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Plant Pathogen Detection Techniques: New Trends

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ABSTRACT: Plant infections are accountable for a significant proportion, around 40%, of the yearly decline in commercially valuable crops. This results in a substantial economic burden and has notable socio-ecological consequences. The primary objective of integrated pest management (IPM) is to mitigate the ecological consequences associated with conventional disease management approaches. This is achieved by the implementation of biological control methods and cultivation techniques that effectively minimize the occurrence of diseases or their associated symptoms. The present and developing techniques for detecting plant pathogens encompass cultivation-based, immunological, and nucleic acid-based approaches. Cultivation-based techniques encompass the deliberate cultivation and subsequent isolation of microorganisms using growth media that are either selective or semi-selective in nature. The aforementioned techniques are characterized by their simplicity, reliability, and lack of dependence on advanced technological apparatus. Nevertheless, these methods are deemed suboptimal as a result of their significant plate count anomaly, time-intensive procedures, and limited capability to identify viral plant diseases. Plant pathogens can be detected utilizing immunological techniques that involve the use of particular antibodies conjugated with enzymes, fluorophores, or nanoparticles. Nevertheless, it is important to acknowledge that these methodologies do possess several limitations, including the suboptimal chemical and physical stability of antibodies, the requirement for refrigeration during storage, and the challenges associated with generating new antibodies. Lateral flow immunoassays (LFIA) are commonly employed in the field of plant pathology for the purpose of detecting plant pathogens. However, it is important to note that these assays possess a restricted capacity for sample loading and are exclusively applicable to liquid samples. The utilization of aptamers as substitutes for antibodies in enzyme-linked apta-sorbent assays and lateral flow devices is feasible; nevertheless, it is important to note that the selectivity and affinity of aptamers can be affected by the circumstances of the sample. Conventional polymerase chain reaction (PCR) is a highly sensitive methodology; yet, it is not without limitations. These include susceptibility to PCR inhibitors, the necessity for a controlled laboratory setting, and an elevated potential for false-positive outcomes. A number of polymerase chain reaction (PCR) variants have been devised with the aim of enhancing the utility of PCR in the realm of plant pathogen identification. Isothermal nucleic acid amplification techniques present a valuable alternative to polymerase chain reaction (PCR)-based approaches. These techniques include loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), hybridization arrays, CRISPR-Cas-based molecular tools, and nucleic acid sequencing methods. Biosensors are intricate instruments that integrate a biorecognition component with a physicochemical transducer, enabling precise and timely identification of volatile organic compounds (VOCs) associated with plant diseases.

Keywords: Pathogens, enzymes, amplification, antibodies, PCR and biosensors.

INTRODUCTION

Plant diseases present a substantial risk to the agricultural sector, resulting in the annual loss of up to 40% of the yield of economically relevant crops (FAO, 2019; Savary *et al*., 2019; Baldi and La Porta 2020). The economic impact associated with these losses is substantial, amounting to an estimated yearly loss of $220 billion. The socio-ecological implications are of considerable importance, given that the worldwide population is projected to reach 9.7 billion by 2050, resulting in a surge in global food demand (FAO, 2017). Agricultural intensification is widely regarded as a superior approach, as it effectively enhances crop productivity. However, it is important to acknowledge that this method can inadvertently contribute to the proliferation of host-specialized diseases. In 2019, the European Commission initiated the Green Deal with the objective of addressing climate change and promoting sustainability within the realms of industry and agriculture. An effective strategy for mitigating these losses involves the implementation of integrated pest management (IPM) techniques, which aim to minimize

the ecological consequences associated with conventional disease management methods. Integrated Pest Management (IPM) encompasses the utilization of biological control methods and agricultural practices aimed at mitigating the occurrence of diseases or symptoms. In cases where it is not feasible to completely eliminate the use of chemical pesticides, it is advisable to employ them judiciously, targeting the specific pathogen of concern and deploying them solely in instances where a genuine hazard is present. The prompt emphasizes the significance of promptly and precisely detecting and identifying infections in order to implement efficient disease control measures, resulting in decreased use on pesticides and a more environmentally friendly approach to agriculture. The objective of this paper is to present a comprehensive analysis of contemporary and developing techniques for detecting plant pathogens. These techniques encompass traditional approaches such as cultivation-based, immunological, and nucleic acid-based detection tactics, as well as new methodologies like biosensors and high-throughput sequencing tools according to McDonald and Steinbruck (2016); Savary *et al*. (2019); Baldi and La Porta (2020).

VISUAL AND SPECTRAL DETECTION METHODS

The field of plant pathogen detection has undergone significant advancements throughout the years, resulting in the availability of several methodologies. Visual detection is a widely employed method; nevertheless, it is limited in its ability to detect latent illnesses or dormant infections (Riley *et al*., 2002). The advent of digitalization has facilitated the integration of imaging and optical or spectral approaches in the field of plant disease detection. Spectral analysis has the potential to be employed across a range of scales, encompassing the examination of high-resolution photographs as well as the utilization of drones to conduct spectral assessments of large fields (Singh *et al*., 2021). The utilization of these sensors has experienced an increase in prevalence within the agricultural sector owing to their compact dimensions, lightweight nature, and affordability (Martinelli *et al*., 2015; Zubler and Yoon 2020). Optical or spectral approaches have several notable advantages, including the capability for real-time detection, the ability to identify biotic stress, and their non-invasive nature. Nevertheless, the process of obtaining data using optical sensors is intricate and necessitates the creation of specialized algorithms, such as machine learning or neural networks. Imaging techniques has the ability to detect biotic stress prior to the manifestation of visible symptoms; nevertheless, they exhibit limitations in their capacity to accurately differentiate and identify specific infections. The integration of imaging techniques with other highly accurate methodologies is important in order to formulate efficacious management strategies. The implementation of stress-detection techniques enables the deployment of more efficient and focused sampling strategies (Martinelli *et al*., 2015; Mahlein, 2016; Zubler and Yoon 2020).

CULTIVATION-BASED METHODS

Cultivation-based techniques are extensively employed in the detection and characterization of plant diseases due to their reliance on the cultivation and isolation of microorganisms on growth media that are either selective or semi-selective in nature. The aforementioned techniques facilitate the proliferation of the specific pathogen of interest while concurrently impeding the growth of surrounding microorganisms (Gopinath *et al*., 2014; Mancini *et al*., 2016; Ferone *et al*., 2020). The confirmation of the identity of the isolates cultivated on the semi-selective growth medium is accomplished by employing morphological, microscopical, biochemical, molecular, or immunological assays (Alvarez, 2004; Figdor and Gulabivala 2011; Mandal *et al*., 2011; Gopinath *et al*., 2014; Mancini *et al*., 2016; Ferone *et al*., 2020). According to Castro-Escarpulli *et al*. (2015), commercial tests such as the analytical profile index (API) systems and Biolog™ microplates demonstrate enhanced reliability and sensitivity. Additional techniques that can be employed are matrix-assisted laser desorption/ionization in conjunction with time-of-flight analysis (MALDI-TOF) and fatty acid profiling. The utilization of DNA barcoding is a common practice in taxonomic identification, as evidenced by the studies conducted by Ahmad *et al*. (2012); Chun *et al*. (2022). Cultivation-based methodologies are characterized by their simplicity, reliability, and independence from sophisticated technological apparatus. These techniques facilitate the differentiation between organisms that are capable of survival and those that are not, enable the measurement of the target pathogen, and provide the ability to detect pathogens with a sensitivity ranging from 10 to 104 colony-forming units per milliliter (CFU/mL). Nevertheless, the efficacy of this approach is compromised by the significant plate count anomaly, its labor-intensive nature, and its limited applicability in identifying viral plant diseases given their dependence on specific host organisms. Regulatory bodies such as the European and Mediterranean Plant Protection Organization (EPPO) provide standardized cultivation-based techniques for the detection of significant plant pathogens. However, these organizations frequently advise the utilization of supplementary DNA-based testing to verify the identity of the pathogen (EPPO, 2022).

IMMUNOLOGICAL METHODS

Plant pathogens can be detected by the utilization of immunological assays, which involve the application of antibodies that are specifically linked to enzymes, fluorophores, or nanoparticles (Alvarez, 2004). The aforementioned assays exhibit a high degree of specificity and possess the capability to selectively target antigens associated with particular pathogenic bacteria. There are two distinct categories of antibodies in immunology: polyclonal antibodies, characterized by the presence of several antibodies that target different epitopes, and monoclonal antibodies, characterized by the presence of a single type of antibody that specifically targets a singular epitope (Alvarez, 2004;

Martinelli *et al*., 2015). In contrast, monoclonal antibodies tend to exhibit higher costs and lower sensitivity compared to polyclonal antibodies (Martinelli *et al*., 2015; Ascoli and Aggeler 2018). Immunological techniques possess the capability to be employed in the detection of bacterial, fungal, and viral diseases by targeting antigenic molecules expressed by all plant pathogens (Venbraux *et al*., 2023). Nevertheless, it is frequently necessary to implement various sample pretreatment procedures, enrichment techniques, and immunomagnetic separation (IMS) methods in order to enhance the sensitivity of the analysis and eliminate any potential impurities. A wide range of immunological assays are already accessible for the detection of infections, primarily employed within clinical settings.

A. Enzyme linked immunosorbent assay(ELISA)

The Enzyme Linked Immunosorbent Assay (ELISA) is a commonly employed immunological method utilized on a global scale for the detection of microbial infections. The ELISA tests, comprising direct, indirect, sandwich, and competitive formats, were introduced in the 1970s as a very efficient and rapid approach (Alhajj and Farhana 2023). The ELISA technique is characterized by its relative simplicity, with a typical duration ranging from one to several hours. Nevertheless, there are many limitations associated with this approach, including the suboptimal chemical and physical stability of antibodies, the requirement for refrigeration during storage, and the need for the development of new antibodies (Sakamoto *et al*., 2018). The enzyme-linked immunosorbent assay (ELISA) has been extensively utilized in the field of agriculture to identify plant infections, including *Xylella fastidiosa*. Furthermore, researchers have made advancements in utilizing ELISA for the detection of several other plant pathogens.

B. Lateral flow immune assays (LFIA)

The LFIA, or lateral flow immunoassay, is a commonly employed immunological technique utilized for the identification of plant pathogens. This test comprises nitrocellulose membrane strips housed within a plastic container, as described by López-Soriano *et al*. (2017). The sample is utilized within the designated application area, where antibodies are specifically attached to the target antigen (Posthuma-Trumpie *et al*., 2009; Koczula and Gallotta 2016; López-Soriano *et al*., 2017; Singh and Singh 2020). The primary antibodies are conjugated with colloidal gold nanoparticles or latex particles, enabling visual identification of the presence or absence of the target antigen. Lateral Flow Immunoassays (LFIAs) are characterized by their user-friendly nature, portability, and affordability, enabling them to deliver outcomes within around 10 minutes. Consequently, these attributes render LFIAs very suitable for point-of-care diagnostic applications (Boonham *et al*., 2008; López-Soriano *et al*., 2017; Singh and Singh, 2020) Nevertheless, their sample loading capability is restricted and confined solely to liquid samples. Yes, the rapid analytical speed and user-friendly nature have resulted in the creation of multiple lateral flow immunoassays (LFIA) for the detection of plant infections. For instance, a polyclonal LFIA has been developed specifically for *Xanthomonas campestris* pv. *musacearum*. This LFIA demonstrated a sensitivity of 105 colony-forming units per milliliter (CFU/ml) and shown a high level of specificity when tested against *X. arboricola* pv. pruni. The European and Mediterranean Plant Protection Organization (EPPO) suggests the utilization of lateral flow immunoassays (LFIAs) as a suitable method for the identification of plant pathogenic viruses. Specifically, LFIAs have been shown effective in detecting viruses such as Tomato spotted wilt virus, Impatiens necrotic spot virus, and Watermelon silver mottle virus. However, it is important to note that positive lateral flow immunoassay (LFIA) tests require further confirmation through the use of enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) procedures in order to prevent the occurrence of false-positive results (EPPO, 200).

C. Aptamers

Aptamers, which are short oligonucleotides possessing a distinct three-dimensional structure, exhibit promising characteristics that make them viable substitutes for antibodies. These characteristics include their facile synthesis, cost-effectiveness, resistance to degradation, compact size, and straightforward labeling capabilities. According to Toh *et al*. (2015), enzyme-linked apta-sorbent assays and lateral flow devices have the potential to substitute antibodies. Nevertheless, it should be noted that the selectivity and affinity of aptamer-based selection for plant pathogen detection can be altered by various sample circumstances. It is worth mentioning that the utilization of aptamer-based selection for this purpose is not yet widely adopted, as evidenced by studies conducted by Komorowska *et al*. (2017); Krivitsky *et al*. (2021).

NUCLEIC ACID-BASED ASSAYS

The detection of pathogenic microorganisms, such as viruses, fungi, and bacteria, relies heavily on the analysis of nucleic acid sequences. PCR, isothermal amplification and hybridization-based approaches are often employed methodologies in the detection of plant diseases. The attainment of high purity in the extracted DNA is of utmost importance in PCR-based tests due to their susceptibility to inhibitors. In the field of plant pathogen detection, it is common for samples to include complex matrices, which have the potential to diminish the effectiveness of the detection process (Lievens and Thomma 2005; López *et al*., 2009). DNA extraction processes can vary in complexity, ranging from straightforward to intricate, and certain methods may not be acceptable for point-of-care applications. DNA-based tests encounter difficulties in distinguishing between viable bacteria and non-viable ones. The particular targeting of RNA can be advantageous; however, the process of extracting RNA from challenging sample matrices is often complex and not consistently efficient. Live/dead probes, which are composed of chemicals that are incapable of traversing the cell membrane, only interact with unbound DNA

molecules, preventing their participation in subsequent amplification processes. However, the efficacy of these probes in distinguishing between dead and live cells varies, which has limited their widespread adoption in plant pathogen detection (Lievens and Thomma 2005; Kralik and Ricchi 2017; Schostag *et al*., 2020).

A. Conventional PCR

The polymerase chain reaction (PCR) is a laboratory technique employed to amplify targeted DNA fragments through the utilization of oligonucleotide primers a DNA polymerase enzyme, deoxyribonucleotide triphosphates (dNTPs), and a thermal cycler (Zhao *et al*., 2014; Shen, 2019). The aforementioned methodology is a specialized and exceptionally sensitive method, with the ability to amplify nucleic acids as few as 3 copies of the target. The limit of detection is contingent upon various factors, including the type of sample, the effectiveness of DNA extraction, and the efficiency of amplification (Kralik and Ricchi 2017). The level of specificity exhibited by the polymerase chain reaction (PCR) is contingent upon the meticulous design of primers that possess a high degree of selectivity, hence minimizing the occurrence of false-positive outcomes. PCR is additionally characterized by its expedited nature compared to traditional culture-based techniques, yielding outcomes within a matter of hours. Nevertheless, PCR-based methodologies possess certain limitations. These include susceptibility to PCR inhibitors, inability to differentiate between viable and non-viable cells, dependence on a controlled laboratory setting, incapability to amplify RNA targets, and an elevated likelihood of false-positive outcomes due to non-specific amplification or contamination (Ward *et al*., 2004; López *et al*., 2009). Numerous polymerase chain reaction (PCR) variants have been devised with the aim of enhancing their applicability in the identification of plant pathogens. One example of a variant is reverse transcriptase polymerase chain reaction (RT-PCR), a technique that incorporates a reverse transcription process to replicate the RNA template into a complementary DNA strand. This methodology enables the identification of genes that are actively transcribed or viral RNA. Multiplex PCR is a laboratory technique that employs two or more primer sets to selectively amplify distinct genetic sequences within a single polymerase chain reaction (PCR) process. This enables the concurrent identification of several target diseases. However, the technique is susceptible to non-specific DNA amplification and the generation of false-positive results as a consequence of the existence of several primer pairs. The process of multiplexing has the potential to reduce sensitivity due to the preferential amplification of a certain target, which may result in the suppression of amplification for other targets. An illustration of this can be seen in the case of a multiplex PCR assay designed to identify two *Phytophthora* spp., which exhibits a detection threshold ranging from 10-100 pg DNA/µl. In contrast, individual singleplex PCRs possess a detection limit of 1 pg DNA/µl. One study conducted by Cui *et al*. (2016) serves as an illustration of a multiplex polymerase chain reaction (mPCR) technique that is capable of detecting several plant diseases. The study showcased the mPCR's notable attributes, including its high specificity and its ability to save time and reduce costs. An further illustration pertains to the assay employed for the identification of cotton pathogens, encompassing fungal, bacterial, and viral targets (Chavhan *et al*., 2023). Nested PCR (nPCR) is an alternative method that utilizes two consecutive rounds of amplification, leading to enhanced sensitivity in detection and increased specificity. Nevertheless, the nested polymerase chain reaction (nPCR) technique is susceptible to carry-over contamination between consecutive reactions and incurs higher costs and requires more labor-intensive procedures. The aforementioned drawbacks can be mitigated through the utilization of multicompartment reaction. These effectively avoid the necessity for subsequent amplification modifications and minimize the potential for carry-over contamination (López *et al*., 2009; Mancini *et al*., 2016; Nair and Manimekalai 2021).

B. Quantitative PCR

Quantitative polymerase chain reaction (qPCR), alternatively referred to as real-time PCR, is a technique employed to quantitatively assess the amplified DNA in real-time throughout the course of the PCR process, as opposed to solely detecting the endpoint. The utilization of fluorescent dsDNA-binding dyes or sequence-specific probes enables the evaluation of the quantity of amplified DNA at each cycle (Postollec *et al*., 2011). Nevertheless, quantitative polymerase chain reaction (qPCR) exhibits certain limitations, including the occurrence of non-specific amplification and the need for melting curve analysis as a means of verifying the absence of non-specific amplification. Sequencing-specific probes, such as TaqMan probes, molecular beacons, and scorpion probes, exhibit enhanced specificity in comparison to dsDNA-binding dyes. Quantitative Polymerase Chain Reaction (QPCR) possesses numerous benefits in the realm of plant pathogen detection. These advantages encompass heightened sensitivity attributed to experimental fluorescence measurements, as well as the utilization of shorter targets ranging from 70 to 150 base pairs. Consequently, QPCR emerges as a beneficial instrument for the timely identification of pathogens (Okubara *et al*., 2005). Additionally, it provides expedited analysis time and has a higher susceptibility to automation. The quantification of pathogens is a crucial factor in disease management, as it enables the establishment of action thresholds in agricultural settings. This, in turn, reduces the need for frequent application of chemical pesticides and promotes a more effective and sustainable approach to disease control. numerous qPCR approaches have been developed for the detection of plant diseases. For instance, one method has been designed specifically for the detection of *Phytophthora cryptogea*. Another qPCR method has been developed for the detection of *P. cactorum* in strawberry samples. Additionally, Verdecchia *et al*. (2021) have described numerous

qPCR methods that are capable of identifying bacterial plant infections.

C. Digital droplet PCR

The technique known as digital droplet PCR (ddPCR) enables the precise measurement of nucleic acids in a given sample, as demonstrated by Hindson *et al*. (2011). The process entails dividing DNA into around 20,000 small droplets composed of water-in-oil. Each droplet contains either no copies or a single copy of template DNA (Hindson *et al*., 2011; Hayden *et al*., 2013; Chen *et al*., 2021). The aforementioned droplets function as discrete PCR reaction vessels, facilitating the amplification of a particular DNA segment corresponding to the target pathogen. The detection of PCR reactions can be accomplished using fluorescent probes or intercalating dyes. By quantifying the number of droplets that contain an amplicon, it is possible to ascertain the quantity of template DNA that is present in the initial sample (Hindson *et al*., 2011; Hoshino and Inagaki, 2012; Chen *et al*., 2021). Digital droplet PCR (ddPCR) offers numerous advantages compared to real-time PCR. Firstly, ddPCR eliminates the requirement for a calibration curve to quantify the target of interest. This alleviates the need for time-consuming and labor-intensive calibration procedures. Secondly, ddPCR exhibits enhanced sensitivity, enabling the detection of low abundance targets with greater precision. Additionally, ddPCR demonstrates increased resistance to PCR-inhibitors, which can often interfere with the accuracy and reliability of real-time PCR results. Lastly, ddPCR reduces the dependence on the amplification efficiency of the PCR reaction, thereby minimizing potential variations and inaccuracies associated with this parameter. The utilization of complicated sample matrices, such as soil, proves to be more advantageous in the analysis of plant pathogen detection, hence providing an additional benefit. Nevertheless, it should be noted that ddPCR remains a comparatively costlier alternative to qPCR, with an approximate 2.3-fold increase in per-test expenses. Additionally, the implementation of ddPCR necessitates a more intricate process, resulting in a time requirement that is 2-3 times lengthier. In addition, it should be noted that droplet digital PCR (ddPCR) has a narrower dynamic range for quantification when compared to quantitative PCR (qPCR). Notable instances of droplet digital polymerase chain reaction (ddPCR) techniques employed in the identification of plant pathogens encompass *Xylella fastidiosa*, *Acidovorax citrulli*, *Tilletia controversa*, and a reverse transcription-ddPCR approach utilized for the detection of peach latent mosaic viroids (Liu *et al*., 2020). Multiplexing applications can also be accommodated by this technology; however, the literature has limited instances of such applications being documented. In general, it can be stated that droplet digital polymerase chain reaction (ddPCR) is a highly important method due to its sensitivity and robustness, particularly in the context of monitoring low titer pathogens inside complicated samples (Maheshwari *et al*., 2021).

D. Isothermal nucleic acid amplification

The utilization of PCR-based techniques for the identification of plant pathogens is widespread; nevertheless, their practical application in the field is constrained by the requirement of thermal cyclers and DNA of exceptional purity (Lau and Botella 2017). Isothermal amplification techniques present a valuable alternative by utilizing strand-displacing DNA polymerases in the absence of thermal cycling equipment. The execution of these procedures can be accomplished utilizing basic apparatus such as heating blocks (Ivanov *et al*., 2021). Loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) are widely employed techniques for isothermal amplification, as documented by Li *et al*. (2018).

E. Loop-mediated isothermal amplification

The Loop-mediated isothermal amplification (LAMP) technique is a commonly employed isothermal amplification assay that involves the utilization of a minimum of four primers, namely two inner primers and two outer primers, in conjunction with a strand-displacing DNA polymerase. The outcome of this process yields a single-stranded DNA molecule that exhibits a distinctive dumbbell-shaped configuration at its termini, facilitating its specific interaction with binding partners (Craw and Balachandran 2012; Lau and Botella, 2017; Becherer *et al*., 2020; Ivanov *et al*., 2021). The experiment is conducted under isothermal conditions at a consistent temperature range of 60-65°C. The duration of the experiment typically yields outcomes within a time frame of 30 minutes, which may vary based on the specific target and primers employed. The LAMP technique offers a number of notable benefits, such as the utilization of uncomplicated apparatus for isothermal amplification and measurement, the ability to be employed in outdoor settings, and a high level of sensitivity. Nevertheless, it is widely acknowledged that the utilization of LAMP is mostly applicable to qualitative assays, as supported by Venbraux *et al*. (2023). A limitation associated with the utilization of LAMP is the intricate nature of the primer design procedure, which may result in the generation of non-specific products and primer dimers. In these instances, the utilization of sequence-specific detection techniques, like as fluorescent probes, may prove to be more appropriate. The preference for carry-over contamination with the amplified product during sample handling and post-amplification visualization is frequently observed. Multiplex loop-mediated isothermal amplification (LAMP) assays can be pursued, albeit with inherent challenges arising from the intricate primer design and the potential for non-specific amplification. The LAMP technique has demonstrated efficacy in the detection of diverse plant diseases, encompassing bacterial, fungal, and viral agents. One example of a molecular test is the SYBR Green-based Loop-Mediated Isothermal Amplification (LAMP) technique, as demonstrated by Boubourakas *et al*. (2009). This method offers the advantage of requiring minimal sample preparation.

The sensitivity of RT-LAMP assays has been demonstrated to be 100 times greater than that of traditional RT-PCR procedures. Moreover, researchers have successfully devised a detection method that combines LAMP technology with smartphones to identify *Phytophthora infestans* and tomato spotted wilt virus. This innovative approach enables swift extraction of DNA and RNA, yielding results in under 30 minutes from the initiation of nucleic acid extraction (Paul *et al*., 2021).

F. Recombinase polymerase amplification

Recombinase polymerase amplification (RPA) is a molecular biology approach that employs a DNA-recombinase, primers, nucleotides, single stranded DNA (ssDNA)-binding proteins, and a strand-displacement polymerase enzyme (Piepenburg *et al*., 2006; Lobato and O'Sullivan 2018). The technique facilitates expeditious amplification of the desired DNA sequence, achieving its final stage within a time frame of 20 minutes. Robotic Process Automation (RPA) exhibits user-friendly functionality, rapid analysis capabilities, and operates at a reduced temperature, rendering it well-suited for point-of-care implementations. Detection is performed at the terminal stage using gel-electrophoresis or a lateral flow device. The utilization of the lateral flow device format proves to be highly advantageous in point-of-care scenarios due to its ability to facilitate prompt identification of amplification products (Lobato and O'Sullivan 2018; Ivanov *et al*., 2021). The sensitivity of RPA is notably great, since it is capable of identifying a range of 1-10 copies of template DNA within a given reaction. The integration of a reverse transcription process can be employed to specifically target RNA templates, including RNA viruses. According to Lobato and O'Sullivan (2018), the multiplexing of RPA can be achieved by utilizing various primer pairs or sequence-specific probes. However, it is crucial to exercise caution and ensure meticulous design in the process. Nevertheless, it has been documented that primer mismatching can occur in DNA sequences that are comparable, leading to the potential for false-positive outcomes. In order to enhance precision, it is imperative to exercise caution during the process of primer design. Researchers have developed RPA assays for the identification of plant diseases, frequently employing lateral flow devices for the detection of amplicons (Mekuria *et al*., 2014).

HYBRIDIZATION ARRAYS

The process of DNA strands forming hybrid complexes with their complementary strands facilitates the efficient identification of infections. Hybridization-based assays, including fluorescence in situ hybridization (FISH) as well as southern and northern blotting techniques, exhibit limited suitability in the detection of plant diseases. Nevertheless, hybridization arrays, such as microarrays and macro arrays, enable the concurrent identification of several diseases. The construction of these arrays entails the immobilization of sequence-specific capture probes on a solid support, utilizing a reverse hybridization methodology (Narayanasamy, 2011). The process involves the extraction of target DNA, followed by the amplification of universal genes. Subsequently, the amplified products are denatured and then hybridized with detector oligonucleotides. Micro- and macro-arrays are two widely recognized types of hybridization arrays. Microarrays consist of a high density of probe DNA patches, whereas macro-arrays have a lower density and employ chemiluminescent labels for detection (Narayanasamy, 2011; Aslam *et al*., 2022). The Luminex xMAP technology employs detector oligonucleotides that are affixed to microbeads possessing distinct spectrum characteristics. Although hybridization arrays offer advantages, such as their ability to detect target pathogens, their usage necessitates prior knowledge of the genetic sequences of the pathogens being targeted. Additionally, the process of utilizing hybridization arrays is both labor-intensive and time-consuming. A number of arrays have been created for the purpose of identifying bacterial, fungal, and viral plant pathogens. However, their utilization has been diminishing as a result of the decreasing expense associated with sequencing (Lievens *et al*., 2003; Zhang *et al*., 2008; Narayanasamy, 2011; Charlermroj *et al*., 2013; Úrbez-Torres *et al*., 2015; Krawczyk *et al*., 2017; Bhat and Rao 2020; Aslam *et al*., 2022).

CRISPR-CAS-BASED DETECTION SYSTEMS

The utilization of CRISPR-Cas-based molecular tools has brought about a significant transformation in the field of molecular biology, enabling the targeted modification of genetic material in diverse organisms (Doudna and Charpentier 2014). Furthermore, their excellent specificity and adaptability have rendered them a subject of investigation in the field of molecular diagnostics. The detection tactics for pathogens are dependent on the process of DNA extraction and the subsequent binding of the Cas protein to a specific DNA motif associated with the disease. This interaction leads to the generation of a detectable signal. These methods exhibit a low cost, great sensitivity, and specificity, while also not necessitating the use of advanced technological equipment. Typically, they exhibit the capacity to deliver outcomes promptly, as the majority of tests may be conducted within duration of fewer than 2 hours. The majority of CRISPR-Cas-based detection methods exhibit sensitivity within the picomolar range. However, the incorporation of pre-amplification of target sequences can lead to a substantial enhancement in sensitivity. Nevertheless, there exist certain drawbacks and obstacles that impede their extensive implementation in practical settings. The potential for multiplexing is constrained, and the utilization of CRISPR-Cas-based diagnostics frequently necessitates laborious sample preparation procedures (Wang *et al*., 2020; Kaminski *et al*., 2021; Huang *et al*., 2022). Pre-amplification procedures are frequently necessary in order to enhance sensitivity and detect pathogens with low titers, hence resulting in increased expenses and analysis duration. Moreover, the presence of single-nucleotide specificity may give rise to false-

negative outcomes, especially in the case of viruses exhibiting elevated mutation rates (Benzigar *et al*., 2021; Huang *et al*., 2022). Recent research by Sharma *et al*. (2021); Karmakar *et al*. (2022) have provided evidence of the potential of CRISPR-Cas-based assays in the detection of plant diseases. These assays have exhibited proof-of-concept in identifying economically significant RNA viruses and fungal infections.

NUCLEIC ACID SEQUENCING METHODS

The utilization of DNA sequencing has emerged as a valuable technique in the identification of microorganisms by the sequencing of distinct genetic markers and subsequent comparison with a reference database (Barghouthi, 2011). The accuracy and reproducibility of this method surpasses that of traditional procedures such as morphological and phenotypic tests (Reller *et al*., 2007; Tewari *et al*., 2011). The utilization of sequencing technologies for detection and identification has been expedited by their evolutionary advancements over the last 15 years. First-generation sequencing technologies, such as Sanger sequencing, provide lengthy reads but have a restricted capability for high-throughput sequencing. The utilization of second-generation sequencers, such as Illumina and IonTorrent, results in the production of short reads with high throughput capabilities. According to Loit *et al*. (2019), the utilization of third-generation sequencing technologies such as Nanopore and PacBio necessitates a substantial upfront financial commitment and the availability of a laboratory setting. Sanger sequencing is considered to be a more appropriate method for confirming the identity of individual isolates following selective cultivation. The utilization of high-throughput sequencing has proven to be advantageous in terms of cost reduction for sequencing, hence facilitating the simultaneous detection of several plant diseases and the analysis of microbial community composition. The repertoire of next-generation sequencing methodologies encompasses metagenome sequencing and amplicon sequencing.

BIOSENSORS

Biosensors refer to devices that integrate a biorecognition component with a physicochemical transducer in order to produce a quantifiable signal upon the interaction with a specific target analyte (Hameed *et al*., 2018; Bridle and Desmulliez 2021). Due to their affordability, user-friendly nature, and rapid outcomes, they are well-suited for point-of-care implementations. Typical instances of transducers encompass electrochemical transducers, mass-based transducers, and optical transducers. Biorecognition elements encompass a range of applications, including nucleic acid probes, antibodies, aptamers, and enzymes. The selection of the biorecognition element is contingent upon the specific transducer employed and the particular target molecule under consideration (Fang and Ramasamy 2015; Hameed *et al*., 2018; Bridle and Desmulliez 2021). The immobilization of biorecognition elements on the sensing surface plays a critical role in ensuring the effectiveness of biosensors. Various methods for immobilization are available, including adsorption-based techniques, covalent attachment, avidin and biotin systems, and self-assembled monolayers (Toh *et al*., 2015; Khater *et al*., 2017; Shahdordizadeh *et al*., 2017; Bridle and Desmulliez 2021). The diverse range of transducers and biorecognition elements facilitates the creation of multiple categories of biosensors designed for the purpose of detecting plant pathogens (Cardoso *et al*., 2022). The study conducted by Freitas *et al*. (2019) presents several notable examples of biosensors utilized for the detection of various plant pathogens. These include a DNA hybridization-based biosensor designed for the identification of *Phytophthora ramorum* in rhododendron leaves, an electrochemical biosensor employing RPA amplification for the detection of *Pseudomonas syringae*, an immunoassay biosensor utilized for the identification of Citrus tristeza virus in infected citrus samples, and a non-invasive volatile organic compound (VOC) biosensor integrated into a smartphone for the detection of late blight in tomato leaves. The biosensors has the capability to identify particular volatile organic compound (VOC) markers, hence enabling precise and timely identification of late blight in tomato leaves and other plant VOCs associated with diseases.

CONCLUSIONS

Plant diseases are responsible for causing a significant reduction in crop output, amounting to around 40% annually in economically significant crops. Consequently, the timely identification of these pathogens is of utmost importance for the implementation of integrated pest management strategies, the promotion of sustainable agricultural practices, and the reduction of reliance on chemical pesticides. Remote sensing technologies have the potential to aid in the identification and localization of stress conditions prior to the manifestation of apparent illness signs. The guidelines established by the National Plant Protection Organization (NPPO) encompass the implementation of PM7 Diagnostic Protocols for Regulated Pests. However, it is important to acknowledge that these protocols are subject to certain constraints, such as the requirement for specialized staff, the associated high costs, and the extended processing times involved. Various techniques, such as cultivation-based, immunological, PCR-based, isothermal amplification, hybridization-based, and next-generation sequencing, are now being developed to overcome these constraints. The selection of an optimal detection method is contingent upon various factors, including the specific pathogen being targeted, the allocated budget, the nature of the sample matrix, and the technological resources that are at one's disposal.

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