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REVIEW ARTICLE

Nanobiosensors for early detection of plant pathogens

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Abstract

Plant pathogens are a major concern in production of crops as they lead to a great loss of food grains. Although several methods are available to manage the diseases and the chemical-based methods are frequently used and sometimes indiscriminate use poses serious problems to the environment. It is, therefore, necessary to detect plant pathogens at an early stage in order to control epidemics. Plant pathogens can be detected using conventional methods such as culture-dependent, biochemical and molecular techniques; however, these methods need advanced technical skills and well-equipped laboratory facilities and are not suitable for *in situ* analysis. Several nanotechnology-based methods are available for plant pathogen detection. Among them, biosensing systems for early detection of the pathogen using nanobiosensor are gaining momentum in field of research on plant pathogen detection. Materials having size ranging from one and one hundred nanometers are known as nanoparticles. These materials have special qualities that can be used to improve agricultural practices. Nanobiosensors are novel integrated systems of biosensors that are made up of a bioreceptor, transducer and a detector on the nano scale size. These nano-inspired biosensors have played a major role in enhancing nature of life through different medical, environmental and quality-control applications globally. Numerous nanobiosensors have been developed, including those for detecting plant infections caused by fungi, viruses and bacteria. This review will contribute to understanding the basics of biosensors and their accessible biosensor based detecting tools and techniques for plant pathogens.

Keywords

application; biosensors; detection; Plant pathogen

Introduction

Since ancient times, plant diseases have been the world's biggest agricultural concern. They cause 20-40% of crop yield losses worldwide, pose major threats to food security and have historically hindered agricultural productivity. Chemical treatment remains the main technique to lower plant disease incidence, if applied often enough, pathogens become less vulnerable. In addition to polluting the environment, excessive spraying can negatively impact soil microbiology. Plant diseases are a major source of crop production constraints and significant financial losses. Pathogenic microorganisms like phytoplasma, viruses, fungi and bacteria, are responsible for disease incidence (1).

Plant pathogens are typically identified using a variety of molecular and immunological diagnostic techniques (2, 3). These diagnostic methods, however, are not always able to identify multiple diseases at once in plants exhibiting signs of unknown pathogens. Rather than identifying the etiology of disease symptoms, these techniques are intended to recognize or determine the existence of particular species causing diseases in plants (2). Numerous possible causative agents such as fungi, bacteria and virus can be concurrently detected by multiplexed detection techniques such as array technology (4, 5). However, because of technological issues, they are rarely commonly employed and require prior knowledge of the agents to be detected.

Thus, effective diagnostic methods for an early detection of diseases caused by the plant pathogens are essential for guaranteeing sustainable food production. To this end, numerous molecular techniques for quick plant pathogen identification have been developed. The visual identification of symptoms, isolation followed by colony identification, enzyme-linked immunosorbent assay (ELISA), fluorescence in situ hybridization (FISH), molecular diagnostic techniques like polymerase chain reaction (PCR) and other techniques have been employed in the previous studies for the identification of the pathogen in plant (6, 7). These methods are not as effective in the early asymptomatic stages. Although these are thought to be result-oriented, the use of classical methods is limited in developing nations because they require a lot of time, specialized equipment and a laboratory setup. Additionally, they require a number of molecular primers and enzymes, which can be costly and have limited shelf life (8). They also take a lot of time, expensive equipment is needed, cross-examination may yield falsely negative results and expert assistance is required. Their inability to access farmers' fields is a significant constraint (9). Numerous possible causative agents can be concurrently detected by multiplexed detection techniques such as array technology. However, because of technological issues, they are rarely employed and require prior knowledge of the agents to be detected.

Thus, effective diagnostic methods for prompt and early detection of diseases caused by the plant pathogens are essential to guarantee sustainable food production. Recently, application of nanotechnology in the field of pathogen detection is gaining momentum in food production and as they become more apparent as potential tools to improve high-throughput analysis, these methods can enhance the sensitivity, accuracy and speed of plant pathogen identification. Additionally, they offer faster, more cost-effective and precise plant pathogen diagnosis.

Plant disease diagnostics use nanotechnology to transform the field and spur the creation of state-of-the-art tools for the prompt coupled with early identification in plant infections. Because of their small size (between 1 and 100 nm), nanomaterials are a great choice for this application because they exhibit unique chemical, photosensitive and electrical properties, as well as better surface-to-volume ratios than their bulk counterparts (10). One promising tool for the detection of pathogens is the expansion and incorporation of molecular diagnostics at a nanoscale level. The use of nanobiotechnology to diagnose the plant diseases is recognised as nanomolecular diagnostics or nanodiagnostics (11). Nano-inspired biosensors have become essential in enhancing nature of life through diverse applications in medical settings, environmental monitoring and quality control worldwide. The progress in nanotechnology has introduced remarkably innovative components that enable biosensors to achieve exceptional performance levels (12). Nowadays, nanobiosensor has become more apparent as a novel component to enhance the analytical process and improve the accuracy, speed and sensitivity of the identification of the plant pathogen. The application of nanobiosensors for faster, less expensive and more precise plant pathogen diagnosis is the main topic of this review.

Despite numerous benefits, molecular detection methods have limitations when it comes to identifying pathogens in materials like seeds and insect vectors at low concentration or during early infection stages. Additionally, cross-contamination with Polymerase Chain Reaction (PCR) reagents can lead to false-negative outcomes by inhibiting the amplification of target DNA. On the other hand, negative outcomes might occur because of PCR-generated fragments due to the cross-amplification from non-target DNA. Another constraint lies in the impracticality of using PCR for on-site plant pathogen detection in the field (3). To address these challenges, recent years have seen the emergence of innovative and portable nanobiosensors, extensively employed as diagnostic instruments in food, environmental and medical analysis. Strategies for pathogen biosensing rely on biological recognition, employing various receptors, such as DNA probes, phages and other agents (13-15). Biosensors are considered as advanced detection tools used for environmental monitoring, pathogen and pesticide residues in food and drink, instantaneous identification of human blood components, identification of airborne pathogens and beverages (16).

Nanobiosensors

A nanobiosensor is composed of a bio-sensing segment along with physiochemical transducer which generates an electric signal upon detecting a specific pathogen or analyte in a solution. The transducer translates the biomolecular interaction into a digital output (17). The biosensors are grouped into electrochemical, optical, thermal or piezoelectric biosensor, based on transducer used in the sensor (18). The bioreceptor, which can take the form of antibodies, DNA, enzymes, tissues or cell cultures, plays a role in recognizing and providing specificity to the sensor. This specificity is achieved through selective biochemical interactions. Over the past few decades, research has shown that biosensing approaches are effective for identifying plant pathogens and achieving meaningful diagnostic results in practical applications. Various sensor-based methods have been employed for detecting different plant pathogens (Table 1).

Significant diagnostic results were obtained through biosensing techniques for the detection of plant pathogens. A microfluidic electrochemical immune biosensor, three times faster than ELISA, was developed to detect bacterial pathogen *Xanthomonas arboricola* (26) and enhanced specificity and sensitivity were achieved. An electrochemical immunosensor was also developed to identify the PPV virus, in which gold electrodes were used along with an anti-PPV polyclonal antibody (27).

Table 1. Biosensors with different sensor components

Target pathogen	Sensor component	Reference
P. syringae	AuNP-ssDNA	(19)
P. lateralis	AuNP-ssDNA	(20)
Aflotoxins	AuNP	(21)
B. thuringiensis	Pt-NP-IgGd	(22)
X. campestris	Si NP-Rubpy-IgG	(23)
R. solanacearum	AuNP-ssDNA	(24)
F. oxysporum	CdSe/ZnS	(25)

Components of biosensors

A nanobiosensor is an analytical device in nanometer scale which is used to detect or measure the biochemical substances. Generally, a nanobiosensor comprises of three crucial segments as shown in (Fig. 1) (28).

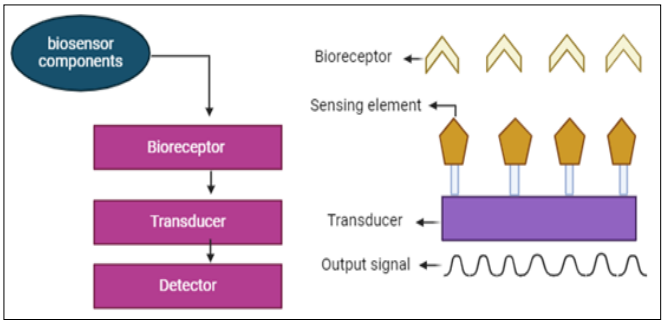

Fig. 1. Components of Biosensor

The main components of a nanobiosensor

Biological Recognition Element: This component is responsible for interacting with the target analyte. DNA/RNA, aptamers, enzymes, and antibodies are examples of common recognition elements that are made to bind to particular molecules in a specific way.

Transducer: The transducer converts the biological interaction into a quantifiable signal. Nanomaterials play a crucial role in improving the efficiency and specificity of the signal transduction process.

Signal Processor: This part uses optical, electrochemical, or mechanical techniques to convert the transducer's signal into a readable output.

The central element of biosensors is the transducer, and it has a transduction mechanism. This mechanism is crucial for transforming the interactions between bioanalytes into identifiable and reproducible signals. It converts the energy from specific biochemical reactions into an electrical form (29). Biological receptors consist of biomolecule-sensing materials *viz*., cell organelles, tissues, antibodies, molecular imprints, enzymes and nucleic acids. These entities which are of biological origin can receive the signals emitted by the sample (30). Upon receiving the signal by the probe material, it is given to the transducer. It functions as an interface by monitoring the external signal in the form of energy. Upon interaction with the sensing material, the signal is converted into a quantifiable electrical signal in the detector. The detector elements then receive this low-energy signal and pass it to a microprocessor for amplification and analysis (31). There are two types of biosensors based on sensing mechanisms.

I. Electrochemical biosensors

In an electrochemical biosensor, the interaction between the analyte and the biosensing element generates a signal, which is then transformed to an electronic signal for the quantitative analysis. An electrochemical biosensor, mainly composed of two systems, consists of an electrochemical transducer and the molecular recognition layer. A transducer transfers biological information from a binding event into an electrical signal, then it will be displayed on a readout device (32). This type of biosensor is able to detect pathogens present in air, water and on seeds and within green houses and open field conditions (33). In electrochemical biosensors, the primary roles of nanoparticles are to immobilise biomolecules, catalyse electrochemical processes, improve electron transport, label bio molecules and act as reactants. There are two types of electrochemical biosensors, one is an antibody-based electrochemical sensor and the other is a DNA-based biosensor (34). A quick and inexpensive immunoassay was developed to identify the capsid protein of the Citrus tristeza virus (CP-CTV). The assay used magnetic beads that were coated in anti-CP-CTV antibodies and horse radish peroxidase (HRP) to separate and capture the virus from sample solutions (35). Subsequently, a disposable microfluidic electrochemical device (DμFED) comprised of an array of immunosensors and built through fast prototyping was utilized to identify the biomarker that was caught magnetically.

Efforts were also taken to detect pathogens using the amperometry approach. The monoclonal antibody anti-CP-CTV was added to the device's electrode. Similar to this, gold nanoparticles (AuNPs) and HRP were used to create a dual amplified electrochemical immunosensor for detecting *Pantoea stewartii* subspecies *stewartii*-NCPPB 449 (36). A modified novel label-free electrochemical immunosensor using a Prussian Blue (PB) electron transfer mediator was electrodeposited onto a carbon nanotube ionic liquid-modified glassy carbon electrode to enable sensitive and reliable detection of the PthA effector protein from the citrus canker pathogen (37). This method improves electroactivity of PB. The immunosensor detects the PthA at various concentrations of antigens using voltammetry techniques and the immunosensor demonstrated excellent selectivity, long-term stability and repeatability, showing significant potential for real sample analysis (37, 38). It was developed using a graphene oxide (GO) based electrochemical platform to detect groundnut bud necrosis and ortho-tospo virus quickly and accurately (39). GO is deposited onto indium-tin oxide (ITO) coated glass substrates to create the immunoelectrode. The electrode is functionalized with anti-GBNV antibodies through EDC-NHS conjugation chemistry, which involves using N-ethyl-N′-(3-dimethylaminopropyl) carbodimide hydrochloride and N-hydroxysuccinimide.

Based on the transducer used, electrochemical biosensors are classified into amperometric, impedometric, potentiometric and conductometric.

Amperometric biosensors are integrated devices that measure the oxidation/reduction processes of the electrically active biological component provided appropriately analyzed quantitative information. This biosensor has advantages including the capacity to fabricate a disposable design for miniature elements for detection at the field level.

Impedometric biosensors detect and quantify analytes by measuring changes in impedance caused by reactions between antibodies and analytes on the electrode surface. Electrochemical Impedance Spectroscopy (EIS) operates by applying small-amplitude sine wave perturbations across a wide frequency range. The resulting signals are then measured as a function of frequency. Impedimetric biosensors are frequently employed for biomass detection by microbial metabolism, based on the metabolic redox reactions of microorganisms (40).

Potentiometric biosensors generate a voltage signal from an analyte's biorecognition. Currently available potentiometric biosensors consist of an immobilized microbe layer coated ion-selective electrodes (pH, ammonium, chloride, etc.) or gas-sensing electrode (pCO_2 and pNH_3) (41). Potentiometric biosensors typically detect the electromotive force (EMF) or electrical potential difference between two electrodes when the current is close to zero using a high-impedance voltmeter (42). A transformer can translate changes in pH, ionic strength, or redox status at the surface into proportionate electrical impulses.

The conductometric biosensor is an analytical tool that can translate a particular biological recognition reaction into electrical conductance (43). In contrast to other biosensor transducer types, conductometric biosensors can be made at low cost using thin-film technology, eliminating the need for a reference electrode (44).

A label-free impedimetric biosensor was developed (45) to detect the nucleic acids of the *Citrus tristeza* virus. Electrodeposited gold nanoparticles (AuNPs) were used to modify the screen-printed carbon electrode (SPCE)-based sensing platform. This improved electrode's conductivity and effectively immobilized thiolated ssDNA probes. By using electrochemical impedance spectroscopy (EIS), the hybridization of the target ssDNA with the probe ssDNA produced an electrochemical change that is measured as a change in impedance value. While supplying the voltage to the cell membrane, oxidation/reduction reactions and molecular interactions on top of elctrode providing opposition to the flow of current, which is the basis for the operation of EIS (46).

Cebula et al. performed a different assay recently to identify *Pseudomonas syringae* pv. *lachrymans* on gold electrodes coated with antibodies using label-free electrochemical sensing (EIS). The group found Psl with a detection limit of 337 CFU/ml and a linear detection range of 10^3 -1.2 × 10^5 CFU/mL (R^2 = 0.992). The assay took 10 minutes to complete and it was 30 times more sensitive than the traditional LAMP approach (47).

Piezoelectric biosensors work on the basis of the ability to measure mass changes brought about by the biomolecular interactions between two substances, such as an antigen and its corresponding antibody (48). This type of biosensor uses crystals, such as quartz, that vibrate in reaction to an electric field. Additionally, a few utilize gold (Au) to gauge the proper angle for which objects exposed to laser light emit electron waves. This is predicated on the notion that the amount the laser's frequency changes will depend on the mass of the material absorbed.

The available non-electrochemical transducers include cantilever-based sensors, quartz crystal microbalance (QCM) and surface plasmon resonance (SPR), which are used to determine biosensing affinity. These methods detect changes in the refractive index that occur when an analyte binds to a metal surface (such as gold), which is often modified with a conjugated ligand's recognition element that can be measured using SPR-based sensors (49). By monitoring a quartz crystal resonator's change in frequency, a QCM-based sensor determines the mass variation of the QCM crystal per unit area. Usually, a recognition element (such as antibodies) is added to the QCM crystal (50). Cantilever-based sensors can detect changes in resonance frequency when the analytes and sensor surface are combined, similar to QCM-based sensors (51). Cantilever-based sensors have been utilized to identify pathogenic organisms because of their capacity to detect small analytes, including proteins and nucleic acids (52).

A surface plasmon resonance (SPR)-based biosensor was created specifically for the purpose of detecting the maize chlorotic mottle virus (MCMV) (49). In their studies, an anti-MCMV layer's antibody had cross-linked on the surface for the specific recognition of MCMV after 11-Mercaptoundecanoic acid was applied to a gold surface to create a self-assembling monolayer. Research examined how detection sensitivity was affected by coupling reaction time and antibody concentration. Nearly two orders of magnitude greater than the current enzyme-linked immune sorbent test (ELISA) approach, the detection limit under this method is 1 ppb. With a dynamic range of 1 to 1000 ppb, the change in the coverage mass is a subject of the MCMV concentration. The developed SPR sensor showed very specific detection for both pure MCMV and crude extracts from real-world materials.

1. *Antibody-based biosensor*

Various immunosensors utilizing antibodies have been created, relying on the interaction between the analyte and antibody. Notably, host plant antibodies and DNA offer distinct advantages and are employed in point-of-care assessments for plant pathogen detection. These antibodies exhibit the capability to specifically recognize target antigens even at minimal concentrations, without producing signals for non-relevant antigens. The crucial attribute of these antibodies lies in their high affinity and minimal interaction with other reagents during the detection process, emphasizing their significance in ensuring the efficient functionality of a biosensor (53).

Over the last ten years, a great deal of research has been done to describe the potential of antibody-based biosensors for the detection of plant pathogens, including *Aspergillus niger*, *Fusarium culmorum, Puccinia striiformis, Cowpea mosaic virus*, *Tobacco mosaic virus, Lettuce mosaic virus*, *Phytophthora infestans*, orchid viruses (50, 54-57). Since the application of nanotechnology-based methods for the manufacture of sensors, antibody-based biosensor technology has advanced significantly in recent years. To quickly diagnose viral infections, gold nanorods (AuNRs) performed by antibodies have been utilized to detect *Odontoglossum ringspot virus* (ORSV) and *Cymbidium mosaic virus* (CymMV). The limits of detection (LODs) for CymMV and ORSV in leaf sap were 48 and 42 pg/mL, respectively (58). QCM technique is also used for the detection of CymMV and ORSV along with the SPR technique. According to

researchers (50), the QCM method could identify each orchid virus at a concentration of just 1 ng. Using a lithographically patterned nanowire electrode position (LPNE) approach, other nano-based materials composed of polymers, such as polypyrrole (PPy) nanoribbon-modified chemiresistive sensors, were created. When the manufactured biosensor was tested for Cucumber mosaic virus (CMV) detection, it showed outstanding sensitivity with a 10 ng/mL detection limit (59). The detection limits of the existing antibody-based biosensors are around two orders of magnitude greater than those of traditional ELISA techniques (60). Biosensors based on living cells have several advantages over standard abiotic materials used in antibody-based biosensor manufacture, such as low detection limit, high specificity, and quick reaction time. It has been demonstrated that the detection limits of the current antibody-based biosensors are roughly two orders of magnitude higher than those of traditional ELISA techniques. Biosensors based on living cells have several advantages over standard abiotic materials used in antibody-based biosensor manufacture, such as low detection limit, high specificity, and quick reaction time. By immobilizing the Vero cells with viral-specific antibodies on their membranes, a novel portable cell biosensor system for the detection of Potato virus Y (PVY), Cucumber mosaic virus (CMV), and Tobacco rattle virus (TRV) was created. There has been a significant advancement in the creation of a transportable plant virus detection system appropriate for in-field use (61).

Due to tireless research, several improvements have taken place in the field of electrochemical biosensors. Electrochemical voltammetric approaches, Electrochemical impedance spectroscopy (EIS) and quartz crystal microbalance (QCM)-based have been utilized worldwide. The basis of the electrochemical impedance spectroscopy operation is the measurement of the resistance to current flow generated by interactions on top of the electrode by redox reactions and molecular interactions upon the application of voltage to the cell membrane (46). This type of impedimetric biosensor uses gold nanoparticles coated on the working carbon electrode to detect the citrus tristeza virus's nucleic acid. Faradaic impedance measurements were employed to assess both the thiolated single-stranded DNA layer and its hybridization with the target single-stranded DNA (45) which has great potential in detecting plant pathogens (47), developed antibody-modified gold electrodes and label-free EIS to detect *Pseudomonas syringae pv. Lachrymans*, which is 30 times more sensitive than conventional LAMP method. Thus, DNA based biosensors offered better reactions than the antibody-based ones. However, some issues in EIS still need clarification regarding whether selected antigens can react with pathogen-specific antibodies. Several researchers questioned the purity of antibodies used in the sensors, sometimes they may react with other substances instead of with the target compounds. This may give false conclusion. Meanwhile, the researcher also pointed out the efficiency of biosensor depends on concentration of ions, temperature, length of immobilization probe and pH (62).

The quartz crystal microbalance-based (QCM) technique is a piezoelectric biosensor with an extremely sensitive micro-mass detection tool. The novel QCM technique represents a label-free and noise sensing structure, exhibiting significant prowess in continuously monitoring the recognition component of a biosensor in real-time (63). QCM sensors include a quartz layer positioned in between operational electrodes, oscillating at a resonance due to variations in mass caused by the target pathogen and biorecognition molecules (such as antibodies or nucleic acids) binding on the surface (64). A significant benefit of the QCM biosensor lies in its exceptional sensitivity, as it can detect minute mass changes in real time (65). By adhering to the antigen-binding principle, immunosensors have been created by immobilizing antibodies on the QCM surface. This can be challenging due to the delicate nature of antibodies, complicating the immobilization process when used as biorecognition materials. The sensitivity of antibodies to certain physical and chemical events, such as ionic strength, temperature and pH can affect their bioactivity and hinder the sensor's performance. To overcome this limitation, various antibody immobilization techniques have been developed, including the use of self-assembled monolayers and Protein A linkers. These techniques create a specialized layer that maintains the bioactivity of antibodies, enabling the effective operation of QCM-based immunosensors (66). Despite these enhancements, the primary concern regarding the finite life span of antibodies requires additional research, Since QCM-based immunosensors may not always be functional, especially in certain disease diagnostics, DNA probes are often used as an alternative bioreceptor in QCM-based biosensors. In this case, the principle involves the immobilization of single-stranded DNA probes on the crystal surface, sequencing with the analyte gene of the pathogen and subsequently obtaining a frequency response.

2. *DNA-based biosensor*

DNA-based biosensors function by utilizing bonding (hydrogen or hybridization bond) between the target DNA sequence and a complementary DNA probe sequence. A DNA fragment known as a "DNA probe" has a nucleotide sequence unique to the target chromosomal region. In this DNA-based biosensor, sensitivity is affected by the rapid degradation of DNA in the environment during quantification of various pathogens (67).

Therefore, there is a need to increase the efficiency of DNA-based sensor for which nano-structured materials such as, refined gold, cadmium sulfide or silver nanoparticles with excellent chemical or electronic characters to enhance the target sequence and amplify the detected signal was developed. The nano structured material is used as a substrate for the adherence of DNA to the sensing surface, thereby enhancing the amount of immobilized DNA, serving a dual purpose by acting as both signal amplifiers and ultimately enhancing the accuracy, sensitivity and speed of diagnostic processes. DNA-based electrochemical sensors provide wide opportunity for on-site detection of plant pathogen in open environment.

The majority of DNA-based electrochemical biosensors for detection of plant pathogens rely on label-free or label-dependent voltametric detection by DNA hybridization (68). The creation of diagnostic tools for fungal plant pathogens has combined DNA-based analyte capture systems with electrochemical methods to quantify the amount of captured DNA (69, 70). The researchers developed a method called microfluidic microarray assembly to identify three fungal plant

pathogens, namely *Botrytis cinerea*, *Didymella bryoniae* and *Botrytis squamosa* simultaneously (71). DNA-based biosensors offer significant advantages, including enhanced sensitivity when combined with nucleic acid amplification techniques, allowing for the detection of plant pathogens before symptoms appear in the host. Some limitations are noticed when DNA biosensors are used like selection and synthesis of specific DNA probes for diagnosing the small DNA sequence of long double stranded DNA (30, 48). An electrochemical DNA biosensor used a recently produced ruthenium [Ru(phen)2(qtpy)]2+ complex as an indicator of hybridization for identification of *Ganoderma boninense,* an oil palm pathogen (72). A conducting nanocomposite of poly (3, 4-ethylene-dioxythiophen) - poly (styrene sulfonate) (PEDOT-PSS) and silver nanoparticles (AgNPs) was used in the sensor in place of a gold electrode (AuE). The modified electrode was used to immobilize a particular sequence of a *Ganoderma boninense* DNA probe, and the hybridization event was seen by measuring the amount of ruthenium complex that intercalated into the hybridized DNA. The recently created ruthenium complex can be applied for routine DNA detection and is a novel redox marker (73). Eventually, nano based electrochemical biosensors were developed for the faster and more appropriate detection of *P. syringae* DNA in plant samples using disposable carbon electrodes printed with a screen (21).

Now, for detecting the bacterial plant pathogens phage based DNA has been developed (33). The primary advantage of this technology is its ability to identify the nucleic acids of only living bacterial cells, thereby reducing the likelihood of false positive results. The biosensor utilizes probe DNA as a biorecognition component on the surface of a paper electrode, combined with GO (oxidized graphene) to improve detection circumstances and sensitivity for identifying false smut of rice (74). In the biosensor, the quantitative measure of hybridization between probe single stranded DNA (ssDNA) and target single stranded DNA is analysed by the electrochemical techniques namely, cyclic voltammetry and linear sweep voltammetry. Recent advances in DNA-based biosensors for enhancement of parallel microarrays and high-capacity outlines connected to innovations in DNA sequencing. Even though much research is done in other areas, such as food quality in electrochemical biosensors its practical application for plant disease identification is in pipeline for detailed investigation. Different types of DNA biosensors are available viz., optical DNA biosensors, piezoelectric DNA Biosensor, strip type DNA sensors and electrochemical DNA biosensors.

a. *Optical DNA biosensors*: An optical DNA biosensor is a compact analytical device that uses light to detect the interaction between a biological material and a substance or analyte. They send the emission signals of the fluorescent labels by using the fiber optics. The output signal is proportional to the concentration of the analyte. Fiber optics are gadgets that carry light from one place to another by a pattern of internal reflections (75). An ssDNA probe is inserted at the end of the fiber to operate fiber-optic DNA biosensors, which then track the fluorescence changes brought on by the double-stranded (ds) DNA hybrid's connection with a fluorescent indicator. The hybridization of fluorescently labelled complementary oligonucleotides was observed by measuring the enhancement in fluorescence. The different types of optical biosensors are molecular beacons, surface plasma resonance, quantum-dot.

b. Molecular beacons : Molecular beacons are oligonucleotides with a stem-and-loop structure that, upon hybridisation, become radiant when labelled with a fluorophore and a quencher. High sensitivity and specificity are features that MB probes offer in addition to their direct monitoring capacity.

c. Surface Plasmon Resonance (SPR) : These biosensors track variations in the surface optical characteristics brought about by the surface binding process, such as changes in resonance angle arising from variations in the interfacial refractive index. SPR is used to immobilise a thiol-modified oligonucleotide onto a gold surface to detect DNA hybridisation (76, 77).

d. Quantum-Dot : The separation of unhybridized DNA is not necessary when using an ultrasensitive nanosensor based on fluorescence resonance energy transfer (FRET) to identify very low concentrations of DNA. This kind of technology uses quantum dots (QDs), which are connected to particular DNA probes to collect target DNA. The FRET donor-acceptor assembly is formed when the target DNA strand attaches to a reporter strand that has been fluorescently dyed (fluorophore-labelled). No fluorescence is produced by unbound DNA strands, but a powerful FRET signal can be produced when even a modest amount of target DNA (50 copies) is bound (78).

e. Piezoelectric DNA Biosensor : The quartz crystal that powers the piezoelectric DNA biosensor oscillates at a certain frequency when an oscillating voltage is supplied. Recently, the piezoelectric approach has become the most attractive because of its affordability, sensitivity and quick and real-time label-free detection (79). The quartz crystal microbalance (QCM) is an extremely sensitive mass-measuring device that allows dynamic monitoring of hybridization events.

f. Strip Type DNA Sensor : Direct detection of DNA hybridization has significant potential impacts on a unique colorimetric detection method based on nanoparticles. In this instance, hybridization results in modifications to the combined functional gold nanoparticles' optical characteristics. For the visual detection of DNA, the dry-reagent strip type biosensor was developed (80, 81).

g. Electrochemical DNA Biosensors : Electrochemical devices play a vital role in DNA biosensing based on predetermined sequences. DNA diagnostics benefit greatly from the devices' enhanced technology and downsizing. DNA hybridization is often detected electrochemically by tracking a current at a given voltage. It was possible to recognize labelled and label-free items using electrical modes. The functionality of DNA biosensors and gene chips is largely dependent on the nucleic acid, and becomes immobile on the transducer surface.

ii. Optical biosensors

Optical biosensors are devices that can measure and identify changes in a material's optical characteristics and convert those changes into an electrical signal which is then measured by the device. Optical biosensors evaluate the interaction between a target analyte and ligand by use of an immobilised biorecognition element, an optical transmission medium, a light source and a signal detecting device. Finally, the amplitude, frequency and phase of the light's reaction to the physicochemical conversion produced by the bio recognition action is measured (82). The techniques like colorimetry,

fluorescence, surface plasmon resonance (SPR), flow cytometry, lateral flow assay (LFA), chemiluminescence and bioluminescence are used in optical sensors (83).

Some of the advantages of optical nanobiosensors are that they are highly sensitive with an extremely low limit of detection (LOD), allowing for precise measurements. Additionally, they can be designed for naked eye readout assays, facilitating simple and rapid diagnostics. Recent advancements in optical technology have reduced the cost of portable lasers, making the testing instruments more affordable (84). Colorimetric, fluorescence-based and surface plasmon resonance-based optical biosensors are commonly used to detect plant pathogens.

The most popular instruments for quickly identifying pathogenic microogranisms in a limited number of samples within 10 to 15 minutes are colorimetric biosensors. They work by changing color. The market is flooded with this kind of sensor. This assay comes in two varieties: solution-based and flat-based. A lateral flow assay is a paper-based sensor with a flat format for colorimetric instruments that is used extensively in laboratories for quick diagnosis. It is also very inexpensive and simple to use.

The sample containing the analyte is put onto the first pad, which is composed of cellulose, the second is made of glass fibre soaked in a bioconjugate solution and the third is the identification and absorption pad, printed with a test line and a control line (68). Due to its vivid hue and lack of need for additional viewing techniques, colloidal gold is currently the most frequently utilized product in commercial lateral flow immunoassays (85). Like the lateral flow test, the solution-based colorimetric sensor works by reacting to the target pathogens through a receptor attached to colloidal gold nanoparticles. When the nanoparticles aggregate, the color changes from red to purple (86).

For a number of plant pathogens, such as Potato Virus X in potatoes (87), *Fusarium* species in maize (88) and *Pantoea stewartii* subsp. *stewartii* (Pss) bacteria in maize (89, 90), Colloidal gold nanoparticle-based lateral flow immunoassays have been produced. To identify the pathogens which cause the late blight of potatoes and tomatoes, a lateral flow biosensor-based gold particle was developed (91). After direct DNA extraction from late blight-infected potato field samples, asymmetric PCR amplification and a biosensor assay were performed, yielding high specificity and a *Phytophthora infestans* genomic DNA with a low detection limit of 0.1 pg ml^{-1} in less than 1.5 hours. This method has the advantages of nanoparticles and hence, universal primer-mediated asymmetric PCR were developed. They are simple to assay, fast to yield results, require a small sample size and provide an instant "point-of-care" diagnostic. However, because of the variety of potential inorganic-biological problems that could result in non-specific adsorption and target analyte annihilation, the accuracy of lateral flow immunoassays is lower than that of other nanotechnology-based methods (92). Lack of high sensitivity is a significant disadvantage shared by all lateral flow assay-based biosensors (93). For this limitation, several authors feel that using magnetic beads as a signal amplification approach is helpful. Target cells can be separated from complex samples and concentrated by resuspending them in any intended assay volume thanks to the original magnetic bead feature. Additionally, chemiluminescent substrates, multiwell plates and quantum dots can be used in place of colloidal gold to address the low sensitivity of lateral flow test strips (86).

The basic idea behind fluorescence-based immunoassays is that target antibodies and molecules labelled with fluorophores or fluorochrome molecules create light as part of their biological recognition process. Reportedly, a unique multiplex identification technique based on a microsphere immunoassay may concurrently identify four major plant diseases: the watermelon silver mottle virus (tospovirus serogroup IV), the Melon Yellow Spot virus (tospovirus), the chili vein-banding mottle virus (potyvirus) and the fruit blotch bacterium *Acidovorax avenae* subsp. *citrulli* (94, 95). This technique's basic idea was to use magnetic microspheres with fluorescence-coded coatings in conjunction with antibodies to capture the target pathogen. The presence of the pathogen was then assessed using R-phycoerythrin-labelled antibodies, which required only one hour of test time. Although highly sensitive detection may be achieved, all immunoassay-based techniques have drawbacks. This involves the preparation of monoclonal antibodies, as previously described for traditional immunoassays (51) and the assay's reliance on the sample and/or environment due to the possibility of antibody cross-reactivity with endogenous and exogenous substances, which could result in false negative results or decreased sensitivity (96).

On the other hand, optical biosensing methods primarily employ surface plasmon resonance-based biosensors, which offer the benefits of label-free, real-time and extremely accurate detection (97, 98, 99). One of the devices' components is a sensor chip, which consists of two glass and liquid layers with a metal surface (like gold). Pathogenic substance flows across the top surface of the chip, going through the bottom or liquid layer and connects to an immobilised ligand to provide the visible light signal at a certain angle. A surface plasmon resonance sensogram is then used to monitor the resulting signal (100). An Odontoglossum Ringspot Virus and the Cymbidium Mosaic Virus are two prevalent and major orchid viruses that may be detected using surface plasmon resonance biosensor-based gold nanorods with no labels (58).

Numerous possible nanomaterials are available with recent advancements in nanotechnology. The ideal surface-to-volume ratios and energies for supporting the immobilization stability of a wide variety and number of biomolecules without affecting their bioactivity are present in gold nanoparticles having 1-100 nm diameters. Furthermore, gold nanoparticles have strong electron conductivity. As such, the dependability, sensitivity and speed of optical and electrical biosensors have been significantly altered by their application (101). Several lateral flow tests, such as *Acidovorax avenae subsp. citrulli* of watermelon, have been developed for the detection of plant diseases. These assays are based on DNA hybridisation to gold nanoparticles (95) and many others. There are several limits and considerations with this rapidly developing method, which are listed below. Pre-emptive diagnostic-guided action in informed diseases management is one of its many possible applications. Colour changes in analytes can be used to detect them both qualitatively and quantitatively using colorimetric sensors. The

resulting optical signal can be detected using a photodetector or seen with unaided eye (102). Later, for the colorimetric detection of *Phytophthora infestans* in tomatoes, a smartphone-based VOC sensing platform was developed (103).

The most popular instruments for quickly identifying harmful microbes in a limited number of samples within 10 to 15 minutes are colorimetric biosensors. They work by changing color. The market is flooded with this kind of sensor. This assay comes in two varieties of lateral flow assays viz., Nucleic acid lateral flow (NALF) and Nucleic Acid Lateral Flow Immuno Assay (NALFIA) and extensively employed in laboratories for rapid diagnostics, utilize a flat format and are available in both solution -based and paper-based versions. The lateral flow assay (LFA) is a paper-based platform that uses test equipment to detect and quantify analytes in complex mixtures. The findings are displayed in 5 to 30 minutes. Because LFAs are simple to produce and have low development costs, they are increasingly being used in a variety of fields where quick tests are necessary. The paper-based sensors are designed for colorimetric instruments, facilitating quick and efficient diagnosis. It is also very inexpensive and simple to use. The sample with analyte is dropped into the first pad, which is composed of cellulose whereas, the second pad is made up of glass fibre soaked in the solution of bio conjugate and the third pad is also known as detection or absorption pad, which is printed with a test and control line (42). A lateral flow biosensor based on gold nanoparticles can identify the pathogen that causes late blight in tomatoes and potatoes, *Phytophthora infestans* (91). This method combines a gold nanoparticle-based lateral flow biosensor with primer-mediated asymmetric PCR, colloidal gold nanoparticle-based lateral flow immunoassays developed for detecting various plant pathogens, including Potato Virus X in potatoes (87). It is very difficult to use the sensors by uneducated farmers at field level and therefore, despite various advantages over conventional techniques, there is need for further research on handling and performance of the biosensors at field conditions.

Conclusion

Nanotechnology plays a crucial role in early detecting plant pathogens, offering a solution to disease management. Nano sensors and devices are used for detecting pathogens before and after infection under both lab and field conditions. In contrast to conventional methods, significant advancements have been made in nanodiagnostic tools, which can be utilized as a quick diagnostic method for the early identification of different plant infections using quick and highly sensitive pathogen probes. Despite the undeniable importance of plant biosensors-based research, the improvement of the research in this area is not elaborate. The early prediction of pathogen needs sensitive nano -based technologies. Nano-based technologies require sensors that can tolerate a wide variety of environmental variations. This requires more investigation into the components of the unique sensor, including its nanoparticles and environmentally resistant materials.

All these are considered as low-cost, highly sensitive, fast and specialised nanotechnologies for pathogen identification under field conditions with a variety of environmental circumstances will be widely used with additional modifications. In future, nanotechnology will play an extensive role in smart agricultural systems. The nano-devices could be used to detect pathogens before symptoms expression and the farmers can take suitable disease management strategies well in advance. Portable nanodevices can detect protein concentrations as low as a few nanograms per milliliter and RNA may be used as a multimodal detection tool to find complicated problems with post-harvest loss. Its application in agriculture could greatly enhance plant health and fight plant diseases, thereby increasing the production of healthy food and meeting demands in an efficient and economical manner. Nanobiosensor based methods can be used to better understand plant-pathogen interactions, which can result in the creation of innovative crop protection techniques. Specific nanodevices and DNA nanodevices can enable accurate plant pathogen monitoring, identification and diagnosis in the early stages of plant disease. In future, the devices will occupy an inevitable role in detection of plant pathogens in the world.

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Authors' contributions

The authors VJN, PTS and IJ prepared and written the manuscript, the authors RA, KR, GK, KAand MV read and corrected the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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