

RECENT ADVANCES IN DETECTION AND DIAGNOSIS OF PLANT DISEASES

Abstract

Plant diseases cause a significant threat to global agriculture, leading to huge economic losses and serious concerns of food security and sustainability. Timely plant pathogen diagnostics can save up to 50% of the total agricultural yield. There are several traditional methods used for identifying plant diseases such as visual observation, microscopy, biological diagnosis, testing in indicator plants or mycological diagnosis, mass spectrophotometry, immunological diagnostics, etc. But these methods for detection and identification of plant diseases have many limitations. Thus, to eliminate the shortcomings in traditional methods and to ensure the promptness and reliability of diagnostics, the advanced technologies including molecular and genomic techniques for the identification of pathogens have been developed. Molecular techniques like Loop-mediated isothermal amplification (LAMP), Next-generation sequencing (NGS) and CRISPR-Based methods play an important role for sensitive and rapid detection. The integration of biosensors and nanotechnology offer rapid, sensitive, and cost-effective disease detection and diagnosis. Remote sensing and imaging technologies provide a bird's-eye view of the entire field or orchard, allowing a quick assessment of plant health. This chapter brings about the recent advancement in the detection and diagnosis of plant diseases which highlights the impact of the same on agriculture and the environment.

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I. INTRODUCTION

Plant diseases pose a significant threat to global agriculture, leading to substantial economic losses and serious concerns of food security. Early and precise detection of these diseases is crucial for proper plant disease management and crop protection. In the recent years, there are remarkable advancements in plant disease detection and diagnosis, driven by developments in technology and innovative research. There are several traditional methods used for identifying plant diseases such as visual observation, microscopy, biological diagnosis, testing in indicator plants or mycological diagnosis, mass spectrophotometry, immunological diagnostics, etc. Visual observation such as screening and an intensive survey revealed that rice production zones of Kashmir, India are affected with leaf blast disease within the average severity range of 3.7-41.3% (Anwar *et al.*, 2009). Biological control agent such as *Trichoderma harzianum* has been found effective against many economically important phytopathogenic fungi. But it is necessary for biocontrol application to develop mass production, formulation and delivery system for an organism that can control diseases in plants (Bhat *et al.*, 2009). Management of brinjal fruit rot caused by *Phomopsis vexans* has been done using fungicides. But fungicide applications are only partially effective under climatic conditions that are favorable for infection (Anwar *et al.*, 2017). However, to eliminate the shortcomings in traditional methods and to ensure the promptness and reliability of diagnostics, the advanced technologies including molecular and genomic techniques for the identification of pathogens have been developed. For example, Serodiagnosis is a sensitive tool that is used to identify species specific molecules; important structural features in vector-pathogen interactions and quantify different crop pathogens (Bhat *et al.*, 2010). Also, the sensitive nature of nanoparticles is utilized to design phytopathogen detection devices which have smart sensing capabilities for use in field (Kashyap *et al.*, 2016). Thus, this chapter explores the recent advancement in the detection and diagnosis of plant diseases, highlighting the impact of these advancements on agriculture and the environment.

II. MOLECULAR TECHNIQUES

The molecular techniques for plant disease detection and diagnosis identify the species which are morphologically similar. This allows the detection of infection before the formation of symptoms. Molecular detection method is precise and accurate along with faster and easier approach. These molecular tools increase the speed of diagnosis with accuracy and efficacy. The success of these nucleic acid-based assays largely depends on highly equipped laboratory facilities for rapid and precise detection (Paudel *et al.*, 2020).

1. Loop-mediated isothermal amplification (LAMP) for rapid detection: Loop-Mediated Isothermal Amplification is a powerful molecular biology technique which is used for rapid detection of RNA or DNA sequences in various crops. It offers several advantages over traditional PCR (Polymerase Chain Reaction) methods, such as it can amplify DNA at constant temperature without thermal cycling.

- **Principle:** LAMP relies on the use of a DNA polymerase enzyme and a set of 4 to 6 primers that target multiple regions of the DNA or RNA sequence of interest. These primers initiate strand displacement amplification, forming looped DNA structures called amplicons. This process occurs isothermally, particularly at a temperature between 60°C to 65°C, thus eliminating the need for a thermal cycler and leads to

exponential amplification of the target sequence within a relatively short period, usually 30 minutes to one hour.

- **Primer Design:** LAMP needs a set of different primers which include:
 - F3 and B3 primers: These are **outer primers** that initiate the amplification process.
 - FIP and BIP primers: These are **inner primers** that form the basis of DNA synthesis.
 - Loop primers: These are **additional primers** that enhance the amplification speed and efficiency.

- **Applications of LAMP in Plant Disease Detection**
 - **Early Disease Diagnosis:** LAMP enables early detection of plant pathogens, even before the visible symptoms appear. This helps in preventing disease spread, allowing targeted chemical application or quarantine measures.
 - **Quarantine and Surveillance:** LAMP is used at entry ports to detect and identify potential plant material which helps prevent the introduction of new diseases to a region.
 - **Monitoring Plant Health:** Regular monitoring of crops using LAMP technology can help farmers and researchers track the prevalence and spread of specific diseases, aiding in developing disease management strategies.
 - **Disease Resistance Screening:** LAMP is used to screen plant varieties for disease resistance, helping breeders in developing more robust and disease-resistant crops.
 - LAMP is an important tool for sensitive and rapid detection of plant diseases, offering benefits such as simplicity, speed, and robustness. It plays a crucial role in plant pathology, helping to mitigate the impact of diseases on agricultural crops and promote food security.

2. **Next-generation sequencing (NGS) for comprehensive pathogen profiling:** Next Generation Sequencing (NGS) is an emerging transformative technology in plant pathology which offers comprehensive along with efficient profiling of plant pathogens. NGS is also known as high-throughput sequencing and enables rapid and cost-effective analysis of nucleic acid sequences of plant pathogens on a large scale. This highlights the genetic diversity, evolution, and virulence factors of plant pathogens. For example, *Phytophthora infestans* genome was sequenced which led to a better understanding of the pathogen and control strategies. NGS is also involved in Metagenomics and Transcriptomics which identifies complex pathogen communities, such as soil borne pathogens. Plant microbiome is also studied using NGS which aids in the development of microbiome-based disease management.

Applications of NGS in plant pathogen profiling

- **Disease Surveillance:** NGS is used for continuous monitoring of plant health in fields, which enables early detection of pathogens.
- **Breeding for Resistance:** Breeders can use NGS to facilitate the development of disease-resistant crop varieties through marker-assisted selection.

- **Biosecurity:** NGS aids in identifying quarantine pathogens and helps to prevent their spread across borders.
- **Precision Disease Management:** NGS data guides precise application of fungicides and pesticides, and other control measures, reducing the environmental impact and optimizing the use of resources.

Next Generation Sequencing has revolutionized the field of Plant Pathology by efficient profiling of plant pathogens. It is an invaluable tool for disease surveillance, management, and research because of its high throughput, accuracy, and versatility. This technology promises to contribute significantly to global food security by enabling more effective control of plant diseases and reducing agricultural losses.

3. **CRISPR-Based methods for Genome Editing and Disease Resistance:** Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is an emerging powerful tool for genome editing. CRISPR-Cas9 has been derived from bacterial immune system, which is used in a wide range of organisms, including plants. There are two main components of this system- a guide RNA (gRNA) and the Cas9 protein. The gRNA targets a specific DNA sequence in the plant's genome, which guides the Cas9 protein to that location. Later, Cas9 induces a double strand break in the DNA, that triggers the cell's natural repair mechanisms.

Enhancing Disease Resistance through CRISPR

- **Targeting Pathogen Vulnerabilities:** Scientists use CRISPR to modify plants to make them less susceptible to pathogens. Also, researchers can reduce the rate of successful infection by disrupting specific plant genes exploited by pathogens.
- **Engineering Pathogen Resistance:** CRISPR allows the introduction of genes from other plants that encode resistance to pathogens. For example, introduction of resistant genes from wild tomatoes to cultivated varieties to combat diseases such as late blight.
- **Fine-Tuning Immune Responses:** CRISPR is used to modify genes to enhance the plant's innate defenses to mount a stronger response when attacked by pathogens.

CRISPR-based methods used for genome editing have ushered in a new era of agricultural innovation, particularly in the case of plant disease resistance. Scientists can develop crops using CRISPR that are more resilient, thus reducing the use of chemicals and promoting sustainable agriculture.

III. BIOSENSORS AND NANOTECHNOLOGY

The integration of biosensors and nanotechnology has revolutionized the field of plant pathology, offering rapid, sensitive, and cost-effective tools for disease detection and diagnosis.

1. **Development of Biosensors for Real-Time Disease Monitoring:** Biosensors are analytical devices that incorporate biological recognition elements (enzymes, antibodies, DNA probes) integrated with nanotechnology and microelectronics, offering the ability to monitor the health of plants autonomously and continuously. They provide instant

feedback on the presence of pathogens or disease-related biomolecules, even before visible symptoms appear on plants, thus enabling precision in disease management. The results are provided within minutes to hours, enabling real-time information. Many biosensors are designed for field use, easily accessible to farmers and scientists for on-site assessment of diseases.

For example, Lateral flow Immunoassay (LFIA) is developed for detection of *Phytophthora spp.* When antigens of this pathogen are present in a plant sample, they bind to specific antibodies on the LFIA strip. This interaction produces a visible signal, i.e., a colored line, within minutes.

- 2. Nanoparticle-based Diagnosis of Plant Diseases:** Nanotechnology involves the manipulation of materials at the nanoscale (1-100 nanometers) often made of substances like gold, quantum dots, or magnetic nanoparticles (nanowires and nanofibers) which are designed to interact with specific plant pathogens, providing highly sensitive and rapid detection capabilities. The Nanopore Sequencing technology allows direct DNA and RNA sequencing for identification of pathogens and their genetic characteristics. The researchers and farmers can precisely target and capture disease causing agents by integrating nanoparticles with antibodies, DNA probes, or other recognition molecules. It helps in offering user-friendly and cost-effective solutions for on-site disease detection in plants.

Gold nanoparticles (AuNP) are used in these diagnostics due to their unique optical properties resulting in a shift in their scattering spectra. These are functionalized with antibodies that recognize antigens or nucleic acid sequences of *Phytophthora infestans*.

IV. REMOTE SENSING AND IMAGING

Remote sensing and imaging technologies provide early warnings, assess disease severity, and support agriculture enhancing food security. This advanced technology uses various sensors, cameras, and drones that capture detailed information about plants and their surrounding environment. One of the key benefits of this technology is its ability to provide a bird's-eye view of the entire field or orchard, allowing a quick and comprehensive assessment of plant health.

Machine Learning and Artificial Intelligence algorithms continue to improve disease detection accuracy, enabling automated diagnosis of plant pathogens. These algorithms are trained on vast datasets of plant images, that enables them to recognize subtle visual cues and patterns indicating the disease presence. They can process and analyze thousands of images of crops and identify disease symptoms far more efficiently than experts. These can also classify images of healthy and infected plants, identify specific diseases or pathogens, and even assess disease severity.

Multispectral and hyperspectral imaging can detect subtle changes in plant pigments and tissue reflectance that may indicate the presence of disease pathogens. These images are analyzed using a unique software to identify and classify diseases based on their spectral signatures. When a plant is infected by a disease, its reflectance properties change

due to alterations in its physiological processes and pigments. **Thermal imaging** reveals variations in plant temperatures which indicates stress or infection. **Drones** equipped with thermal cameras efficiently scan large agricultural areas, providing valuable insights into concerned areas.

Along with early detection, remote sensing and imaging also aids disease management and control. The specific locations of infected plants are pinpointed, by which farmers can take actions such as applying pesticides or adjusting irrigation to mitigate the spread of disease. This approach saves time and resources along with reduction in environmental impact of farming practices.

V. CONCLUSION

These advances in detection and diagnosis of diseases of crops have transformed the way we approach crop protection and agriculture. These innovations, ranging from molecular techniques to artificial intelligence and nanotechnology, offer more accurate, efficient, and timely methods for identifying and managing plant diseases. As the world faces growing challenges in food production and climate change, these advancements are instrumental in safeguarding crop yields, reducing chemical inputs, and ensuring global food security. Continued research and collaboration between scientists, farmers, and technology developers are essential to further harness the potential of these cutting-edge tools for the benefit of agriculture and the environment.

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