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Plant Pathogen Detection and Diagnostic Laboratory Techniques in Digital Agriculture – A Review

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Abstract

As the global population approaches nine billion by 2050, there is a need for better and efficient food production systems to cater to the growing population. While improving crop production, there is an urgent need to develop a better understanding of the extent of global crop losses caused by plant-pathogen infestations. The diversity and acquired resistance of plant pathogens is on the rise as these pathogens have learnt to overcome the resistant genes in crops. Climate change has also led to a variety of plant diseases, compounded by both natural and human-induced alterations in climate conditions. This necessitates a better review of existing integrated disease management strategies for long-term agricultural sustainability. Sustainable disease management strategies could enhance agricultural productivity, food safety, food quality, and economic prosperity of farmers. In this regard, the on-farm detection, diagnosis, and treatment of plant pathogens are the crucial steps in combating plant disease, which is the focus of this review article. Several laboratory methods offer portable, cost-effective, and rapid detection of pathogens. As new and traditional methods become standardized, the low cost and ease-of-use of these methods and tools for smooth adoption in fields is the next challenge for the agricultural industry. Here we discuss the molecular biology diagnostic approaches and nucleic acid amplification methods as applied to plant and soil samples. These laboratory detection and diagnostic tools and methods for plant pathogens are discussed in the context of on-farm technology use and agricultural sustainability.

Introduction

Plants and pathogens co-exist in nature with complex interactions with the environment [1-7]. The environmental conditions, including water content, temperature, humidity, sunlight, soil health, weather, carbon dioxide, and other gas emissions play crucial roles in influencing plant disease development. Addressing emerging plant diseases necessitates prioritizing pathogen exclusion through rigorous plant quarantine measures as the initial step toward safeguarding food security in both developed and developing nations [5-12]. Additional strategies such as inter-cropping, crop rotation, cover crops, pesticide use, pathogen-host interactions, and sustainable pest protection measures are also needed.

The conventional understanding of host-pathogen interactions is becoming outdated in the face of these emerging plant diseases, emphasizing the need to improve traditional detection methods and explore novel approaches tailored to new pathogen strains [7-20]. Over the past century, the accuracy and precision of plant disease detection have relied solely on traditional methods, which are often slow and ineffective, highlighting the urgent need for improvement and modernization.

Preserving the genetic and phenotypic traits in crop plants is paramount for improving crop yields in a sustainable manner [20-23]. Plant breeding experts aim to improve crop varieties which prioritize incorporating traits associated with growth, biotic and abiotic resistance, and plant immunity. These natural traits are often preserved in wild-type versions of these crops who have accumulated resistance over time. However, over time the crop varieties often fail to retain these beneficial traits which encourages plant diseases. Utilizing molecular-level genetic methods can facilitate the introduction of desirable traits from wild-type relatives into cultivated plant varieties [22-28].

The initial stages of any contemporary plant-based inquiry involve identifying and diagnosing the responsible pathogen. Realizing the damaging role of plant pathogens in crop yields, there is a demand to achieve quick, sensitive, and specific detection and diagnosis. To meet these requirements, laboratory methods have improved over the past years from simple observation of disease symptoms on plant leaves and roots to more advanced techniques capable of detecting pathogen constituents, their byproducts or their effects [28-38].

Plant Pathogen Laboratory Detection Methods

Here we discuss the established diagnostic approaches, including optical microscopy and other molecular-level techniques [11-21]. Microscopy was the oldest method used to detect and identify plant material and plant pathogens [4]. Technological advancements in microscopy have continuously improved the resolution, ease-of-use, and cost of the microscopy systems. For example, electron microscopy has helped plant pathologists to unravel the structure and functioning of various viruses and their symbiotic relations with plant hosts [4].

Following the manifestation of plant disease symptoms, numerous methods are employed for disease detection. Two primary techniques include ELISA, which relies on pathogen-produced proteins, and PCR, which targets specific DNA sequences of the plant pathogen. In ELISA-based plant disease detection, antibodies produced against microbial proteins (antigens) are extracted from animals and labeled with fluorescence dyes and enzymes to detect host-pathogen interactions. ELISA, introduced in the 1970s, remains the most widely used immunodiagnostic technique. However, its sensitivity varies depending on sample types and volumes. Although ELISA utilizes antibodies against many pathogens, monoclonal antibodies developed through hybridoma technology offer increased specificity. PCR is a cost-effective nucleic acid-based diagnostic tool and is extensively used to detect and amplify specific nucleic acids present in raw samples. The plummeting cost of sequencing has facilitated obtaining nucleotide sequences for numerous PCR amplicons, which has helped to identify other strains of known pathogens.

Real-time PCR, an advanced molecular detection method, offers superior accuracy, sensitivity, and specificity compared to conventional PCR [22-28]. It involves monitoring amplicon accumulation in real-time using fluorescently labeled primers or amplicons, eliminating the need for downstream processes like agarose gel electrophoresis. Despite PCR-based molecular methods' limitations, LAMP has emerged as a viable alternative to conventional PCR. It can amplify target DNA at very

low copy numbers within a short timeframe, offering rapid and sensitive detection capabilities. While PCR's requirement of a thermocycler limits its application where resources are scarce, the development of isothermal DNA amplification techniques has led to the creation of simple, rapid diagnostic methods suitable for point-of-care testing.

Limitations of Molecular-based Detection Techniques

However, molecular-based techniques have their own limitations which create hurdles in technology adoption [25-32]. These methods demand considerable laboratory time and involve complex sample pre-preparation steps. In addition, these methods are laborious and require specific chemical reagents tailored for specific pathogen under study. Furthermore, low concentrations of the initial samples (in soil, water or plant tissue) and pathogens can lower the sensitivity and impede effective pathogen detection from samples. Moreover, the possibility of false negatives and false positives due to target DNA sequence degradation or subpar reagent quality can compromise the experimental result reliability. Additionally, the substantial expenses associated with the laboratory methods, chemicals, and reagents in molecular detection methods dampen their technology adoption in agricultural farming. Consequently, spectroscopic techniques present themselves as promising alternatives to molecular-based techniques for reliable plant pathogen detection.

Non-destructive methods for Plant Pathogen Detection

Recent research emphasizes new automated nondestructive methods for plant disease detection that promises scalable and efficient large-scale disease monitoring adoptable in large farmlands. Advancements in spectroscopic methods have standardized disease detection with various techniques explored for detecting early and late-stage diseases. These methods include microscope imaging, fluorescence spectroscopy, (near) infrared spectroscopy, and nuclear magnetic resonance spectroscopy. Spectroscopic methods facilitate crop disease monitoring due to their potential in imaging large farmlands for digital agriculture

Fluorescence spectroscopy measures the fluorescence emitted by the sample after ultraviolet spectrum excitation [11-21]. Laser-induced fluorescence is commonly used to monitor plant stress and physiological states, as well as nutrient deficiencies. Non-imaging spectroscopy methods rely on leaf pigment, chemical component, and structural feature optical properties. Spectra collected from the samples are employed in various remote sensing detection methods for pathogen identification and detection.

Despite conventional techniques in plant pathology, there is a market demand from users for alternate plant disease detection methods targeting a diverse ranges of pathogens [30-34]. While ELISA and real-time PCR methods are available, gas sensors and optical sensors offer better results, particularly for identifying asymptomatic infections. Remote sensing technologies efficiently compartmentalize diagnostic results with spatial resolution, enhancing agricultural

sustainability and safety by reducing pesticide use. DNA fingerprinting, utilizing molecular genetic methods, identifies unique patterns in plant pathogen DNA samples.

Optical Sensors and Biophotonics

In recent years, a number of novel rapid, inexpensive, efficient, and reliable approaches have emerged, such as lateral flow microarrays and metabolomics for detecting plant metabolites [5-21]. Gas chromatography (GC) and mass spectrometry (MS) systems identify the biochemicals emitted by plants which can serve as biomarkers for specific disease detection. Biophotonics, an emerging technique, efficiently detects plant pathogens using ELISA.

Optical biosensor technologies analyze the optical spectral signatures correlating with plant stress, water availability, and plant growth. Optical technologies do not need any direct sample collection, which helps in remote and non-destructive image collection to determine disease state. Optical technologies provide non-destructive assessment of plant material which eliminates the need for direct sample collection from fields. Spectral imaging methods have been implemented using drones, such as hyperspectral remote sensing, and provide rapid large-area vegetation or crop assessments [30-34]. However, spectral imaging system sensitivity varies depending on the imaging system in use. As an example, aerial or drone-mounted systems offering broad area coverage but limited spatial resolution.

Advancements in optical biomarker-based technologies with integrated artificial intelligence tools offer key advantages such as non-invasive imaging, no sample destruction, and scalability to monitor large acres of land [11-25]. Optical detection's high throughput nature guides other technologies for follow-up or confirmation, crucial for large-scale disease detection to prevent epidemics. Traditional fungal identification methods, although tedious and time-consuming, have evolved with PCR-based approaches becoming the gold standard. Remote sensing methods indirectly detect plant pathogens by monitoring vegetation conditions and analyzing radiation changes used by plants. Remote sensing has been successfully applied to monitor plant stress conditions from plant material such as leaf pigments, chlorophyll amount, and water content in leaves and stalk.

Conclusion

As the global population approaches nine billion by 2050, there is a need for better and efficient food production systems. Plant pathogens present challenges, including depleting resources and increasing plant disease epidemics, and necessitate sustainable disease management strategies for agricultural sustainability and development. Plant pathogens, posing real threats to global agriculture, highlight the importance of strategies considering societal, economic, and ecological factors. Sustainable disease management strategies enhance agricultural productivity, food quality, and economic growth. Plant pathologists could focus their efforts on understanding plant disease mechanisms and improving management systems for stable crop health and food security. The

detection, diagnosis, and treatment of pathogens are crucial steps in combating plant disease, requiring rigorous exploration and implementation of innovative methods. Isothermal amplification methods offer portable, cost-effective, and rapid detection, but onsite technology development challenges remain in cost-effectiveness and affordability. As new and traditional methods become standardized, the low cost and ease-of-use of these methods and tools for smooth adoption in fields is the next challenge for the agricultural industry.

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