RECENT TECHNIQUES IN PLANT DISEASE DIAGNOSTICS FOR SUSTAINABLE AGRICULTURE - A REVIEW

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Abstract

There has been a growing need for rapid, on-site detection of plant diseases in the agricultural field. The desire is to identify pathogens using portable, handheld devices or tools which are cost-effective and easy to operate by minimally-trained personnel. Existing pathogen detection technologies are limited to the identification of known plant pathogens with low to medium accuracy. There is a need to invent smart techniques and methods for the rapid detection of plant pathogens in a quick and easy way so that timely decision support can be provided to farmers to protect their plants against pathogens. There are a number of advanced biotechnology techniques being developed and tailored for agriculture, such as single cell genomics and gene editing techniques. The biosensing techniques have shown progress with stronger focus on commercialization potential of microfluidics. There is a general realization to develop tools beyond laboratory settings that adequately address the end user needs for on-the-spot testing. This review covers the abovementioned topics covering the recent tools and techniques for field testing of plant pathogens that are cost-effective, sensitive, and easy-to-use.

Introduction

In the past, identifying the type of plant typically involved providing a list of potential bacterial, fungal, and viral diseases associated with it [1-12]. Most of these plant pathogens took days or months to show symptoms which made traditional laboratory detection techniques inadequate for diagnosis and treatment [7-10]. However, rapid pathogen detection has become increasingly critical due to a multitude of factors such as geopolitics, climate change, international border security, multidrug resistance, and threats of new outbreaks [5-14].

Diagnostic and detection tools are critical for on-field pest management to limit the spread of plant diseases and to improve better selection of cultivars and help make informed decisions for the control of plant diseases [15-28]. Early detection of plant pathogens on the field will also help in meeting the stringent export/import regulatory requirements of plant materials and products. New tools and technologies are needed for prompt and accurate diagnostics of plant pathogens, both the conventional pathogens and emerging pathogens or emerging variants of known pathogens. The emerging pathogens need smarter assays to screen potential genes and genetic materials that confer resistance to diseases [27-35]. This will require significant research in multiple areas of engineering and biotechnology, including those in sample preparation, sample purification, chemical screening, biodefence, plant growth, and food safety [21-28].

The diagnostics of plant pathogens and their variants in the field is especially critical with important economic ramifications in food safety and security in production plants, food extraction, manufacturing plants, and storage facilities [32-40]. New sensors are also needed which have high accuracy, accessibility, and test flexibility comparable to standard laboratory assays which can integrate multiple steps from sample preparation, sample extraction, and sample detection while minimizing the number of manual steps. The desired sensors are needed to perform in varied experimental conditions with better portability and accessibility for end users.

The value of field detection of plant pathogens for disease diagnosis is being recognized. This need is driven by farmers and planters who envision new test devices and platforms which are catering to the demands of the field and pathogens under test [32-40]. While there are already standard laboratory equipment and assays with desired accuracy and sensitivity, there are very limited number of portable and easy-to-use devices and platforms that are commercialized for mass production and field testing. There is progress being made in this area to improve the sensitivity, cost, operability, and ease-of-use in agriculture [30-36].

Microscopy and Culturing Techniques for Plant Pathogen Detection

In the conventional methods used in laboratories, the pathogen is visually observed under a microscope for any disease symptoms [32-39]. Microscopy is cheap and easily available with low-technology skills needed to operate the microscopes. However, it is difficult to differentiate subtle differences between disease symptoms or pathogen variants using microscopes. The disease symptoms are an after-effect which is a delayed response compared to early identification of pathogens that is needed. Besides microscopy, the other method involves culturing the pathogens in laboratory settings which requires specialized equipment, culturing media, reagents, and trained personnel. Culturing inherently requires 24-48 hours for the pathogen to replicate sufficiently to observe the desired effects on culture plates [11-29]. Culturing techniques can be beneficial as the gold standard for pathogen detection but may not be practical for rapid detection of pathogens on-site.

ELISA and PCR Techniques for Plant Pathogen Detection

Two common detection systems for plant pathogens are based on (a) enzyme-linked immunosorbent assay (ELISA) and (b) polymerase chain reaction (PCR)-based methods [23, 26-37]. In ELISA systems, antigens bind to capture antibodies immobilized on a prepared surface [23]. The antibodies bind to the antigen, which generates a signal to confirm the presence of a specific plant pathogen. ELISA systems are used to detect plant pathogens and analyze more than one sample in parallel.

PCR technique is employed to identify the DNA of specific pathogen [23]. In this process, we extract the DNA of the plant pathogen, amplify it to create yield millions of copies, and conduct a DNA detection step. The PCR systems face multiple challenges in sample preparation, DNA extraction, and temperature control. The desired capability of detecting multiple samples has challenges on regulating the chemical flow and automating the experimental steps. While these challenges are addressable to some extent, there has been progress in making portable PCR systems with applications to agriculture and plant pathology [23]. There has been progress in

integrating sample preparation steps into PCR systems. To extract the DNA content from cells, a number of cell lysis methods have been developed and tested, including electrical, temperature-based, and chemical methods. Thermal energy could be applied to cells in localized areas to enable cell lysis [1-12].

Volatile Organic Compounds as Sensing Elements for Plant Diseases

Another detection technique involves identifying the volatile organic compounds (VOCs) emitted by plants. VOCs are organic chemicals that have a high vapor pressure at room temperature, meaning they evaporate into the air easily. VOCs are emitted by a wide array of products and processes, and they can have short- and long-term adverse health effects. The VOCs are influenced by pathogens [30-39]. This makes it a non-invasive method for monitoring plant diseases by monitoring the VOCs. Various system design for electronic nose chips integrated with gas sensors are employed to detect VOCs. Algorithms can be developed to differentiate the VOCs emitted by normal and infected plants [13, 23]. These gas sensors employ advanced gas sensing materials with improved accuracy and lower costs compared to few years back.

The sensing of Volatile Organic Compounds (VOCs) emitted by plants presents several challenges due to the complex nature of these emissions and the diverse range of compounds involved. Here are some key challenges in sensing VOCs from plants: (i) Variability in Emissions: Plants emit a wide variety of VOCs, and the types and quantities of compounds can vary significantly depending on factors such as plant species, developmental stage, environmental conditions, and stressors. This variability makes it challenging to develop sensing technologies that can detect and quantify the diverse range of VOCs emitted by different plant species under various conditions. (ii) Low Concentrations: VOC emissions from plants are typically present at very low concentrations in the atmosphere, often in the parts per billion (ppb) or even parts per trillion (ppt) range. Detecting and accurately measuring these low concentrations of VOCs require sensitive and selective sensing techniques capable of distinguishing target compounds from background noise and interference. (iii) Interference from Background Compounds: In addition to VOCs emitted by plants, the atmosphere contains a complex mixture of other volatile compounds from sources such as anthropogenic activities, microbial processes, and natural emissions. Interference from background compounds can pose challenges in accurately identifying and quantifying plant-derived VOCs amidst the background noise. (iii) Temporal and Spatial Dynamics: VOC emissions from plants can exhibit temporal and spatial variability, with emission rates fluctuating over time and across different parts of a plant or plant canopy. Monitoring these temporal and spatial dynamics requires sensing technologies capable of capturing real-time data with high temporal and spatial resolution. (iv) Selectivity and Specificity: Many VOCs emitted by plants are structurally similar to compounds found in the atmosphere or produced by other sources, making it difficult to distinguish plant-derived VOCs from background compounds. Developing sensing technologies with high selectivity and specificity for plant-derived VOCs is essential for accurate detection and quantification. (v) Non-intrusive Monitoring: In some applications, such as field studies or monitoring of natural ecosystems, it may be desirable to perform non-intrusive monitoring of plant VOC emissions without disturbing the plants or their surrounding

environment. Developing non-intrusive sensing techniques that can remotely detect and quantify plant-derived VOCs presents additional challenges.

Addressing these challenges in sensing plant-derived VOCs requires interdisciplinary research efforts involving expertise in analytical chemistry, sensor technology, plant biology, atmospheric science, and data analysis. Advances in sensor development, data processing algorithms, and field deployment strategies are essential for improving our understanding of plant VOC emissions and their ecological and environmental roles.

Various steps are involved in accomplishing the tasks of pathogen detection, such as sample separation from raw plant material, sample isolation, and sample sensing by binding of target molecules using antibodies or magnetic particles with appropriate binding molecules. Physical separation techniques have been demonstrated for separating desired molecules using techniques such as filtering, centrifugation, electrophoresis, and on-chip dielectrophoresis. Some of these techniques have yet to show their commercial potential, and more experimentation is needed to harness their true potential as field assays.

Binding Assays for Plant Pathogen Detection

After the step of sample preparation, the target pathogens are detected through binding reactions, typically the binding between antigen and antibody or the binding between ligand and receptors [33]. Antibodies production is expensive and their physical and chemical stability is generally unreliable for field applications. An alternative to antibodies are aptamers that come with lower costs and better chemical/physical stability [31-38].

Microfluidic Chip Technologies for Plant Pathogens

Microfluidics and portable chip assays can play a vital role in on-site, field testing of plant pathogens in agriculture [23, 27-38]. There are challenges in making robust microfluidic platforms. The key challenges involve obtaining relatively pure samples, fluid evaporation in microscale chambers over long time, sample cross-contamination, and the stability of biochemical reagents and molecules [2]. The long-term storage and shelf-life of these microfluidic chips is also a concern. Microfluidics need to be designed beyond research applications and for commercialization as the end goal, which will entice new research directions that are practical, field-ready, and built for sustainability and business acumen in the area. There is rapid progress being made in biotechnology to improve the sensor sensitivity, selectivity, and accuracy which will help bolster the interest around microfluidics.

Sample preparation is a critical first step where microfluidics can make significant contributions to DNA sequencing platforms that are portable and low cost. For plant samples, sample preparation is very important to enrich the sample, concentrate the genetic material of pathogens, and eliminate the other biological content. For sample preparation, different approaches have been demonstrated such as electrophoresis, binding to microscale and nanoscale beads and particles, and membrane filters. Additionally, microfluidics allows to stack multiple layers of chips and membranes for refined sample preparation and purification.

Current Limitations of Recent Detection Techniques

Within the scope of plant bioinformatics and genomics, the challenge lies in analyzing the huge databases. The databases are searched for known host and pathogen relationships. This approach works for known pathogens but may not work for emerging variants of pathogens for which the host-pathogen relationships are yet unknown. Detecting new pathogens using bioinformatics would have simplified the molecular experiments for detection, provided smart data analytics tools are developed.

One limitation of these techniques is their limited capability for multiplex detection of different samples [23]. Most techniques require special, validated assays for single targets, and are difficult to expand for multiplex sensing, which is becoming more important with emerging variants of plant pathogens. Most next-generation multiplex detection techniques are very expensive to run many tests for the average customer. One method is called "label multiplexing" where the plant sample is exposed to different molecular probes where each type of probe is placed in a separate channel. There is, however, a limit to the finite number of channels and probes that can be placed in the assays. PDMS microfluidics offer the possibility of parallel processing with small reaction times and temperature regulation for thermal cycles.

Conclusion

The need for faster and accurate detection techniques and tools is clear and established in the agricultural community. We envision the next-generation tools to integrate the best of several areas into one integrated multi-chip system combining sample preparation, thermal cycle generation, sample extraction, and disease detection [23]. Next-generation DNA sequencing techniques with genomics and metabolomics can enable multiplex handling of different samples but come with high costs. The cost barrier needs to be overcome for mass usage of these technologies. However, validation of new platforms regarding specificity, sensitivity, and cost-effectiveness is important with proper characterization using plant samples and comparisons with gold standards. Portable field-applicable technologies have the ability to both complement and aid laboratory and greenhouse experiments in plant pathology.

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