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Current and emerging molecular technologies for the diagnosis of plant diseases – An overview

Mohammad Malek Faizal Azizi[1], Noor Hani Mardhiah[2], Han Yih Lau[1*]

[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia.
[2]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor.

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ABSTRACT

Plant diseases caused by numerous pathogens such as bacteria, viruses, and fungi are responsible for substantial economic losses in the agricultural industry worldwide. Specific, sensitive, and efficient diagnostic tools have been developed worldwide to mitigate and prevent the pathogenic threat. The diagnostic tools have revolutionized from classical methods to more advanced molecular diagnostic approaches such as enzyme-linked immunosorbent assay (ELISA), conventional polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP), biosensor, and next-generation sequencing (NGS). Hence, this review describes the current and emerging molecular diagnostic tools to distinguish and identify pathogens in crops.

* Corresponding author

E-mail: hylau@mardi.gov.my (Han Yih Lau)

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1 Introduction

Agricultural production is pivotal to meet the increasing food demand, and it becomes the backbone of industrial and developing countries. As agricultural activity rapidly expands to feed the growing world population, plant diseases and pests significantly affect food production and quality. Diseases and pests may exacerbate yield losses of up to 30% of the world's total annual economy owing to lost food production in the billions of dollars (Savary et al. 2017; Rizzo et al. 2021).

Therefore, it is necessary to define the problem and seek remedies to manage diseases. A vast number of plant pathogens such as fungi, bacteria, viruses, nematodes, oomycetes, and parasitic plants are responsible for a range of serious plant diseases, and they have been categorized as quarantined pathogens globally. Appropriate disease diagnosis and early detection are imperative to overcome disease outbreaks (Fang and Ramasamy 2015). Years of experience coupled with advanced technologies, render the development in various disciplines, including phytopathology, ecology, taxonomy, molecular biology, and immunology. Scientists work on various plant pathogens, pests, invasive species, and organisms associated with species. With the advancement of equipment and laboratory facilities, the reliability of recent techniques can be compared and combined with conventional plant diagnostics. The emergence of accurate diagnostic assays is essential to identify and monitor pests and plant diseases efficiently, where better plant health monitoring can be ensured with the developed diagnostic products. Inaccurate identification and diagnosis could consequently affect disease control and lead to a waste of time and resources. Therefore, specific, rapid disease diagnosis and early detection are paramount in disease management to prevent the establishment and dispersal of pests and pathogens effectively and to minimize the subsequent impact (Myerson and Reaser 2002; Piombo et al. 2021).

Before developing modern and high-tech techniques for plant pathogen detection, traditional methods such as visual symptoms inspection and laboratory tests, including physiological, biochemical, chemical, and pathogenicity tests, were adopted to identify plant pathogens and diseases (Lau and Botella 2017). However, these methods can only be performed once severe damage were observed on the infected plant. Thus, further treatments for the infected plant might not be successful since it has been severely damaged by the disease. Traditional methods are considered irrelevant and insufficient in terms of accuracy, precision, and sensitivity of plant disease diagnosis. Traditional methods are also inexpensive and time-consuming, especially in the analysis process, which requires expertise (Skottrup et al. 2008).

To bridge the gap, scientists begin to develop effective molecular-based techniques such as plant disease diagnostic tools and kits that could improve decision-making in control management and overcome many shortcomings of the traditional assays. Molecular-based diagnostic tools have emerged rapidly over several decades and have recently moved towards a new era in plant diagnostic technologies. The evolution of diagnostic technologies has enabled effective optional tools with relevance to plant disease diagnosis. Herein, this review summarizes the current and emerging advanced molecular techniques of plant disease detection that could be exceptionally impactful in detecting pathogens and plant diseases.

2 Molecular-based Techniques used for early stage plant disease diagnostic

2.1 ELISA

ELISA and enzyme immunoassay (EIA) are solid-phase assays that deploy antibodies labeled with enzymes, reacting with a substrate to generate a color change, detecting and quantifying the amount of a specific substance in a sample (Ichiki et al. 2013). This technique has been broadly employed as a diagnostic tool in clinical and plant pathology. Sakudo et al. (2006) found that invasive techniques such as ELISA effectively diagnose viral infections but are not ideal in cost-effectiveness, speed, and accuracy. For instance, a rapid dot- ELISA developed by Wang et al. (2012) to diagnose rice plants infected by the southern rice black-streaked dwarf disease virus (SRBSDV) using anti-SRBSDV rabbit antiserum was found to be highly reliable, sensitive, and specific to the disease. Furthermore, Ichiki et al. (2013) demonstrated that the double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) method is straightforward to use for routine diagnostic RSV detection in insect vectors. Monitoring the rate of virulent insect vectors in early summer (before rice planting season) is pivotal for predicting rice stripe, a financially devastating disease. This method is simple, as up to 96 insect samples can be processed concurrently using plastic multi sticks. Besides this, in China, the wheat dwarf virus (WDV) was detected accurately in WDV-infected wheat plant tissue crude extracts using ACP-ELISA and dot-ELISA (Zhang et al. 2018). In 2020, Klap et al. (2020) revealed that tomato fruits exhibiting viral-like symptoms of marbled yellow spots in Israel were caused by the tobamovirus ToBRFV and the potexvirus PepMV using the ELISA technique as one of the serological tests.

Though ELISA has many advantages when used for pathogen detection, but this technique also has various drawbacks like the sensitivity of ELISA is not as high as that of many other molecular methods, and the assay takes up to 24 hours to complete (Stackhouse et al. 2020). Further, due to the sophistication of the technique and the high cost of culture cell media required to obtain a specific antibody, ELISA can also be quite expensive and labor-intensive (Sakamoto et al. 2018). Although polyclonal antibodies are effective at detecting pathogens, they are not always

sufficiently specific. Monoclonal antibodies, on the other hand, are more specific but also more costly. While developing antibodies against plant viruses has been extremely successful due to ELISA's high sensitivity for viruses, it is significantly less effective against more complex organisms such as fungal plant pathogens (McCartney et al. 2003). Due to the low sensitivity of this application, it will be ineffective at detecting bacteria (Fang and Ramasamy 2015). As a result, ELISA is considered ineffective for detecting fungal and bacterial pathogens that cause rice diseases. Additionally, due to a lack of specificity, antibodies used in ELISA can react to a variety of strains with clearly distinct symptoms and are unable to distinguish them precisely (Scala et al. 2018).

2.2 Conventional PCR

Polymerase chain reaction (PCR), developed by Kary Mulis in the mid-1980s, is one of the greatest molecular biology achievements (Mullis and Faloona 1987). Specific qualification of plant pathogens can be performed by technological advances of PCR to overcome the drawbacks of traditional methods. The PCR technique requires several reagents and components, which include the DNA template, *Taq* polymerase, two primers, deoxynucleotide triphosphates (dNTPs), buffer solution, and bivalent cations (Mg^{2+} or Mn^{2+}), and also monovalent cation (K^{+}) (Figure 1).

It has been also widely used for the detection of a plant pathogen. PCR products can be visualized through gel electrophoresis analysis. However, the PCR sensitivity can be influenced by the nucleic acid extraction method and target DNA variability (Bastien et al. 2008). PCR manages to amplify a small amount of DNA due to its high sensitivity, and it is widely used for detecting pathogens that have a long latent period between infection and the development of the disease's symptoms. Nevertheless, the limitations of conventional PCR include susceptibility to contamination and the lack of robustness (Boonham et al. 2008). This technique also has very poor resolution, i.e., about 10-fold because agarose gel could not resolve yield variabilities. However, it is more specific and sensitive compared to traditional methods for pathogen detection in plants. To date, conventional PCR remains one of the most commonly used techniques in detecting plant pathogens.

For instance, Azizi et al. (2019a,b) employed a conventional PCR technique for the identification of *Pantoea ananatis* and, *P. stewartii* subspecies *indologenes* as the causal agents of bacterial leaf blight (BLB) disease of rice in Malaysia. Likewise, in Southern Karnataka, India, PCR was also deployed to characterize *Ralstonia solanacerum* isolates that cause bacterial wilt in tomatoes (Shweta et al. 2018). Besides that, Murugan et al. (2020) have also successfully identified *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) isolates from infected tomatoes using PCR technique.

However, there are some limitations to using the PCR technique. One of the most significant disadvantages of this method is the high risk of contamination, which results in a longer time required to obtain the result. Amplicons can be easily dispersed throughout the environment using this method (Hajia 2018). As a result of false-positive results, it is critical to isolate the work environment from the external environment and to establish work disciplines. Although the early theory stated that the PCR's ability to detect target sequences should be less than 10 per sample, researchers have rarely been able to design a test that meets this detection level in different types of protocols. The method's detection level is

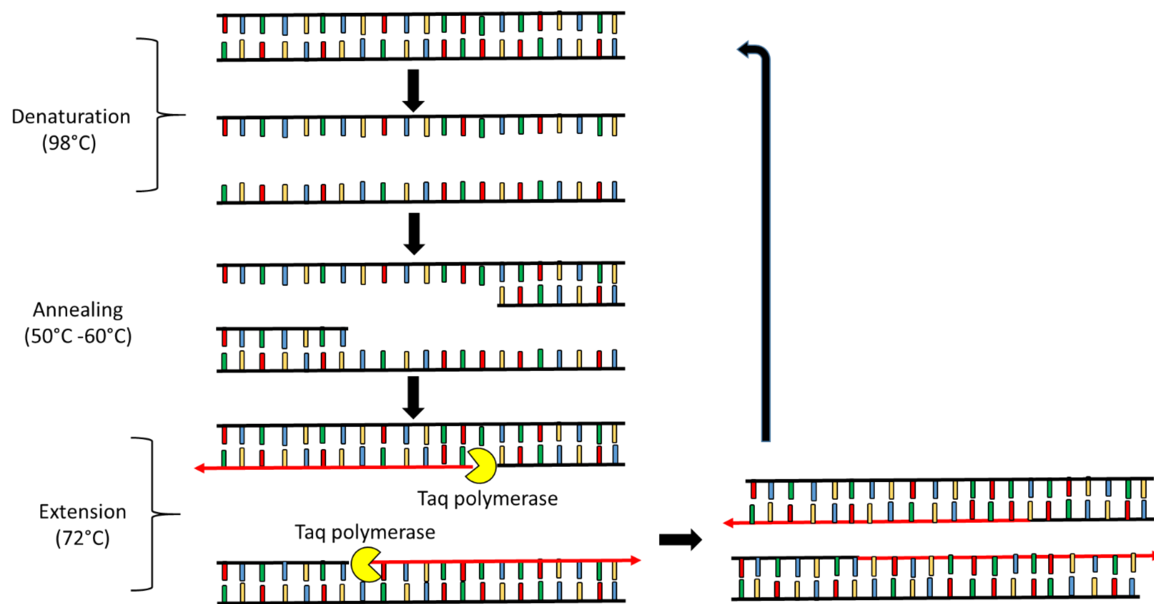

Figure 1 Schematic of conventional PCR

currently much lower than the level mentioned above, and its sensitivity has decreased as a result of interference of various factors. The absence of a standard genomic purification method, the use of low-quality materials, and an inability to optimize the test are all significant influencing factors. However, as working conditions change, test sensitivity decreases. So, continuous quality control of utilized facilities should be considered (Panteghini and Forest 2005; Hajia 2018).

2.3 Real-time PCR

Real-time polymerase chain reaction (RT-PCR), also known as quantitative polymerase chain reaction (qPCR), developed by Kary Mullis in the 1980s, is a significant improvement and revolutionary PCR technique (Mullis 1990). The RT-PCR technique has captured the attention of researchers globally and represents a groundbreaking alternative to other molecular techniques. It is one of the most powerful applications among the most rapid and sensitive detection and can reduce cross-contamination risk (Vincelli and Tisserat 2008; White et al. 2011). Furthermore, this technique could detect the minimum amount of DNA pathogens from infected plant tissues and insect vectors (Crosslin et al. 2006). Real-time PCR is performed in a thermal cycler with the capacity to illuminate each sample with a beam of light of at least one specified wavelength and the detection of the amplicon through the use of oligonucleotide probes that emit fluorescence during amplification (Figure 2).

The PCR protocol cycle in which the amount of significant fluorescence emitted during amplification is directly proportional to the amplified DNA/RNA present in the sample. This cycle is termed as quantitation cycle (Cq) or cycle threshold (Ct).To date, real-time PCR has been widely applied by scientists in detecting plant pathogens, including bacteria, oomycetes, fungi, nematodes, and viruses(Lievens et al. 2006).

For example, Carneiro et al. (2017) developed a TaqMan RT-PCR on the *TEF 1*-α gene to detect *Fusarium fujikuroi*, which causes the bakanae disease in different rice tissues. The technique successfully detected and quantified *F. fujikuroi* from rice culms, leaves, roots, and seeds during the analysis. Similarly, He et al. (2020) described a rapid, highly sensitive, and simple DNA-based real-time polymerase chain reaction (RT-PCR) assay for screening resistant tea plant cultivars and identifying differences in pathogen aggressiveness within and among Colletotrichum species isolated from infected tea. This technique has replaced lesion size assessment at the infection sites on the leaves that lack sensitivity and accuracy in quantifying pathogen growth (He et al. 2020). Further, *Dickeyafangzhongda*t caused bleeding canker disease in pear in China was detected and identified using TaqMan real-time PCR assay based on an elongation factor G (f*usA*) gene (Tian et al.2020). This technique was successfully performed as the first detection technique for tomato leaf curl New Delhi virus (ToLCNDV) (Luigi et al. 2020). However, one of the drawbacks of real-time PCR is that it might fail to identify biological

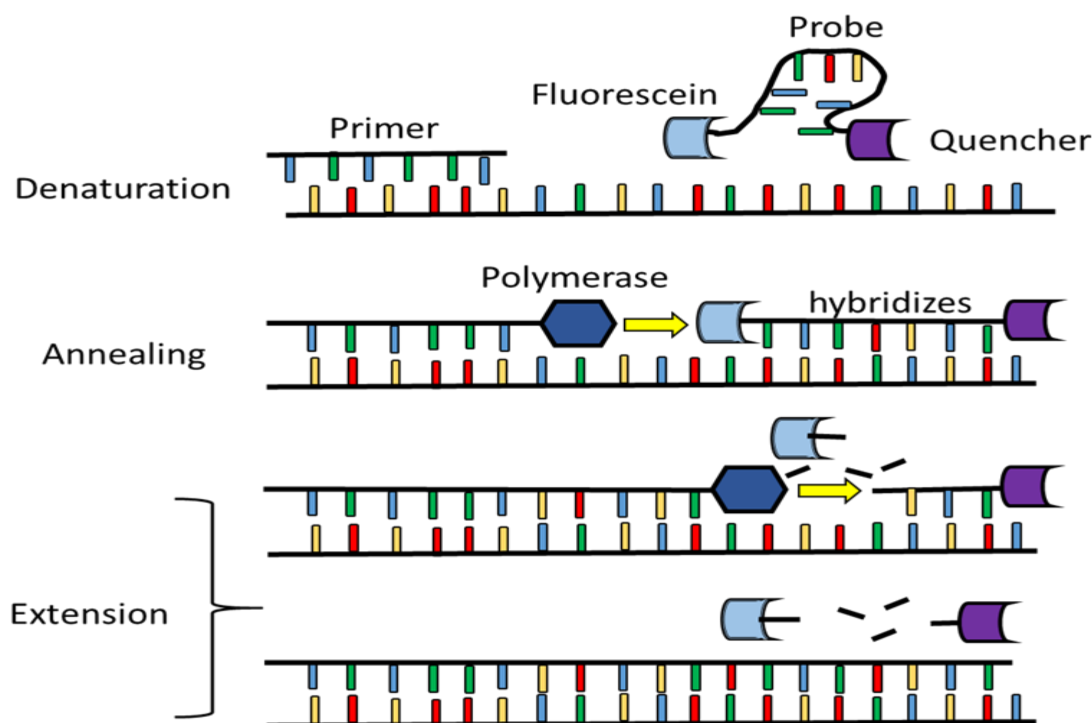

Figure 2 Schematic diagram of Real-time PCR

processes such as alternative splicing since it is performed on small DNA fragments. This method also requires the usage of costly equipment and reagents (Gachon et al. 2004).

A significant disadvantage of qPCR is the requirement for prior sequence data for the desired target gene; consequently, qPCR can be used to target only known genes (Smith and Osborn 2009). However, it will be difficult to target unknown genes because genome or gene fragment sequences from cultured organisms and/or clone libraries are derived via PCR using primers based on current sequence knowledge (Smith and Osborn 2009). As a result, access to the unknown target gene is inevitably limited when compared to analyses of previously characterized sequences.

2.4 LAMP

LAMP is one of the successful novel techniques widely employed as an alternative technique to PCR due to its rapidity, simplicity, practicality, and cost-effective equipment. LAMP is a method for amplifying specific DNA sequences initially established by Notomi et al. (2000) to detect hepatitis B virus (HBV) by amplifying a specific DNA region of HBV under isothermal amplification. This technique adopts four different primers (forward and reverse internal and external primers) that recognize the six distinct sequences within the target HBV viral DNA. LAMP consists of four main different primers, including two inner primers (FIP and BIP) and two outer primers (FIP and BIP) [a forward outer primer (F3) and a backward outer primer (B3)]. The amplification relies on auto cycling strand and high DNA strand single-stranded displacement activity that contains a loop structure, which is performed under isothermal conditions at 60°C–65°C for 45–60 minutes (Lafar et al. 2020). The amplification process is comprised of three important steps: production of the starting material, cycling amplification, and elongation and recycling (Figure 3) (Hardinge and Murray 2019).

LAMP is considered a more versatile technique due to the detection of the amplicon using three optional methods: turbidimeter, colorimetric detection, and agarose gel electrophoresis or real-time fluorimeter platform (Waliulla et al. 2020). Despite the advantages of this method, the drawbacks of LAMP include the high risk of primer dimer formation and it requires the use of a heat block to maintain the temperature at 65°C (Rani et al. 2019).

The LAMP assay has been established in plant pathology for the detection of various plant pathogens. Recently, Karimi et al. (2020) reported that the genus and species-specific PCR primers can detect and discriminate *Colletotrichum* and *C. Nymphaeae* from other fungal species in pure culture and assays of diseased using the LAMP technique than PCR assay due to its higher sensitivity and specificity. Likewise, the LAMP assay rapidly detected *Pseudomonas syringae* pv. *Tomato* (*Pst*) in artificially and naturally infected tomato leaves and stem tissues in the field without laboratory work (Chen et al. 2020). The sensitivity of the LAMP assay was claimed as similar to qPCR and even 100 times more sensitive compared to RT-PCR in detecting *tomato brown rugose fruit virus* (ToBRFV) (Sarkes et al. 2020).

Additionally, the advancement of the LAMP technique has its own set of constraints. Cross-contamination is the most serious issue,

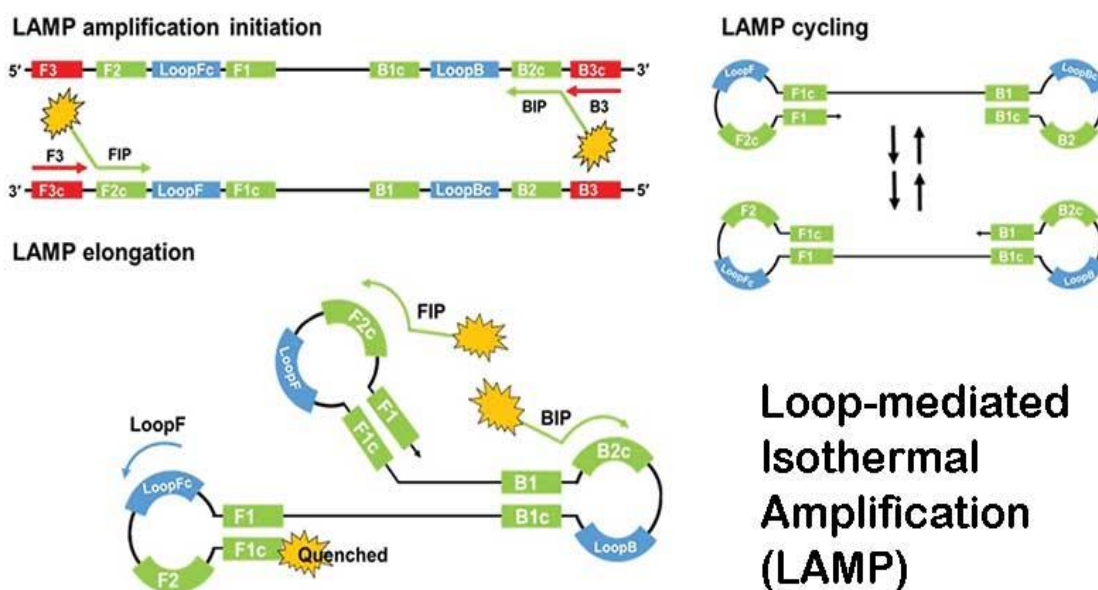

Figure 3 LAMP amplification initiation, cycling and elongation (Hardinge and Murray 2019).

owing to the technique's high sensitivity, particularly following the amplification step and the fact that certain variants require the opening of reaction tubes for amplicon detection (Mori et al. 2006; Tomlinson et al. 2007; Tomlinson and Boonham 2008). Because LAMP amplification products do not degrade readily, the possibility of carry-over contamination exists. As a result, proper handling and practice are necessary to avoid the risk of cross-contamination. The relative complexity of assay design is also a significant hurdle, as each assay requires up to six primers, in comparison to PCR, which only requires two primers (Tomlinson and Boonham 2008). Amplification time affects the duration of the LAMP process. Francois et al. (2011) discovered that the shortest duration for amplification is between 60 and 120 minutes, and a 180-minute negative control reveals amplification (Dhama et al. 2014).

2.5 Biosensor

Biosensors are of great importance due to their capability to resolve a potentially wide range of analytical problems and challenges in various fields, including agriculture and food safety, medicine, pharmacology, security, environmental monitoring, etc. The biosensor was first developed by Clark and Lyons (1962) using electrochemical detection of oxygen or hydrogen peroxide to measure glucose in biological samples. Since then, biosensors have been progressively developed with innovative techniques involving electrochemistry, nanotechnology, to bioelectronics (Vigneshvar et al.2016). According to the International Union of Pure and Applied Chemistry (IUPAC) recommendations (1999), a biosensor is described as a device that deploys the integration of specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds, commonly by electrical, thermal, or optical signals. Biosensors are analytical devices comprising a biorecognition element coupled with a transducer and converting the recognition event into some measurable readout/analytical signal (Figure 4).

The development of pathogen biosensor strategies is based on biological recognition using receptors on antibodies, DNA probes, phages, and others. Biosensors play an important role in detecting pathogens as they provide more rapid and specific detection than conventional methods. Antibody-based biosensors offer sensitive and rapid analysis for a wide range of pathogens, including bacterial, fungal, and viral species. For instance, in 2019, a highly sensitive Surface Plasmon Resonance (SPR) immunosensor was successfully developed for early detection of *Pseudocercospora fijiensis*, the causative agent of the banana black Sigatoka disease. It consists of a polyclonal antibody covalently immobilized on a gold-coated chip using the EDC/NHS method via a mixed self-assembled monolayer (SAM) of alkanethiols (Luna-Moreno et al. 2019). The immunosensor technique is also considered an alternative method for fig mosaic virus (FMV) detection due to its advantages such as high sensitivity, simplicity, accuracy, and low cost over conventional methods (Haji-Hashemi et al. 2019). Conversely, the deployment of antibody-based techniques may show cross-reactivity due to limited specificity, which sometimes produces false-negative results (Franken et al. 1992; Lau et al.2014; Lau and Botella 2017). DNA-based biosensors provide advantages over antibody-based techniques, mostly in terms of sensitivity. Lau et al. (2017) successfully developed a DNA-based biosensor combining recombinase polymerase amplification (RPA) with nanoparticle electrochemistry to detect *P. Syringae* infected with *Arabidopsis thaliana* and infection detection before the appearance of disease symptoms. Nevertheless, the DNA biosensor can be rapidly degraded and needs specific storage and analysis

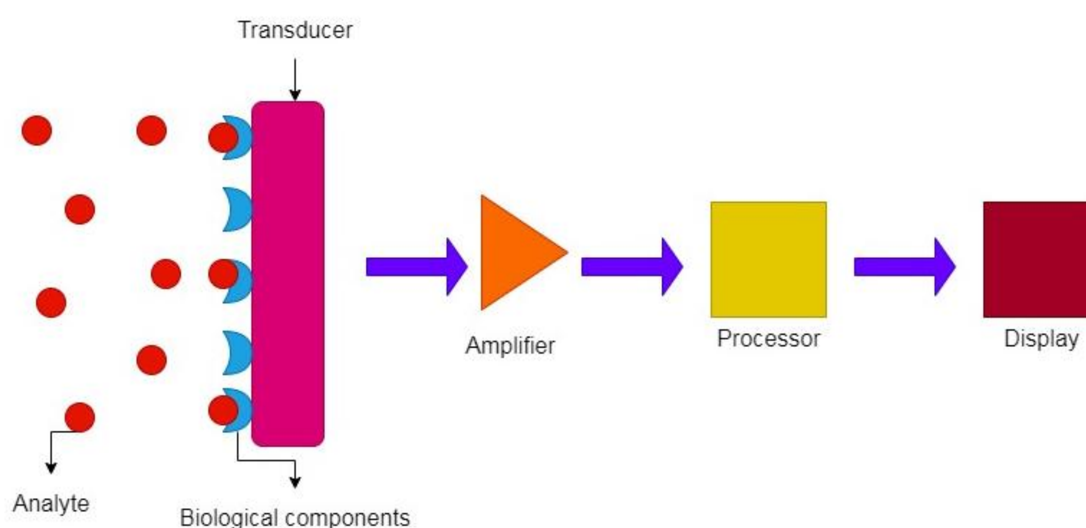

Figure 4 Schematic diagrams of generic components of a biosensor and its processes

conditions to maintain DNA stability and its attachment to the transducer (Peña-Bahamonde et al. 2018). Admittedly, the effectiveness of a DNA biosensor might be significantly impacted by changes in temperature or pH (Koyun et al. 2012). Specifically, in the case of temperature, the sensitivity of DNA biosensors is dependent on experimental temperatures, as this is caused by a hybridization process occurring between the probe and the target molecules that occurs at optimum temperatures, which must be determined before the deployment of the sensor (Kavita 2017; Rahman et al. 2017). In terms of pH conditions, a buffer with potassium or sodium phosphate is required to generate the highest signal at pH 7.0 to improve the sensor's effectiveness (Kavita 2017; Rahman et al. 2017).

The advancements in electrochemical DNA biosensors demonstrated in previous studies for the detection of rice diseases offer many advantages, including simplicity, cost-effectiveness, and enhanced sensitivity and selectivity under optimized conditions. However, because DNA biosensors are prone to degradation, they must be stored and analyzed under conditions that ensure DNA stability. Because DNA is easily degraded, they require special storage and analysis conditions to keep the DNA stable and attached to the transducer (Peña-Bahamonde et al. 2018). Additionally, temperature and pH can affect a DNA biosensor's effectiveness (Koyun et al. 2012). For example, the sensitivity of DNA biosensors is temperature dependent, as the probe hybridizes with target molecules at optimal temperatures that must be determined before sensor deployment.

2.6 Next generation sequencing (NGS)

Traditionally, molecular detection techniques greatly relied on the Sanger sequencing technology, which was based on chain termination (Sanger et al.1977). Due to its high efficiency and low radioactivity, this technique was employed as the primary technology in the "first generation" of laboratory and commercial sequencing (Liu et al. 2012). Though this technology is efficient for sequencing short DNA fragments, it is tedious and ineffective for sequencing large DNA fragments.

The advances in the next generation sequencing (NGS) technology, or also called massively parallel sequencing, between 2004 and 2014 have filled the gap and transformed the sequencing technology in aspects of massively parallel analysis approaches capturing millions of short-read sequences in a much shorter time, high throughput, and dramatically facilitated genome sequencing at a lower cost (Kanzi et al. 2020). The rapid evolution of NGS platforms reduced the cost of sequencing gigabase pairs of nucleic acid from $1,000 to $10 (Ronholm 2018). The NGS technology has opened new molecular biology opportunities, including whole-genome sequencing (Walker et al. 2013), transcriptomics (Oono et al. 2013), metagenomics (Qin et al. 2010), epigenomics (Cruickshanks et al. 2013), proteomics applications such as ProteinSeq (Darmanis et al. 2011), and single-cell sequencing (Navin et al. 2011). The relatively recent NGS development is an invaluable technique for multiple biological disciplines, including plant pathology (Behjati and Tarpey 2013; Díaz-Cruz et al. 2019). The limitations of the approaches above, specifically for helmpathogen diagnosis, can be addressed using this technology (Díaz-Cruz et al. 2019). The combination of this technique with advanced bioinformatics has fuelled innovative ways for a more rapid detection, identification, and elucidation of the causal agents of new and emerging diseases (Chalupowicz et al. 2019). NGS entails numerous DNA-related steps, including DNA isolation and fragmentation, library preparation, massively parallel sequencing, bioinformatics analysis, and variant/mutation annotation and interpretation (Qin 2019).

Advanced sequencing methods in NGS commonly adopt massively parallel signature sequencing, colony sequencing, pyrosequencing, and sequencing by oligonucleotide ligation detection (SOLID) (Rajesh and Jaya 2017). Though NGS can rapidly generate a large amount of data, data analysis is a great challenge. Several free software programs are available for the assembly of NGS data (e.g., SOAP de novo, Abyss, Velvet, and bowtie). Even so, these programs require a proficient and expert researcher in the deployment of command-line driven applications (Kehoe et al. 2014). NGS has been used in the rapid identification of plant pathogens that induce severe diseases. New generation sequencing was adopted to detect *Sarcococca* blight-causing novel fungal pathogen, *Calonectria pseudonaviculata*, in ornamental plants using IlluminaMiSeq. A 51.4 Mb genome of the two host isolates revealed unique single nucleotide polymorphism for the two isolates and identified both as *C. Pseudonaviculata* (Malapi-Wight et al., 2016). RNA-seq based NGS was used to study field pathogenomics and gain insight into the emergence of *Puccinia striiformis* f. sp. *tritici* (PST) populations as the causal agent of wheat yellow (stripe) rust in wheat and triticale (Hubbard et al. 2015).

Next-generation sequencing necessitates the use of sophisticated bioinformatics systems, as well as fast data processing and large data storage capabilities, all of which can be prohibitively expensive (Pabinger et al. 2014; Di Resta et al. 2018). The ability to purchase next-generation sequencing equipment is a common occurrence in academic institutions. However, many institutions lack the computational resources and staffing necessary to analyze and clinically interpret the data (Di Resta et al. 2018).

3 Conclusions and future prospects

The occurrence of emerging plant pathogens continues to become a major threat to ecosystems, food security, and the global economy. Furthermore, important factors such as globalization,

increased human mobility, climate change, and vector and pathogen evolution have encouraged the spread of invasive plant pathogens. Thereby, accurate and early diagnoses of the pathogen are critical to providing measure control strategies. The advances of molecular diagnostic tools for plant disease detection have witnessed unprecedented development in the recent decade in combination with modern technology techniques. This review has highlighted the recent and most notable molecular diagnostic tools for plant disease detection. Due to rapid advances in molecular diagnostic approaches in the recent decade, great development in various useful technologies such as biosensors and NGS has continuously emerged for plant disease detection. All techniques mentioned in this review have contributed to the rapid, sensitive, and specific detection of plant pathogens. However, available plant pathogen diagnosis tools remain the major challenges/limitations that should be deliberated in selecting the best tools for detecting plant pathogens.

The continuous development of technological advances in plant disease detection will improve plant diagnostics for the early detection and containment of quarantine pathogens and better-integrated management tools to combat the disease. The detection of plant-pathogen can also be useful in the future for some regulation purposes, e.g., plant import and export screening procedures, where only plants that are free from pathogenic diseases are allowed to be imported from and exported to other countries. Thus, it will help to prevent plant diseases from spreading to other regions or countries. Identifying plant pathogens is also beneficial to plant breeders, helping them to develop disease-resistant varieties. Furthermore, plant pathologists hoped to see more advanced molecular techniques in plant disease detection that provide alternatives to available options and encourage growth in agriculture and the global economy. Although extensive studies have been conducted on developing molecular detection tools for detecting and identifying plant diseases, farmers' involvement is also vital in identifying plant diseases. Farmers may play an active role in identifying diseases affecting their crops in the early stages. Therefore, it is important to develop new user-friendly techniques that are not complex and do not require experts to handle them. Farmer-scientist collaboration is valuable to combat diseases and generate relevant techniques in plant disease detection more efficiently. Efforts and largely tacit knowledge from both farmers and researchers may contribute to remarkable innovations and improvements used in detecting plant diseases.

Human beings live in an era of technological transformation, which is rapidly reshaping lifestyles and erasing distinctions between the physical, digital, and biological realms. Cyberinfrastructure, big data management, and data mining capabilities require painstaking planning and collaboration to amass critical resources for research studies. While these cyber technologies continue to evolve, the fundamental challenges associated with rice crops will persist. However, since knowledge has become unrestricted, the solutions will be more in-depth and can be solved more quickly. The development of high-throughput sequencing (HTS) technology has transformed research into detecting and identifying plant pathogens in recent years. The development of novel methods for detecting and identifying phytopathogens has been accelerated by the advent of HTS technologies. Due to the ability to sequence multiple organisms concurrently, HTS/NGS methods enable the detection of multiple organisms in a sample. Virus discovery is the most advanced application of HTS/NGS in plant pathology. The technique is rapidly gaining acceptance as a gold standard for determining the aetiology of a novel or uncommon viral symptoms in diagnostic settings. In the short term, HTS/NGS is also an extremely promising technique for screening propagation material for quarantine or certification purposes, particularly for plant viruses, where benefits could be discovered with only minor modifications to existing techniques, but with significant technical and quality control challenges. Simultaneous sequencing is possible with HTS/NGS methods, allowing for the detection of multiple organisms in a sample. The most advanced application of HTS/NGS in plant pathology is virus discovery. The technique is rapidly gaining acceptance as a standard method for determining the aetiology of a novel or uncommon viral symptoms in a diagnostic setting. In the short term, HTS/NGS also represents a highly promising technique for screening propagation material for quarantine or certification purposes, particularly for plant viruses, where benefits could be discovered with only minor modifications to existing techniques, but with technical and quality control challenges. HTS/NGS technologies generate significantly more genomic data than conventional molecular techniques. This can aid researchers in gaining a better understanding of the genomic diversity within a species (or at a lower taxonomic level), resulting in more precise taxonomic assignment for unambiguously identified pathogens, more precise assessment of the impact of a pathogen's genetic diversity, and more targeted molecular test design. HTS/NGS has developed into a technique for sequencing fungal genomes without prior knowledge of the pathogen's sequence. It can be used to identify both novel and emerging infections of rice, as well as previously identified pathogens. The molecular techniques discussed in this study are precise, effective, laboratory-based, and require sophisticated tools for rice pathogen identification.

Author contributions

MMF and NHM conceived the idea and wrote the manuscript. HYL critically reviewed the manuscript. All others have approved it.

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Conflicts of Interest

The authors declare no conflict of interest.

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