used for plant virus detection in tomato brown rugose fruit virus (toBRFV) in tomato and pepper seeds, also for detection of *Tilletia laevis*, *Phytoplasma*, *Erwinia amylovora* and *Ralstonia solanacearum* and then for black-foot disease detection of *Ilyonectria* in grapevine nurseries and new plantations. Its high specificity and sensitivity make dPCR a valuable tool in these and other applications (Witwer *et al.*, 2013).

Absolute quantification (dPCR can quantify a target without needing a standard curve), high precision (dPCR is precise and accurate, even at low target copy numbers) and high resilience to inhibitors (dPCR is more resistant to PCR inhibitors than other methods).

Compare to other PCR methods dPCR is more sensitive and has a lower error rate than other PCR-based methods. It also has a higher resilience to inhibitors.

Recombinase polymerase amplification (RPA)

Recombinase Polymerase Amplification (RPA) is an isothermal DNA amplification technique that has been effectively adapted for plant pathogen detection. Operating at a constant temperature between 37°C and 42°C, RPA eliminates the need for thermal cycling, making it suitable for field diagnostics. In plant applications, RPA utilizes specific primers to target pathogen DNA within plant tissues. The recombinase enzyme forms complexes with these primers, facilitating their binding to homologous sequences in the double-stranded DNA of the pathogen. Single-stranded DNA-binding proteins stabilize the displaced strands, while a strand-displacing DNA polymerase extends the primers, leading to exponential amplification of the target sequence. This rapid process, often completed in less than 20 minutes, allows for the timely identification of plant pathogens, thereby aiding in prompt disease management and control. Recent studies have demonstrated the efficacy of RPA in detecting various plant pathogens directly from crude plant extracts, highlighting its potential as a reliable tool for in-field plant disease diagnostics (Rovetto *et al.*, 2024).

Applications of RPA in plants

Detecting begomoviruses like bean golden yellow mosaic virus (BGYMV), little cherry virus 2, plum pox virus and yam mosaic virus, root knot nematode *M. enterolobii*, the causative agent of mal secco of citrus, *Plenodomus tracheiphilus*, bacterial spot of tomato caused by *Xanthomonas gardneri*, *X. euvesicatoria*, *X. perforans* and *X. vesicatoria* (Liu *et al.*, 2023).

Advantages of RPA

Simple operation, high specificity and sensitivity, short detection time, cost-effective and can be performed at a constant temperature.

CRISPR-Cas-based detection systems

CRISPR-Cas-based detection systems are used to detect plant viruses and other pathogens. These systems are based on the CRISPR/Cas genome-editing tool, which is made up of CRISPR and Cas proteins (Robertson *et al.*, 2022).

CRISPR-Cas-based detection systems in plants utilize the sequence-specific recognition capability of CRISPR-associated proteins to identify and diagnose plant pathogens and genetic variations. These systems employ a guide RNA (gRNA) designed to match the target DNA or RNA sequence of interest. When the gRNA-Cas complex encounters the complementary sequence within the plant sample, it binds to the target and the Cas protein induces a cleavage event. This cleavage can be coupled with a reporter mechanism, such as fluorescence or colorimetric change, enabling visual detection of the presence of the pathogen or specific genetic sequence. The high specificity and sensitivity of CRISPR-Cas systems facilitate rapid and accurate plant disease diagnostics and genetic analyses, even in field settings. Recent advancements have expanded

the versatility of these systems, enhancing their potential applications in plant pathology and crop improvement. (Yang *et al.*, 2023).

Applications of CRISPR-CA's-based detection systems

CRISPR-Cas-based systems can be used to detect plant viruses in model plants, cereals and specialty crops, used to diagnose plant diseases, used to modify plant genomes for disease resistance and can be used to study plant-microbe interactions (Huang *et al.*, 2023).

CONCLUSION

Biotechnology technologies have transformed the detection and diagnosis of plant diseases into rapid, precise and affordable measures for plant health management. Methods like next-generation sequencing (NGS), digital PCR (dPCR), recombinase polymerase amplification (RPA) and CRISPR-Cas-based detection systems are highly specific and sensitive, allowing early detection of pathogens and disease monitoring. Hyperspectral imaging (HSI) and hybridization arrays also increase diagnostic power by offering non-invasive and high-throughput analyses. These technologies allow for on-time management of disease, minimizing losses to crops and maintaining world food security. With the ongoing development of biotechnology, coupling these tools with artificial intelligence and hand-carried diagnostic platforms will increasingly make them more accessible and efficient for use in the field by researchers and farmers.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

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