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REVIEW ARTICLE

Research advances and applications of biosensing technology for the diagnosis of pathogens in sustainable agriculture

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Abstract

Plant diseases significantly impact the global economy, and plant pathogenic microorganisms such as nematodes, viruses, bacteria, fungi, and viroids may be the etiology for most infectious diseases. In agriculture, the development of disease-free plants is an important strategy for the determination of the survival and productivity of plants in the field. This article reviews biosensor methods of disease detection that have been used effectively in other fields, and these methods could possibly transform the production methods of the agricultural industry. The precise identification of plant pathogens assists in the assessment of effective management steps for minimization of production loss. The new plant pathogen detection methods include evaluation of signs of disease, detection of cultured organisms, or direct examination of contaminated tissues through molecular and serological techniques. Laboratory-based approaches are costly and time-consuming and require specialized skills. The conclusions of this review also indicate that there is an urgent need for the establishment of a reliable, fast, accurate, responsive, and cost-effective testing method for the detection of field plants at early stages of growth. We also summarized new emerging biosensor technologies, including isothermal amplification, detection of nanomaterials, paper-based techniques, robotics, and lab-on-a-chip analytical devices. However, these constitute novelty in the research and development of approaches for the early diagnosis of pathogens in sustainable agriculture.

Keywords Microorganisms · Detection · Sensitivity · Nanomaterials · Serological techniques

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Introduction

Phytopathogens are one of the most important causes of plant diseases and pose a threat to the success of the agriculture industry and the safety of agro-based products worldwide. The main causes are old and new emerging plant diseases, including those caused by fungi, bacteria, viruses, and other pathogens such as phytoplasma. Their widespread behavior, severity, and incidence present a vital risk to the sustainability of global food sources (Roberts et al. 2006; Savary et al. 2012; Priyanka et al. 2016). In recent years, global food security, determined by the balance of global food production and demand, has become a major international concern (Senauer and Sur 2001; Keinan and Clark 2012). An increase in food production prices caused a worldwide disaster that produced governmental and financial issues in several developing nations (Rosset 2008; Ali et al. 2020). It was projected that over a period of 40 years a 70% increase in food production will be required by 2050 due to the increase in the human population (Miles et al. 2017). Despite the lack of sufficient evidence for the financial damage, the total cost of damage caused by plant diseases associated with plant pathogens in Georgia, USA, was estimated and has been described and their management expenses reached 647.2 million dollars in 2006 and then continued to increase to 821.85 million dollars in 2013 (Martinez 2011). Crop production losses due to infections by plant pathogens range from 20 to 40% (Savary et al. 2012).

Plants show various symptoms on stems, leaves, and fruits due to infectious plant diseases (Al-Hiary et al. 2011). These signs and symptoms are significant for visual identification as a conventional initial stage for the diagnosis of infectious plant diseases. However, visual identification of such signs cannot be solely used to identify the existence of plant pathogens in the primary stage of infection when a crop disease does not lead to the manifestation of visible symptoms. Early diagnosis of phytopathogens plays a significant role in monitoring plant health. It permits the control of plant pathogens in the field throughout the growth phases of plant infection and the management of greenhouse systems. Additionally, it decreases the hazard of the transmission of phytopathogen-associated diseases and prevents the need for the establishment of measures for new pathogens, particularly quarantine laws at national borders (Vincelli and Tisserat 2008; Miller 2009). Several methods for the diagnosis of infectious diseases in plants have been commonly used as direct laboratory-based methods for further visual examination of plant signs and symptoms in field conditions. Such methods include deoxyribonucleic acid (DNA)–based techniques and immunoassays for the identification of pathogen-derived nucleic acids and proteins from diseased crop materials (López et al. 2009). Additionally, indirect methods have focused on examination of volatile organic compounds (VOCs) that enable plants to counter pathogens as a defensive mechanism (Scala et al. 2013; Gaggiotti et al. 2020). Many recent studies have published comprehensive VOC observation techniques for the identification of infectious diseases in plants (Fang and Ramasamy 2015; Martinelli et al. 2015).

Numerous previous reports on the molecular-based examination have highlighted the effectiveness of plant infection diagnosis and plant disease detection, including DNA-based techniques, mostly involving polymerase chain reaction (PCR); quantitative PCR (qPCR) is also known as real-time PCR, nested PCR, immunocapture PCR (IC-PCR), and reverse transcription PCR (RT-PCR), followed by DNA hybridization detection. However, all PCR methods have shown sensitivity and specificity in the regulation of the hereditary content of fungi, viruses, and bacteria (Gutiérrez-Aguirre et al. 2009; Li et al. 2009; Ruiz-Ruiz et al. 2009; Coy et al. 2014; Lin et al. 2018). Instead, serological assays, also known as immunoassays, including direct dot-blot immunoassays (DTBIA), lateral flow devices (LF), and enzyme-linked immunosorbent assays (ELISA), have been used to detect the infectious phytopathogenic antigen (Vidal et al. 2012a; Djelouah et al. 2014; Ding et al. 2016; Madufor et al. 2018). Serological assays using antibodies (monoclonal) show a high specificity for virus detection in plants as shown in Table 1, with a considerable number of plant samples tested and on-site phytopathogen detection coupled with tissue print ELISA and LF systems (Vidal et al. 2012b). In contrast, DNA-based detection techniques are more accurate, specific, and sufficient to detect one target pathogen and highly effective for the detection of multiple targets. The disadvantages of these molecular techniques have limitations in detecting plant pathogens, false-negative results, time-consuming efforts, requirement of technical personnel for handling, low titers in the early detection of pathogens, and the lack of potential application in on-site phytopathogen detection (Martinelli et al. 2015).

Emerging and innovative biosensor techniques are widely used as diagnostic approaches for agricultural (plants and nurseries), environmental, clinical, and foodborne pathogens (Khater et al. 2017; Rani et al. 2019). The biosensor system includes biological recognition via transducers and active receptors such as antibodies, enzymes, DNA probes, and phage-based biosensors (Fig. 1) that allow the identification of an analyte by particle interactions (Singh et al. 2013; Sadani et al. 2019). Biosensors represent the end products of a fast-growing field, engineering and integrated systems, and computer or digital science to meet the crucial demand for its application in several fields (Belkin 2003; Wilson et al. 2005; Vaseghi et al. 2017). This review summarizes the advanced biosensor tools for the diagnosis of pathogens in agriculture industries, such as calorimetric, electrochemical, piezoelectric, potentiometric, optoelectric, amperometric, immuno, and acoustic. Decreased disease-associated damage during growth periods, harvest and postharvest processes, high output and validation of agricultural production and

Table 1 Various biosensing techniques employed for pathogen detection in sustainable agricultural systems

Techniques	Target pathogen(s)	Detection limit	Reference
Paper-based immunosensors	*Phakopsora pachyrhizi*	2.2 ng mL^{-1}	(Miranda et al. 2013)
DNA optical fiber sensor	*Brettanomyces bruxellensis*	12.5 ng μL^{-1}	(Cecchini et al. 2012)
Surface plasmon resonance biosensor	*Phakopsora pachyrhizi*	800 ng mL^{-1}	(Chen et al. 2005)
Voltammetric enzyme-based biosensor	*Cucumber mosaic virus*	0.5 ng mL^{-1}	(Jiao et al. 2000)
Surface plasmon resonance biosensor	*Cymbidium mosaic virus*	48 pg mL^{-1}	(Lin et al. 2014a)
DNA hybridization voltammetric system	*Sugarcane white leaf disease*	4.7 ng μL^{-1}	(Wongkaew and Poosittisak 2014)
DNA hybridization voltammetric system	*Trichoderma harzianum*	10–19 mol L^{-1}	(Siddiquee et al. 2014)
Electrochemical based biosensor	*Banana streak virus*, *Banana bunchy top virus*	50 fM^{-1}	(Tang et al. 2007)
AuNPs aggregation-based biosensor	*Pseudomonas syringae*	0.5 ng μL^{-1}	(Fang and Ramasamy 2015)
Fluorescent based biosensor	*Pantoea stewartii* subsp. *Stewartii*	103 cfu mL^{-1}	(Zhao et al. 2014)
Quartz crystal microbalance biosensor	*Saccharomyces cerevisiae*	104 cells mL^{-1}	(Dickert and Hayden 2002)
Quartz crystal microbalance biosensor	*Potato virus x*	2 ng mL^{-1}	(Drygin et al. 2012)
Fluorescent approach	*Botrytis cinerea*	1 fM^{-1}	(Boonham et al. 2007)
Fluorescence-based bacteriophage assay	*Escherichia coli*	10–100 ng mL^{-1}	(Goodridge et al. 1999)
FRET-based quantum dot immunoassay	*Phytophthora* species	103 spores mL^{-1}	(Kattke et al. 2011)
ELISA-based PCR	*Escherichia coli*	100.00 ng mL^{-1}	(Daly et al. 2002)
Electrochemical impedance spectroscopy	*Phakopsora pachyrhizi*	385 ng mL^{-1}	(Mendes et al. 2009)
Microfluidic microarray device	*Botrytis* species	0.2 ng of DNA	(Wang and Li 2010)
Surface plasmon resonance biosensor	*Salmonella typhimurium*	4 log CFU mL^{-1}	(Oh et al. 2017)
Surface plasmon resonance biosensor	*Pseudomonas*	7.09 log CFU mL^{-1}	(Mudgal et al. 2020)
Quartz crystal microbalance biosensor	*Campylobacter jejuni*	1.30 log CFU mL^{-1}	(Masdor et al. 2019)
Quartz crystal microbalance biosensor	*Staphylococcus aureus*	7.41 log CFU mL^{-1}	(Noi et al. 2019)
Amperometric biosensor	*Streptococcus agalactiae*	1–7 log CFU mL^{-1}	(Arachchillaya 2018)
Lateral Flow immunoassays	*Acidovorax avenae* subsp. *citrulli*	48 pg mL^{-1}	(Zhao et al. 2011)

sustainability, and advancement in the level of detection and prevention of infectious diseases in plants are important. This review also discusses advances and limitations of biosensor techniques, which will assist scientists and researchers in the progress of fast, accurate, and inexpensive plant pathogen detection methods with high specificity and sensitivity.

DNA-based biosensor system for crop pathogen detection

DNA-based biosensors involve complementary sequence hybridization among the target DNA sequence and the immobilized probe. The probe is a sequence of DNA that is generated to obtain the objective sequence. Depending on the redox response, the electrochemical biosensors are based on the movement of DNA towards electrons among electrodes after hybridizing the target to an immobilized capture probe. Label-free electrochemical biosensors are based on impedance, conductivity, and current changes (Batchelor-McAuley et al. 2009; Cui et al. 2018a). The DNA biosensor is over 10,000 times more sensitive than conventional PCR, which is capable of sensing 13 pg of *Pseudomonas syringae* in plant tissues (Lau and Botella 2017). Recombinase polymerase amplification (RPA) is used with gold nanoparticles to increase the objective DNA detection probe for signal improvement (Zou et al. 2017). However, these biosensors that are needed for nucleic acid detection also have limitations, and it is challenging to extract nucleic acids, which are at risk of degradation and contamination and are also difficult to amplify. This technique presents risks with negative or false-positive results and is incapable of separating dead and live pathogens or toxins. Moreover, biosensors based on enzymatic and piezoelectric DNA are highly sensitive and are easily affected owing to changes in the environmental pH (Batchelor-McAuley et al. 2009). However, the coupling of biosensors with a DNA extraction dipstick is possible (Zou et al. 2017).

Voltammetric DNA hybridization detection

Based on voltammetric, amperometric, and impedimetric detection of cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square-wave voltammetry (SWV), a label-free DNA hybridization system may be observed

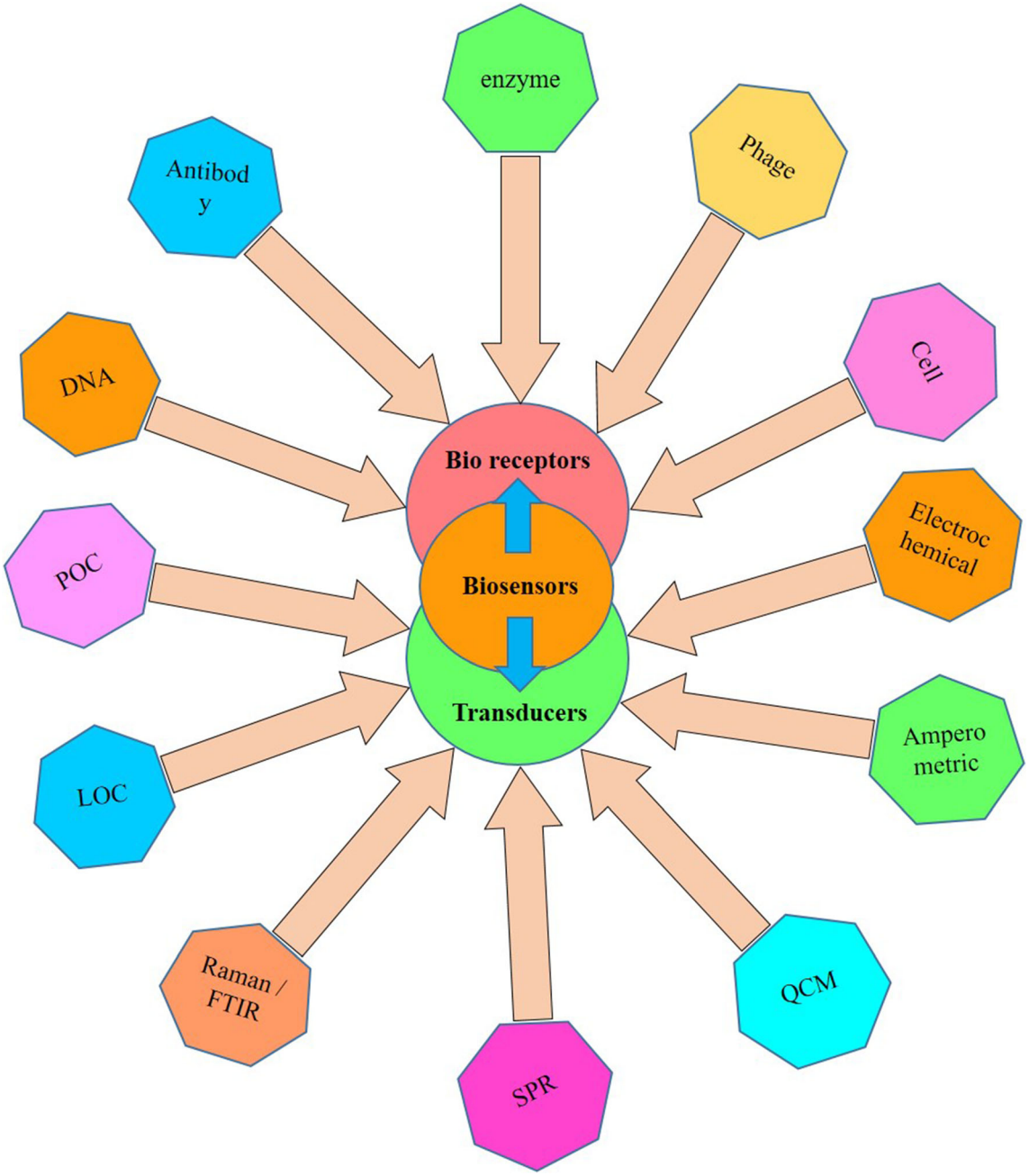

Fig. 1 Illustration of current and potential prospective detection techniques for sustainable agriculture

(Lillis et al. 2006; Gao et al. 2007; Lin et al. 2018). Label-free detection methods are used for revealing the picomolar concentration of DNA from the plum pox virus (PPV) using glassy carbon electrodes (Grabowska et al. 2014). Osteryoung square wave voltammetry (OSWV) electrodes were used to detect target-matched immobilized and monitored DNA-based pathogens (Grabowska et al. 2014) (Table 1). Voltammetric methods aid in the analysis of the number of electrons which move to change the hybridization response and in the detection of the 22-mer to 42-mer matching objective chromosome sequence of PPV using an array with a concentration of 10–50 pg/mL. PPV was detected according to a recent report on healthy leaf and infected foliage samples, but this method was not described for other plant pathogens (Wongkaew and Poosittisak 2014).

Nanocomposite membranes enhanced with gold electrodes made of zinc oxide nanoparticles (ZnO NPs) and chitosan (CHIT) were also used to develop biosensor systems based on the MB redox indicator for identification of nucleic acids using voltammetric methods. Techniques using such approaches and compounds increase the efficiency, biocompatibility, and electrochemical conductivity of the control samples of the tested DNA. Most recently, the function of soil-borne fungi (*Trichoderma harzianum*) was reported

(Siddiquee et al. 2014). Studies were conducted through DPV, the method of hybridization between *Trichoderma* DNA and its paired probe control on the nanocomposite-developed electrodes. The manufactured DNA biosensor detects crude DNA from control samples of fungal mycelia with high replicate ability, with a maximum detected concentration of 10^{-1} mol/L (Siddiquee et al. 2014). Label-free DNA-based hybridization using hybridization indicators and voltammetric devices is highly beneficial and inexpensive, and no labeling process is needed, thereby providing an opportunity of analyzing fewer sample volumes. Isothermal amplification systems and nanoparticles have the potential to be valuable methods for the diagnosis of plant pathogens.

Enzyme-based biosensors for plant pathogen detection

In previous years, serological methods such as ELISA were the most popular and widely used for the detection of plant and clinical pathogens (Rossier and Girault 2001). Enzyme immunoassays have been paired with electrochemical detection techniques to diagnose both clinical and plant infectious diseases with higher sensitivity and selectivity (Paternolli et al. 2004). The electrochemical enzyme-linked immunoassay (ELISA) combines enzyme catalysis with elucidation of enzyme-substrate reactions with gradual H_2O_2 decrease, followed by electrochemical techniques that reduce response through voltammetric and amperometric approaches (Lee et al. 2005; Noori et al. 2020). The techniques utilize amperometric, conductometric, potentiometric, and impedimetric electrochemical biosensors based on electrical factors such as current high potential, conductivity, and impedance (Fig. 2). Another key characteristic is their low cost and ease of application in the detection of plant pathogens (Ray et al. 2017; Adachi et al. 2020). Stable voltammetric points are achieved by changing the pH of both electrolyte solutions and enzymatic reactions. Subsequently, alkaline phosphatase (AP) and horseradish peroxidase (HRP) as enzymatic labels have different varieties of proper substrate molecules to attain the obligatory sensitivity (Thompson et al. 1991; Jiang et al. 1995). Although biosensing devices are highly sensitive, limited enzyme accessibility combined with antibodies presents a major drawback. Furthermore, enzymes and their products are considerably influenced by the pH of the electrolyte results, thus presenting a further disadvantage in the circumstance of enzymatic responses occurring in a similar medium as the last electrochemical dimension (Khater et al. 2017). A recent clear competitor to conventional cumbersome technologies, such as the dichromate colorimetric method, is the bioelectrochemical system (BES), including microbial electrolysis cells (MEC) and microbial fuel cells (MFC) for biological detecting (Xu et al. 2021).

The ELISA system based on HRP uses two separate substrates of HRP, namely o-phenylenediamine (OPD) and o-aminophenol (OAP) to detect plant viruses such as the cucumber mosaic virus (CMV) (Jiao et al. 2000). This indirect approach involves the following three main steps: (i) use of immunoreactive secondary antibodies labeled with HRP, (ii) use of viral antigens immobilized to purified CMV, and (iii) incubation with appropriate antibodies to detect CMS. Recent enzymatic products were tested with a hanging mercury electrode using linear voltammetry scanning. ECEIA technology uses CMV and detection limits have been reported to be four to ten times higher than those observed with ordinary spectrophotometric ELISA, with detection limits as low as 0.5 ng/mL using OAD as a substrate with the following four different plant-pathogen viruses: potato virus Y (PVY), turnip mosaic virus (TuMV), tobacco mosaic virus (TMV), southern bean mosaic virus (SBMV), and tomato aspermy virus (ToAV) (Khater et al. 2017). Gold nanoparticles have recently been used as tags to increase analytical signals and to improve immunoassay sensitivity. For example, ECEIA first uses gold nanoparticle tags loaded with HRP-labeled antibodies to diagnose infections with the plant bacterial pathogen *Pantoea stewartii* sub spp. Stewartii (PSS) (Zhao et al. 2014) (Table 1). Apart from this technique, PSS antigens can also be sensitively and precisely identified against other plant pathogens, such as *Cercospora* leaf spot on rice, *Fusarium* wilt, dark spots, and *Cruciferae* leaf streaks (Khater et al. 2017).

Surface plasmon resonance techniques

The method of surface plasmon resonance (SPR) is based on observing the shift in the refractive index of a biosensor surface as the ligand biomolecules interact. Biosensors based on SPR have previously been used to detect pathogenic plant and food microorganisms that cause food spoilage, plant infections, and loss of yield (Bergwerff and Van Knapen 2006; Dudak and Boyacı 2009). The major advantage of this approach depends on the ability of the biosensors and their ability to effectively track and measure the response to biological affinity as illustrated in Fig. 3. The tobacco mosaic virus (TMV) was identified using the first SPR-based biosensor (Altschuh et al. 1992) and was tested using the SPR method (Skottrup et al. 2008). For the diagnosis of phytopathogens, such as plant viruses, a marker-free biosensor was developed based on SPR for *Puccinia striiiformis*, *Fusarium culmorum*, *Phytophthora infestans*, and *Phakopsora pachyrhizi* (Boltovets et al. 2002; Skottrup et al. 2007, 2008; Mendes et al. 2009).

Several SPR-based biosensors can be used and developed for tracking phytopathogens using DNA probes, aptamers, and antibodies (Wang et al. 2006; Candresse et al. 2007; Lautner et al. 2010). In current years,

Fig. 2 Illustration of different types of electrochemical biosensor systems

unlabeled SPR immunosensors were developed using gold nanorods (AuNRs) for the detection of two viruses in the orchard (cymbidium mosaic virus (CymMV) and odontoglossum ringspot virus (ORSV)). Orchard virus-specific antibodies have been modified with AuNRs as the detection layer, which shows a wider spectral range and helps eliminate color interference problems caused by sample matrices (Lin et al. 2014b). This methodology has shown high limits of detection of 48 and 42 pg/mL for ORSV and CymMV, respectively (Lin et al. 2014b). The use of SPR-based biosensors also has limitations, as nonspecific adsorption of surface detection must be carefully controlled (Khater et al. 2017).

Quartz crystal microbalance–based biosensors

The quartz crystal microbalance (QCM)–based immunosensors have high sensitivity and can be used to perform label-free detection. They are considered piezoelectric biosensors. Several applications for the detection of foodborne

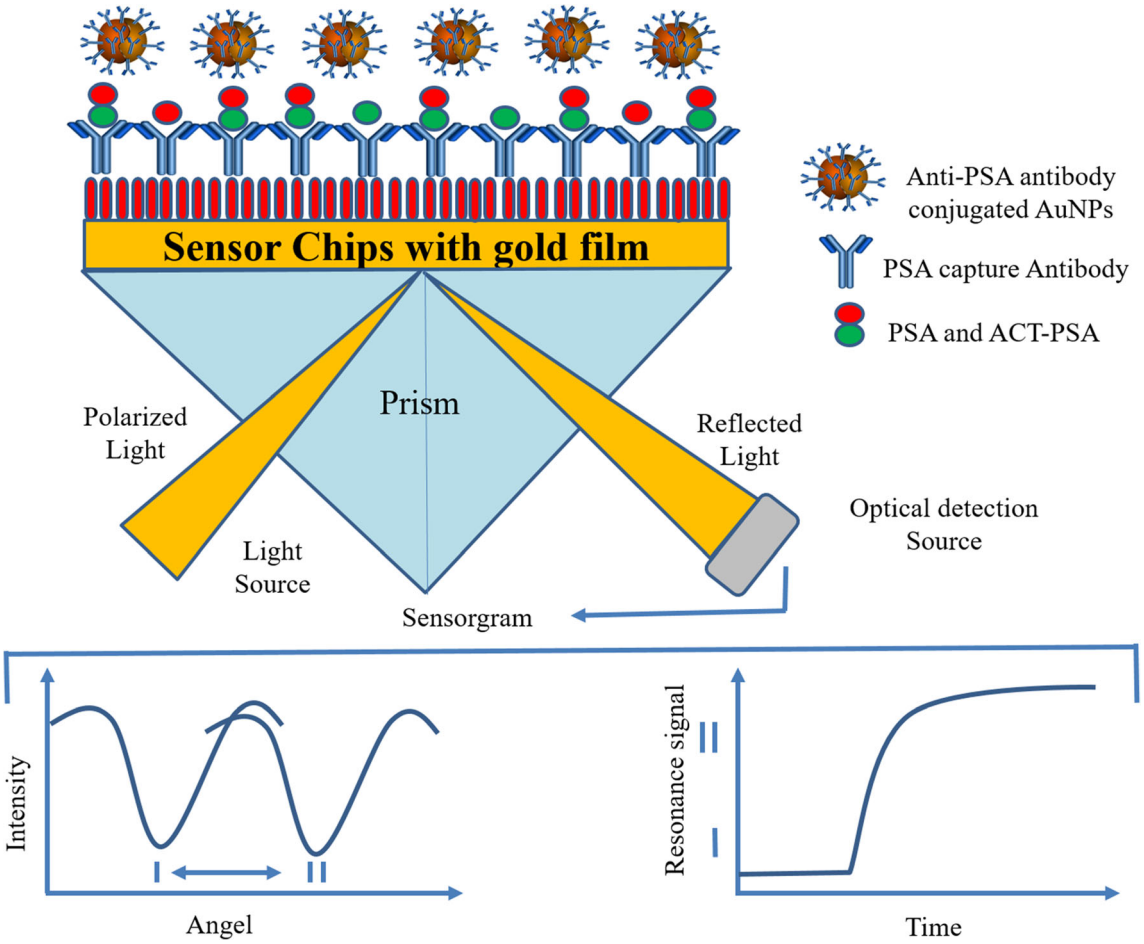

Fig. 3 The schematic representation of a surface plasmon resonance biosensor

and phytopathogens as well as clinical and environmental analyses have been described (Pohanka et al. 2007; Bragazzi et al. 2015). QCM-based biosensor techniques have considerably improved and have several applications in microbiological detection. Similar to the SPR-based biosensor, the QCM-based biosensor is a label-free methodology that has the advantages of automatic detection and simple pretreatment, which are beneficial for comparison with some labeling techniques (Fig. 4). Early detection of *Escherichia coli* O157:H7 using QCM techniques established a suitable selectivity (Chen et al. 2018b; Poitras and Tufenkji 2009). QCM-based biosensors have been used for the first time in botanical garden virus detection (Eun et al. 2002), and several piezoelectric immunosensors based on QCM have been applied for the phytopathogen detection (Skottrup et al. 2008; Khater et al. 2017). Three different types were recently described as multiple detection techniques for plant pathogenic bacteria (Papadakis et al. 2015). A biosensor for genotoxicity dependent on *Acinetobacter baylyi* ADP1 was previously designed for the detection of genotoxins in polluted soil and groundwater and has been reported to be more robust than *E. coli*–derived genotoxicity biosensors in terms of feasibility, upkeep, and storage (Jiang et al. 2015). Recently, Cui et al. (2018b) has developed a biosensor for cytotoxicity, *A. baylyi* Tox2, which in response to toxic compounds constitutively expresses bioluminescence and decreases its strength (Cui et al. 2018b).

The latest techniques of QCM immunosensors have focused on self-assembled monolayers (SAMs) for the diagnosis of plant infections with the maize chlorotic mottle virus (MCMV). Using mercaptoundecanoic acids, mercaptopropanoic acid, and antibodies specific to MCMV, SAMs were observed on the gold surface of the QCM sheet shown in Table 1. This QCM biosensor showed a sensitivity close to that of ELISA and displayed detection limits of 250 g/mL. Furthermore, verified immunosensors showed considerable selectivity against similar viruses such as the wheat streak mosaic virus (WSMV) and maize dwarf mosaic virus (MDMV) (Khater et al. 2017). Another recent magnetic bead separation method for the detection of *Salmonella typhimurium* by QCM-based biosensors showed a detection limit of 100 CFU/mL in less than 10 min (Ozalp et al. 2015). Despite the resonant frequency and high sensitivity, the measurement of the QCM-based biosensor is strongly influenced by environmental conditions. This technique also has

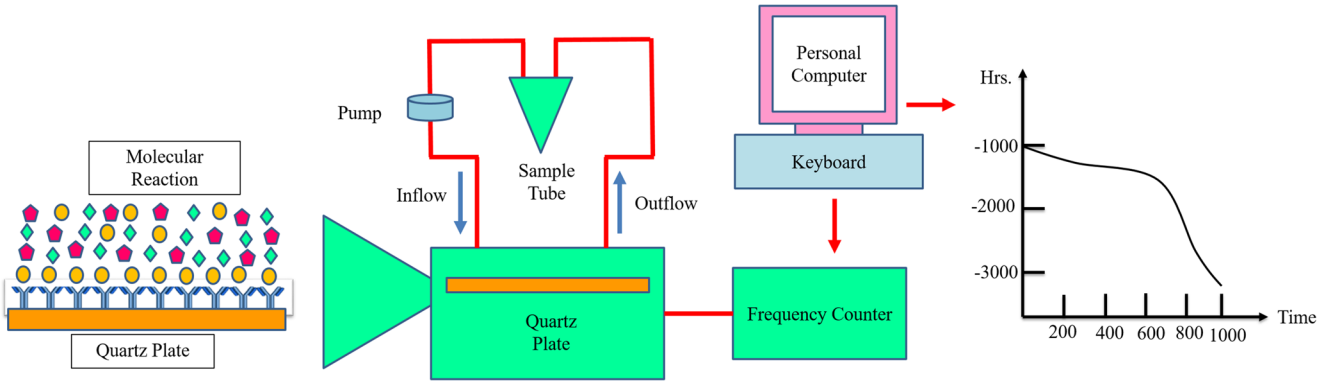

Fig. 4 The diagram representation of a quartz crystal microbalance biosensor

noteworthy issues to be solved for point-of-care applications and suggests that further research on early infection with a plant pathogen is needed.

Aptamer-based biosensor system

Aptamers can be used to target the complete cell or a part of cells such as proteins, toxins, or enzymes. An aptamer-based system is more aptly compared with antibodies due to its high specificity, low cost, and speed of detection. The aptamer is developed by several iterative systemic evolutions of ligands by exponential enrichment (SELEX) from approximately 10^{15} different sequences. Moreover, aptamers are highly flexible and have great potential and useful binding capacities (Seok Kim et al. 2016). Aptamers can be used with other detection systems, including SPR, chemiluminescence, electrochemical, fluorometric, optical, colorimetric, and magnetic devices. Numerous nanomaterials can be used in aptamer sensors to develop increased specificity and sensitivity (Khedri et al. 2018). However, aptamers have some disadvantages, such as the inability to efficiently target small site molecules, the inability to detect small variations between large particles, and their limited applicability to various plant pathogens (Wang et al. 2012). Additionally, the evolution of the old SELEX method was tedious, expensive, inefficient, and time consuming. Nevertheless, the new high-throughput graphene oxide–based monitoring methods and automatic microfluidic–based SELEX overcome the disadvantages of traditional SELEX systems (Lin et al. 2014b).

Recently, aptamer sensors have been used in combination with QCM and SPR diagnostic systems, which are highly sensitive. When used in combination with NP, they can detect human thrombin at 0.1 attomolar concentration, thereby improving detection ability in a single generation. They are used in conjunction with aptamer sensors for isothermal amplification (He et al. 2014). Clinical pathogens such as *Staphylococcus aureus*, *Lactobacillus acidophilus*, and *Salmonella enterica* can be detected by an aptamer nanobiosensor with graphene oxide (GO) on a microfluidic chip. The detection is done in 10 min and at 11 CFU/mL (Zuo et al. 2013). Fang et al. (2014) established a lateral aptamer flow sensor with no extraction of DNA to detect ten *Salmonella enteritidis* colonies at POC by isothermal amplification of the strand displacement (Fang et al. 2014). Biosensors based on aptamers can be successfully used for the detection of several foodborne and clinical pathogens, including influenza A and B viruses, human immunodeficiency virus-1 (HIV-1), hepatitis C virus, *E. coli* DH5α, and *Mycobacterium tuberculosis* strains (Wang et al. 2012). Aptamers can also be used to detect β-lectin, an allergen developed by the *Lupinus albus* plant.

These biosensors have not been commonly used in agriculture for the identification of plant pathogens. Balogh et al. (2010) first developed aptamers for plant virus coat proteins in 2010, with two apple stem pitting virus (ASPV) strains of MT32 and PSA-H as their targets (Balogh et al. 2010). A double-oligonucleotide sandwich enzyme-linked oligonucleotide assay (DOS-ELONA) was later developed by using western blotting and SPR to produce aptamer reactions (Lautner et al. 2010). These biosensors continue to have limitations in the detection of plant pathogens in agriculture. The adaptable nature of aptamer sensors and their potential utility in combination with other detection devices such as nanomaterials are an excellent selection for plant pathogen detection (Seok Kim et al. 2016).

Isothermal amplification methods

In the area of molecular diagnostics, isothermal amplification methods were an advance and DNA can be amplified without a thermocycler. Several isothermal methods have been used to diagnose plants, such as loop-mediated amplification (LAMP), helicase-dependent amplification (HDA), strand-displacement amplification (SPIA), isothermal-multiple-displacement amplification (IMDA), recombinase polymerase amplification (RPA), extension amplification reaction and nicking, and multiple

amplification of displacement. Most of these are mainly used in combination with microfluidic technology, slip chip, rotating disk, and microarray to improve the applicability and productivity of the assay in different fields (Dimov et al. 2008; Shen et al. 2011). RPA and LAMP can be widely used in plant diagnostics because of their wide variety of applications and speed, and thus have the greatest potential for early diagnosis of plants (Zhao et al. 2015; Sun et al. 2016).

Recombinase polymerase amplification

The latest isothermal technology is recombinase polymerase amplification (RPA), which has the advantage of room temperature amplification, and the maximum output occurs at 37–42 °C. RPA consists of three polymerase enzymes (recombinase single-stranded DNA binding protein (SSB) and displacement of strands) and uses 32–36 nucleotide length primers. In the presence of the ATP molecule, the recombinase scans the double-stranded target sequence to bind the primer at the homologous site and open up the double-helix structure stabilized by the SSB protein. The recombinase can be decomposed by ATP hydrolysis, and then polymerase replaces the strand to connect complementary nucleotides to the primer sequence to form a new DNA strand (Piepenburg et al. 2006; Patil et al. 2011; Daher et al. 2015). Although RPA loopholes occur with DNA extraction and primer design, the effectiveness of the RPA method is strongly affected by the length of the primers. It was recently stated that 3′ end primers with mismatched alignments lead to decreased efficiency of RPA (Daher et al., 2015).

The RPA method was tried to process crude plant extracts to decrease the difficulty related to DNA extraction, but the production was inconsistent. Despite these drawbacks, RPA has drawn people's attention in diagnosis due to its speed, selectivity, sensitivity, and low cost. Within 30 min, RPA technology can detect fewer DNA copies and detect different targets in a single reaction (Babu et al. 2017; Qian et al. 2018). The RPA method can be used to identify plant pathogens in the field, such as *Pseudomonas syringae*, *Candidatus* Liberibacter asiaticus (*C*Las), and *Agrobacterium* spp. (Zhang et al. 2014; Wee et al. 2015; Ghosh et al. 2018). To improve the signal efficiency, RPA can also be used in combination with a surface-enhanced Raman spectrometer (SERS). It is reported that this spectrometer can identify as little as one copy of plant pathogens, i.e., *Pseudomonas syringae*, *Fusarium oxysporum*, and grapevine spores (Lau et al. 2016). For plant pathogens used in plants on POC, especially in the early detection of foreign pathogens during the invasion, there is great potential for developing RPA analysis for plant pathogens.

Loop-mediated amplification technique

The loop-mediated amplification technique (LAMP) is a form of target amplification that uses 6 primers in the target sequence. These primers are very unique to 8 various target locations (Notomi et al. 2000). After adding RTase (RT-LAMP) enzyme, LAMP is suitable for DNA/RNA genome. Using fluorescent probes to modify LAMP made it a real-time isothermal amplification technique that can quickly and easily perform complete quantification of pathogen DNA (Tomita et al. 2008; Zhang et al. 2013; Katoh et al. 2020). However, LAMP has some disadvantages, such as the need to maintain a temperature of 65 °C for a hot water bath or heating block and the configuration of 6 pairs of primers (Fukuta et al. 2003). Even in the presence of contaminants and inhibitors, the potential benefits of using this technology for plant diagnostics are easier to apply and have durability and high specificity. In the previous years, LAMP has played an important role in plant diagnosis. According to recent reports, it has a sensitivity of up to five to six copies of DNA target sites (Wu et al. 2016) and can be applied to diagnose *C*Las, *Phytophthora ramorum*, and *Erwinia amylovora* pathogens (Keremane et al. 2015).

Lab-on-a-chip and paper-based system

Lab on a chip (LOC) is a technique based on a chip used to identify pathogens and various biochemical reactions. Thermoplastic, silicon, glass, and poly-dimethylsiloxane can be used in the composition of POC. The LOC system plays a significant role in signal amplification, processing of samples, and data analysis (Rohrman and Richards-Kortum 2012; Luka et al. 2015). Rapid microbial detection can be performed by LOC with a smaller sample volume. The LOC system has shown higher sensitivity compared with ELISA assays and detects 0.1 pg/μL and 10^3 CFU/mL of *Xylella fastidiosa* and *Phytophthora infestans*, respectively (Chiriacò et al. 2018; Zhan et al. 2018). Owing to its sensitivity, the LOC system has some limitations because of the requirement of stable polymer and label-free assays, which impede target site detection pathogens (Julich et al. 2011). Furthermore, improvement is required in LOC devices for the indirect use and quick diagnosis of phytopathogens in field conditions and various functions, as illustrated in Fig. 5.

In addition, by using permeable absorbent fiber paper (pore size 1–10 μm), the production of 2D- and 3D-potential microfluidic paper analysis equipment (μPAD) has overwhelmed the blocks related to traditional LOC equipment, thereby reducing costs associated with production. The μPAD system has a strong ability to manage large quantities of fluids and can operate on capillary action or the principles of lateral flow. These are easy to handle and inexpensive. These devices have four basic components, namely a

conjugate pad (for the sample and label binding), a sample pad made of cellulose (for sample loading), an indicator pad (for test and control lines), and an absorption pad (for sample and label binding) (for the absorption of the remaining liquid). The existence of the cellulose groups of hydroxyl (–OH) and carboxyl (–COOH) helps to immobilize many molecules (Mahato et al. 2017). In 1950, an immunochromatographic strip was used to measure the glucose concentration in urine, which was the first paper-based dipstick assay (Rani et al. 2019). Initially, μPADs provided only colorimetric (qualitative) readings; however, later, with advancements in hardware-software technologies and wireless communication, target quantitation could be performed using a detection system based on cell phones (CP) (Syedmoradi and Gomez 2017; Syedmoradi et al. 2017). On-site *Phytophthora* spp. and *Ralstonia solanacearum* can be detected through commercially available paper-based immune strips (Champoiseau et al. 2009). However, further progress in μPADs will lead to the creation of a "pocket-sized" detection platform for pathogen diagnostics in plants (Martinez 2011; Mahato et al. 2017).

Phage-based biosensor systems

Bacteriophages are viral genomes enclosed by coat proteins (DNA or RNA) that infect and propagate via bacterial cells. Phage-based technology was used early for the treatment of plant bacterial infections. However, phage approaches have also been applied to detect plant infectious diseases or detect phytopathogens, with the binding affinity of bacterial cells, carbohydrates, or proteins. The relation between the target bacterial cell and the phage carries a new response to change and is translated by the sensor into a measurable signal. Phage-based biosensors are user-friendly, cost-effective, responsive, efficient, precise, and dynamic and have a long life cycle owing to their constancy in number at high temperatures. These biosensors should demonstrate the capability of multiple infections in pathogens (Yue et al. 2017). These techniques also restrict the required sample preparation process and have low applicability for the diagnosis and detection of unculturable bacterial and fungal pathogens (Singh et al. 2013). However, a phage-based biosensors system can be used and established to detect foodborne pathogens (Yue et al. 2017).

Label-free electroluminescence (ECL) can detect *Pseudomonas aeruginosa* (56 CFU/mL^{-1}) in less than 30 min. Phage PaP1, isolated and collected from sewage in hospitals, is used. The surface of glassy carbon electrodes was made to capture *P. aeruginosa* by depositing phage-conjugated carboxy graphene. Carbon-based nanomaterials have thus emerged as potential candidates for the production of next-generation miniaturized biosensors due to their interesting chemical, physical, and electrical properties and have been integrated into electrochemical biosensors for extremely

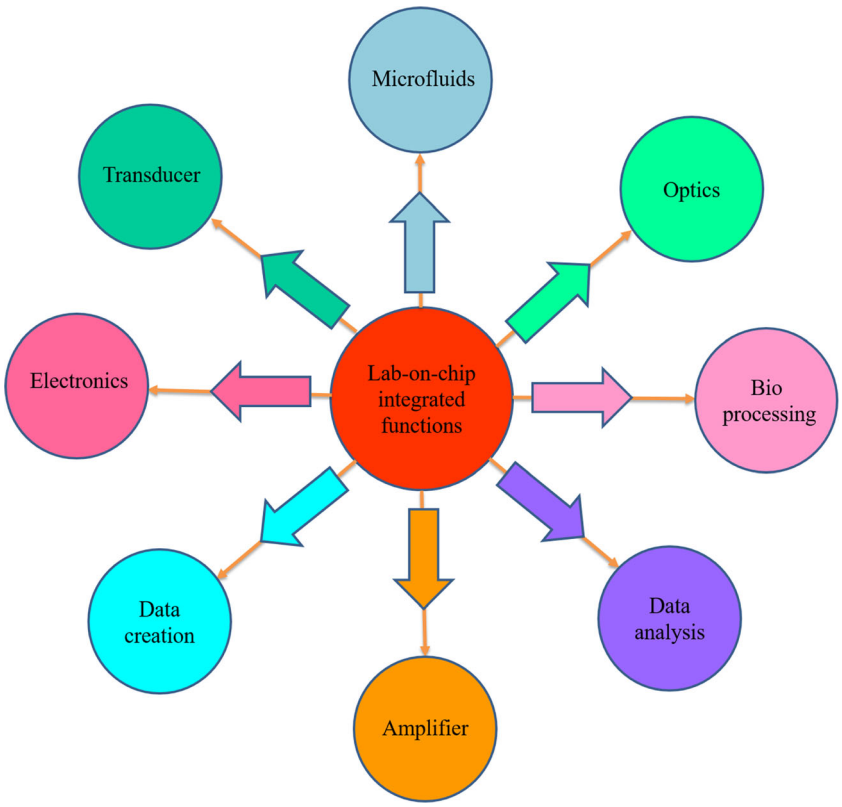

Fig. 5 Integrated functions of a lab-on-chip device

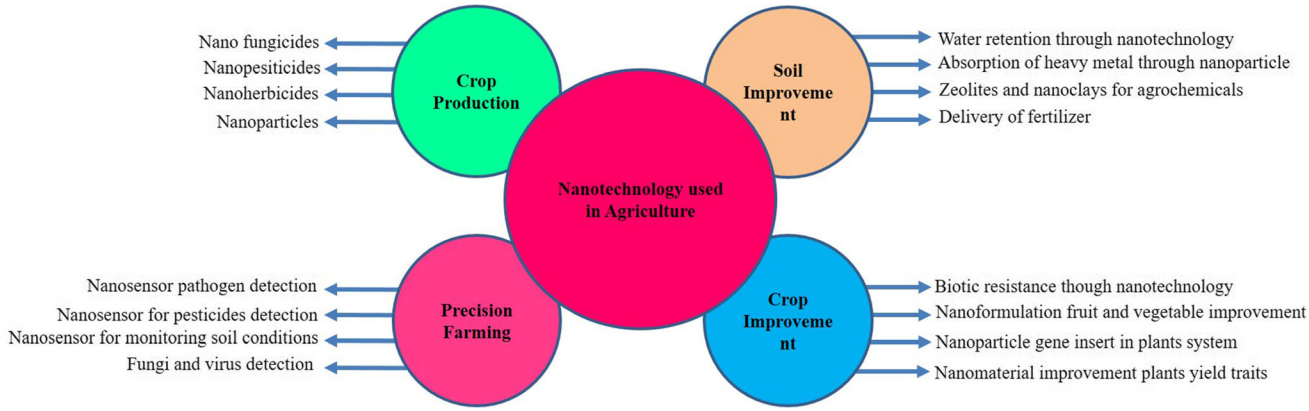

Fig. 6 Various applications of nanotechnology in sustainable agriculture

sensitive and selective detection of different analytes (Ramnani et al. 2016). The development of non-conductive complexes that prevent interfacial electron transfers induces an increase or decrease in the ECL signal attention of *P. aeruginosa*, thus preventing the spread of ECL vigorous molecules (Yue et al. 2017). Another similar study inoculated phage DNA into bacterial cells to bind bacterial organisms and propagate them over several virions. Host DNA is occupied by the genome of the virions, and the genome remains temporarily inactive until the stimulated host reproduces. Studies on phage-based biosensors for endotoxin and pathogen detection have been reported. Different analysis techniques combined with phage-based probes can be used to diagnose specific target sites. Some phage-based biosensors can be directly and effectively used for pathogen detection in tomato plants (Park et al. 2013a) and fresh milk (Balasubramanian et al. 2007).

Phage-based magnetoelastic (ME) biosensors have been established to detect plant pathogens, such as the surface of spinach leaves and tomato plants infected by *Salmonella typhimurium*. The detection limit of this biosensor is 1.94 CFU/leaf (Park et al. 2013b). Although less research on plant pathogens is being conducted in the field, phage biosensors are more responsive and steady than qPCR (Park et al. 2013b), thereby highlighting the potential of a phage-based biosensor for the early diagnosis of phytopathogens (Rani et al. 2019). Plant-based bioreactors using wood chips or plant residues as carbon sources are widely used because they are easy to use, simple in activities, and low in cost, and can reduce a load of pollutants in surface water from agricultural underground drainage and groundwater drainage (Wang et al. 2019).

Nano-biosensors for plant pathogen detection

The application of nanotechnology in agricultural research has shown an important role in the reduction of pesticide (insecticides, fungicides, and bactericides) particles from the standard 5 μm to 100 nm. At present, the use of nanomaterials allows the application of pesticides, thereby eliminating food safety concerns caused by the misuse of pesticides (Zhao et al. 2014). Several research reports note that nanofabrication approaches focus on the unparalleled growth of information and a deep understanding of its properties, performance, and incorporation into multifunctional devices with editing nanomaterials (Ariga et al. 2011; Sakakibara et al. 2011; Yaman 2011). Different carbon nanomaterials (CNMs) can be used for a variety of applications due to the rapid growth of nanotechnology. The most commonly used CNMs in the world are carbon nanofibers (CNFs), multi-walled carbon nanotubes (MWCNTs), single-walled carbon nanotubes (SWCNTs), fullerene (C60), and graphene (Ghorbanpour and Hadian 2015). However, more research has shown that both biology and the environment have adverse effects on CNMs (Chen et al. 2016). Additionally, CNTs have been affecting photosynthesis and have other consequences. Tao et al. (2013) investigated the sub-lethal effects on *Scenedesmus obliquus* of nanocrystalline C60 as nC60, and showed that nC60 repressed plant growth rate and decreased chlorophyll gratified at sub-lethal concentration in photosynthesis. By inhibiting plant growth and influencing vegetative material absorption and the degradation of plant parts, CNMs can have poisonous effects on crops (Chen et al. 2018a).

Nanosensors were commonly used for the identification and assessment of crop pathogens. For example, the plant pathogen *Xanthomonas axonopodis* pv. *vesicatoria* produces a bacterial spot on tomato and pepper plants and can be observed by fluorescent silica nanoparticles (FSNP) conjugated with antibodies (Etefagh et al. 2013). Nanoparticles of copper oxide (CuO) were used to detect *Aspergillus niger* fungi (Dubertret et al. 2001). Furthermore, water- and soil-borne pathogens were detected by using silver-based nanoparticles (AgNPs). Thus, by using a nanosensor, we can predict and manage plant diseases in agriculture to an acceptable level. Early diagnosis of the plant infectious diseases is vital to

hinder disease transmission across the country and decrease yield damage (Strange and Scott 2005; Miller 2009). The newly developed QD techniques can also detect phytopathogens (Arya et al. 2005; Khiyami et al. 2014). QDs are semiconductor nanoparticles that can be used for visual inspection as labels on proteins or DNA (Duhan et al. 2017). A technique has been established for the identification of beet necrotic yellow vein virus vectors using resonance energy from QD fluorescence (Safarpour et al. 2012).

A technique to classify the beet necrotic yellow vein virus vector using QD fluorescence-resonance energy has been developed and can also be used to detect highly sensitive *Phytoplasma aurantifolia* on lime (Rad et al. 2012; Safarpour et al. 2012). Transfer-related developments in biosensors systems have been made in recent years based on nanomaterials, in which nanomaterials have been used to change the sensor electrodes. Many studies can identify dangerous pathogens as a result of this development (Madufor et al. 2018). Biosensors are commonly used as diagnostic instruments for enhancing pathogen identification and the environment in foods. They allow the development of pathogen detection technology for different microbial cultures (Khater et al. 2017). Biosensor strategies have been developed using existing knowledge of receptors, counting DNA probes and biosensors based on antibodies (Singh et al. 2013; Khiyami et al. 2014). Numerous earlier revisions have discussed the identification of nano-particles for plant diseases such as those caused by fungi (Chartuprayoon et al. 2010), viruses (Yao et al. 2009; Chartuprayoon et al. 2013), and bacteria (Boonham et al. 2008).

Robotics techniques for agriculture

Major scientific improvements in agriculture in the last few decades have completely altered several processes in livestock and crop production systems (Fountas et al. 2020). These developments are mainly concerned with minimizing operational and production costs, reducing the environmental effects, and maximizing the entire production cycle. One of the most important issues in a crop production system is labor-intensive operations (Pedersen et al. 2006). These are predominantly field tasks (e.g., disease control, intra-row weed control, and sensitive fruit harvesting), which are harder to accomplish with old-style field machines and therefore require human labor (Adachi et al. 2020). This has led to increasing demand for automatic tractors and robotic platforms for crop field operations currently in the research phase of development (Blackmore 2007).

Detection of disease is primarily a graphical task; hence, totally, robots are integrated with vision-based systems. The detection accuracy is defined as the ratio of detected diseased plants to the total number of infected plants. All vetted robots use color cameras, which are easy to use and incur low costs. A multispectral camera is the second-most commonly used type of camera, which is affordable and requires more sophisticated computational power (Schor et al. 2016). With color cameras, all device configurations with multispectral/hyperspectral sensors are mixed. However, the use of more complex structures does not always achieve the best outcomes. The use of color cameras showed high precision when detecting powdery mildew in greenhouse pepper plants of colored pepper and tomato spotted wilt virus (TSWV), whereas the use of multispectral cameras for these two diseases had an accuracy of 80% and 61%, respectively (Schor et al. 2017). Another method was introduced which fused a series of sensors to detect *Xylela fastidiosa* in olive trees (Rey et al. 2019).

Simple disease detection robots are primarily based on image data from red-green-blue (RGB). Using a machine vision system focused on requirements for artificial cloud lighting conditions, powdery mildew was found in strawberry plants and an accuracy of 72–95% was achieved under two different lighting conditions (Mahmud et al. 2019). Furthermore, eAGROBOT is used to detect a series of diseases; the accuracy rate for cotton plants and peanut plants is approximately 90% (Pilli et al. 2015).

In summary, robot technology for disease detection is in its infancy. According to current information, the following three key problems related to disease detection through robots are (I) the lack of image databases for each detection/classification model for each disease; (II) non-uniform lighting conditions in the field; and (III) slow processing of images, especially when large volumes of images are used, such as hyperspectral (Zheng et al., 2019). As open-access agricultural databases become accessible and new methods of data synthesis are presented, the lack of available image datasets is increasingly being addressed (Barth et al. 2018). At the same time, lighting systems for the identification of various diseases, such as powdery mildew (Mahmud et al. 2019), are being developed.

Concluding remarks

Plant diseases continue to threaten important commercial plants and cause significant global economic losses. Food shortages and pathogen-associated food production damage also pose significant challenges for global food safety. When new pathogens are introduced into a country or region, early detection in the nursery helps disease management, reduces disease transmission, and increases the chances of successful eradication. Plant pathogens must be identified early before being exported to agricultural science and forest production methods, as well as to commercial and home gardens that use plants to make the natural environment. Conventional techniques used in symptomatic plants to confirm the identity of

plant pathogens include in vitro plate culture, ELISA, biological indexing, PCR, and dot blot hybridization. These tests have been confirmed to be specific, sensitive, and to some amount reliable; however, they have evident disadvantages, for example, requirement of skilled operators and complex equipment, thereby making it impossible to use POC for most routine testing approaches. Although a portable ELISA test strip assay has been developed to detect plant pathogens in the field, sensitivity- and specificity-related problems exist. Recent developments in micro- and nanotechnology have made it possible to build biosensors to assess infections of pathogens in crops using antibodies and DNA as biosensing receptors to solve these difficulties.

Furthermore, most DNA biosensor methods are focused on the determination of DNA hybridization events, including fluorescent, electroluminescence, and colorimetric approaches. Despite the sensitivity and selectivity benefits of DNA biosensors due to excellent gratitude properties, sample treatment requirements still suffer from their in-field application (e.g., DNA extraction). However, using methods of SPR, voltammetric, label-free impedance, fluorescent and QCM detection, antibody-based scenarios have been developed. Developing sensitive and specific advanced diagnostic techniques would allow early detection (including in asymptomatic plants) and decrease the impact of plant pathogens on the agricultural production systems. In this review, new high-throughput technologies focused on laboratory and field-care technologies and types of equipment were tested for use in nurseries. Although laboratory-based technologies (such as SERS and ddPCR) are expensive, they are specific and sensitive and need professional operators. Moreover, allowing collective multiple pathogen detections per sample is also a significant benefit of developing laboratory-based techniques.

However, as they are not portable and require trained personnel, biosafety officials or industry regulators can use them effectively to prevent pathogens from entering an area or to restrict the transportation of infected materials to protect nurseries from further spread. Unqualified personnel, on the other hand, can easily use immediate detection technologies such as biosensors, isothermal amplification technology, nano-bio sensors, paper-based techniques, and LOC, thereby requiring less processing and simple readings. Continuous study has increased the specificity of their LOD, and the low sensitivity of some POC systems remains a bottleneck for the identification of low-level pathogens from asymptomatic plants in the nursery. Importantly, the throughput of the POC method is lower than that of the emerging laboratory-based method, and robotics methods are emerging methods in on-site pathogen detection. Finally, we conclude with the convergence of existing biosensing mechanisms with new developing nanotechnology and DNA sequencing technologies. Overall, the full probability of biosensor technology must be realized to address the challenges of plant pathogen detection.

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Data availability As this is a review article, data availability is not applicable. However, the resource pool can be provided on request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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