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Advanced Detection Techniques for Identification of Plant Pathogens

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SUMMARY

Plants are attacked by various fungi, bacteria, viruses and nematodes throughout their life cycle. Despite several advancements in crop protection measures against almost all plant pathogens, they still continue to cause significant damage to the majority of economically important crops around the world. Therefore, the most crucial step in managing diseases is early detection, which is followed by diagnosis. Several techniques of plant pathogens detection are discussed here along with recent advances such as DNA/RNA probe technology, polymerase chain reaction (PCR) amplification of nucleic acid sequences, post amplification techniques, isothermal amplification based methods and RNA-sequence based next generation sequencing techniques, which paves a new way for pathogen detection methods.

INTRODUCTION

Precision in disease diagnosis and pathogen identification is crucial because without it, we are unable to comprehend the disease and, in many cases, control it. Establishing the existence of a certain target organism in a sample, even asymptomatic individuals, is the aim of detection. Diagnosis relates to the identification of the nature and cause of the disease problem and thus deals with plants showing the symptoms. The risk of unintentional introduction of exotic pests and pathogens is increased by growing globalisation and the international trade in plants and plant-based products. Therefore, the significance of diagnostic testing has multiplied in the current era. Rapid and precise diagnostic tests are necessary to monitor the establishment of novel pathogen variations in addition to identifying new invading species. In the face of threatening diseases emerging as a result of climate change or other environmental shifts, or as a result of new agricultural methods, improved techniques are essential to ensure food security. Detection of pathogens may be carried out either by employing conventional or relatively modern methods.

Methods of plant disease diagnosis:

Plant disease diagnostic methods can be divided into two parts:

1. Conventional methods
2. Modern detection techniques

1. Conventional detection methods:

Conventional methods mostly include identification of the pathogens by visual observations, studying cultural characteristics and microscopic study. Traditional methods for diagnosing fungal diseases have relied on visible symptoms following fungal infections, such as fungi propagules like conidia, sclerotia, or mycelia on the outsides of flora, or symptoms of fungal diseases brought on by fungal pathogens after infection. While in case of bacteria, enrichment media which encourages the growth of some bacteria while refusing the other kind of bacterial genera are generally used. Widely used conventional methods include isolation and culturing, re-inoculation, microscopic techniques and biochemical tests (Sharma and Sharma, 2016). However, conventional detection techniques have a number of drawbacks, including the fact that culturing can frequently take days or weeks, which is problematic when rapid and high throughput detection is needed.

2. Modern detection techniques:

Newer methods that are increasingly being applied to the diagnosis of plant pathogens include, DNA/RNA probe technology, polymerase chain reaction (PCR) amplification of nucleic acid sequences, post amplification techniques, isothermal amplification based methods and RNA-sequence based next generation sequencing techniques. These methods have a number of potential advantages over traditional diagnostic

procedures, including the fact that they are more precise, quicker, and accessible to individuals without specialist taxonomical knowledge.

PCR based methods: For the detection and diagnosis of plant diseases, PCR is a widely used and highly sensitive method. By repeatedly cycling through denaturation, annealing, and elongation at various temperatures while using particular oligonucleotides (primers), deoxyribonucleotide triphosphates (dNTPs), and a thermostable Taq DNA, PCR enables the amplification of specific DNA sequences into millions of copies. Agarose gel electrophoresis or alternatively, colorimetric or fluorometric assays are then used to identify the amplified DNA fragments. The target pathogen is present in the sample if a certain DNA band of the expected size is present. For the detection of plant pathogens, some PCR-based approaches include RT-PCR, Real Time PCR, Multiplex PCR, and Nested PCR.

Reverse Transcriptase-PCR (RT-PCR): The RT-PCR technique combines cDNA synthesis from RNA templates with PCR to offer a quick, reliable method for analysing gene expression. When working with a low concentration of target RNA, RT-PCR is frequently employed to detect or measure the expression of mRNA. Using TMV and ToMV specific primers by RT-PCR, Kumar *et al*. (2011) reported tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) infection in pepper and tomato.

Multiplex PCR: The multiplex PCR detects simultaneously several bacterial pathogens in a single PCR reaction. Several primers are used in the multiplex PCR, and various targets must produce products of different sizes in order to distinguish them from one another using an agarose gel. Roy *et al*. (2005) simultaneously detected 7 different viruses in citrus by using multiplex PCR.

Real time PCR: Plant pathogens can be quickly and precisely detected and quantified using real-time PCR in an automated reaction. This method eliminates the need for post-reaction analysis, such as gel electrophoresis, by monitoring products online as they accumulate during each reaction cycle in a closed tube format. Using TaqMan probes, it is a highly sensitive approach that can even identify minute amounts of DNA from pathogens. Additionally, it offers the precise amount of the target gene in processed samples and aids in differentiating between closely related species. Koroleva *et al.,* (2022) detected paddy bacterial leaf streak pathogen using real time PCR method.

Nested PCR: It is a variation of PCR that reduces nonspecific product amplification due to the extra primer binding sites. Two PCR reactions using two sets of primer pairs are used to accomplish this process. The primer used in the second round is internal to the primers used in the first round of PCR. The number of templates for the second reaction is often increased by the first reaction. In the initial round of PCR, degenerated primers are typically used. Then an aliquot of reaction is placed into a fresh tube for the second round of PCR with the primer that anneal with first amplification. Twig blight and crown rot of pomegranate are emerging diseases in pomegranate cultivation that are caused by *Pilidiella granati*. While the sample included as little as 10 pg of *P. granati* DNA, a nested PCR technique enhanced both sensitivity and detection of *P. granati* and made it possible to identify the causal agent (Yang *et al*., 2017).

DNA/RNA probe based methods: Fluorescence in-situ hybridization (FISH) uses combination of microscopy, hybridization of DNA probes and target gene from plant samples to detect microorganisms. Plants contain pathogen-specific ribosomal RNA (rRNA) sequences, and FISH can assist identify these sequences to detect pathogen infections in plants. FISH can be used to detect fungi, viruses, and endosymbiotic bacteria that infect the plant in addition to bacterial pathogens. The practical limit of detection, however, is in the range of around 103 CFU/mL. FISH could also be used to identify yet-to-be-cultured microorganisms to look into complex microbial communities in addition to detecting culturable microbes that cause plant diseases (Kaur and Sharma, 2021). Cardinale *et al*. (2018) evaluated the degree of bacterial infection in olive trees and the function of bacterial aggregates in vessel occlusions using the EUB338MIX FISH probe, which was universal bacterial Cy3-labeled (red), and the KO 210 FISH probe (green), which was specifically Cy5-labeled for *Xylella fastidiosa*.

Post amplification based techniques:

Microarray: Photolithography is used to manufacture DNA microarray chips by etching a large number of individual DNA/RNA sequences as capture probes. In a single experiment, each nucleic acid fragment is specific for a DNA or RNA sequence. The biochip preparation is done with glass, nylon, or other polymers. Up to 70,000 short and distinct DNA fragments can be found on a single microarray chip. Short synthesised oligonucleotides of 30–50 bp or PCR products can be used as arrayed probes. When the arrayed chips hybridise with fluorescently tagged DNA/RNA in a test sample, numerous fluorophores disclose the presence or absence of the target sequence in the sample when read with laser technology. This method is highly effective for detection of several viruses when plants are infected by them simultaneously.

Isothermal Amplification Based Methods:

LAMP (Loop mediated isothermal amplification): LAMP, or loop-mediated isothermal amplification, is a single tube method for DNA amplification and a less expensive alternative to diagnose certain plant diseases. Isothermal amplification is carried out at a constant temperature and does not require a thermal cycler, unlike the polymerase chain reaction (PCR) technology, which carries out the reaction with a series of alternating temperature steps or cycles. Kant *et al*., (2021) detected *Pseudomonas syringae* pv. *pisi* in pea via LAMP assay.

NASBA (Nucleic Acid Sequence Based Amplification): A dual function reverse transcriptase/DNA polymerase, RNA polymerase, RNase H, and a target-specific primer with a T7 promoter are used in this method. An RNA:DNA hybrid fragment with a T7 promoter is initially generated by this set of enzymes and primers, which targets a particular RNA transcript. This hybrid's RNA is destroyed by RNase H, and the DNA is extended to create a dsDNA fragment containing a T7 promoter, acting as a template for T7 RNA polymerase to produce more RNA transcripts. Plum pox virus was detected by using NASBA method (Olmos *et al*., 2007).

RNA sequence based - Next- generation sequencing (NGS): Sequencing is the application of a technique that aids in identifying the number and arrangement of nucleotides in a certain organism's DNA. A form of DNA sequencing method known as next-generation sequencing (NGS) uses parallel sequencing of several small DNA fragments to identify sequences. When compared to sanger technique, high-throughput technology is significantly faster, generates a large number of data, and is also more affordable. Next-generation sequencing technologies provide unique and quick methods for characterising and profiling mRNAs, short RNAs, transcription factor regions, chromatin structure, DNA methylation patterns across the entire genome**.**

List of sequencing platforms as per generations:

Generation	Platform	Avg. read length (bp)
First	ABI Sanger	400-900
Second	454	100-700
	Illumina	150-300
	SOLiD	75
	Ion Torrent	200-400
Third	PacBio	1300-13,500
	Oxford Nanopore	9545

CONCLUSION

Pathogen detection in plants is crucial for ensuring secure and sustainable agricultural production. The methods that can detect pathogens quickly and accurately have seen substantial development in recent years. The expansion of sensitive and precise recognition approaches for pathogens will benefit from the breakthroughs in research that will come from the sequencing of many plant pathogen genomes, particularly in the current era of proteomics. There are many different detection technologies available today, but before using any of them in experiments, significant questions must be answered which includes their cost, testing frequency, sensitivity, accuracy, and robustness. Despite many novel technologies being accessible, challenges remain to recognize unculturable pathogens, to detect cryptic species. Pyrosequencing and next-generation sequencing techniques will also offer intriguing solutions to widen the scope of molecular detection studies. Nevertheless, modern technology can offer valuable information in the deliberate prevention of plant diseases when combined with other conventional techniques, which they should support rather than replace.

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