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Electrochemical and Piezoelectric-Based Nanosensor: A Cutting-Edge Technology for Plant Disease Detection

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Abstract

In agriculture, plant diseases cause considerable crop losses, amounting to a substantial financial burden. Innovative solutions are essential for minimizing these losses and enhancing agricultural sustainability. Nanosensors represent a promising technology that offers precision, speed, and cost-effectiveness in the detection of plant pathogens. They utilize various nanomaterials to interact with target pathogens, enhancing the sensitivity and specificity of detection. Nanosensors employ a variety of nanomaterials, including nanoparticles (e.g., AuNPs, AgNPs), nanowires, carbon nanotubes, graphene, and quantum dots. These nanomaterials offer unique properties like high surface area, electrical conductivity, and optical characteristics, making them suitable for diverse sensing applications. This article explores two types of nanosensors: Electrochemical Nanosensors (ECN) and Piezoelectric Nanosensors (PZN), shedding light on their mechanisms, applications, and advantages, owing to their profound utility in plant pathogen detection.

Keywords: Plant Pathogens, Detection, Electrochemical-based Nanosensor, Piezoelectric-based Nanosensor

Introduction

On a global scale, insect pests and plant diseases collectively account for a staggering 27% of crop losses, with insect pests responsible for 14% and plant diseases for 13% of this detriment. The financial impact of this agricultural challenge is profound, with the annual cost of crop losses estimated at a staggering 2 trillion dollars (FAO, 2018). In order to minimize the devastating crop losses inflicted by plant pathogens and to promote the broader goals of agricultural sustainability, accurate, cost-effective, rapid, and dependable diagnostic methods are absolutely imperative. Such methods would not only facilitate the early detection and assessment of pathogens but will also aid in advancing the overall resilience and productivity of agricultural systems. The aforesaid needs could be met by nanosensors, used for the detection of plant pathogens which represent a cutting-edge technology that offers significant benefits for agriculture and plant disease management. These highly sensitive devices are designed to detect and monitor the presence of plant pathogens at the nanoscale level, providing precise and rapid detection. Nanosensors typically utilize various nanomaterials, such as nanoparticles (NPs), nanowires, and nanotubes, to interact with the components of plant pathogens such as protein, DNA, etc. These nanomaterials can be functionalized with specific ligands, antibodies, or aptamers that have a high affinity for the target pathogen (Sharifi et al., 2020). When the target pathogen binds to these functionalized nanomaterials, it induces a detectable signal or change in the sensor's properties. Common detection methods include electrical conductivity changes, colorimetric changes, fluorescence, and surface plasmon resonance. DNA probes and other biorecognition receptors attached to NPs, several nanobiosensors have been developed to have an edge over conventional methods such as Fluorescence Resonance Energy Transfer (FRET) nanosensors, Surface-Enhanced Raman Scattering (SERS) Nanosensors, Electrochemical Nanosensors (ECN), and Piezoelectric Nanosensors (PZN) (Kwak et al., 2017). For instance, Gold NP-coated Quartz crystal microbalance exhibited high sensitivity and specificity for *Maize chlorotic mottle virus* (MCMV) with a detection limit and time of 250 ng ml^{-1} and 2 h, respectively (Huang et al., 2017). This short article discusses the details of nanosensors in plant pathogen detection, focusing on Electrochemical-based nanosensors (ECN) and Piezoelectric-based nanosensors (PZN) exploring their working principles, applications, and advantages.

Types of nanosensors for phytopathogen detection

A number of nano structure-based sensors have been developed which are used to diagnose and detect plant pathogens, such as gold nanoparticles (AuNPs) based nanosensors, Aluminium nanoparticles (AlNPs) based nanosensors, Quantum dots, carbon-based nanosensors, silica nanoparticles-based nanosensors, carbon nanotubes nanosensors, Silver nanoparticles (AgNPs) based nanosensors, copper nanoparticles (CuNPs) based nanosensors, etc. (Mokhtarzadeh *et a.*, 2017). However, based on bio-recognition principles and signal transduction mechanisms, nanosensors are categorized into four types (Kwak et al., 2017) *viz.*, Fluorescence Resonance Energy Transfer (FRET) nanosensors, Surface-Enhanced Raman Scattering (SERS) nanosensors, Electrochemical-based nanosensors (ECN), and Piezoelectric-based nanosensors (PZN).

Advantages of Nanosensors

There are several advantages of nanosensors over conventional methods such as high sensitivity (can detect low pathogen concentrations), rapid detection, high specificity reducing false positives, ease of use, high portability, reduced resource usage, and enhanced detection limit in detecting different kinds of pathogens such as bacterial, fungal, viral pathogens, etc. Moreover, on-site detection of pathogens is possible, thus field application of nanosensors is an excellent tool to make crop cultivation more sustainable and safer by minimizing the use of agrochemicals. However, there are certain challenges that need to be addressed. Firstly, the high cost of developing and manufacturing nanosensors limits their widespread adoption. Secondly, extensive testing is required to validate the accuracy and reliability of nanosensors in different environmental conditions and against various pathogens. Thirdly, regulatory approval and standardization are necessary for broad adoption, especially in agriculture, which somehow impedes its swift utilization. Importantly, the long-term environmental impact of nanosensors or any other nanomaterials is under the purview of criticism. Thus, more investigation needs to be carried out in the toxicological aspects.

Electrochemical-based nanosensors

Electrochemical-based nanosensors (ECN) are a very efficient technique to detect crop pathogens at an early stage. ECN reads the chemical information of a sample and converts the data into an analytical signal. It can examine any biological sample by virtue of its capability of direct translation of biological events to an electric signal. The mechanism of ECN is simple, the cost of development is comparatively low, easy to miniaturize, has high sensitivity, and is capable of direct data analysis. The most commonly used nanostructures for the development of ECN are nanotubes, silica-based NPs, TiO_2 NPs, ZnO NPs, etc. which improve the performance in terms of both sensitivity and selectivity with an extremely low limit of detection.

Mechanism

ECN is composed of three types of electrodes, brief descriptions of them are as follows (Fig).

1) **Working electrode**: The working electrode is a crucial component in the redox process of an electrochemical cell. It serves as the interface where the electrochemical reactions occur, facilitating the conversion of chemical reactions into electrical signals. Working electrodes can be constructed using various materials, ranging from high-cost elements like platinum, mercury, gold, and silver to more cost-effective options such as glassy carbon, carbon paste, and screen-printed electrodes. In biosensors and electrochemical sensors, the working surface of the electrode is often modified with biomolecular receptors, such as enzymes, nucleic acids, antibiotic dyes, and metal ions. These receptor molecules are immobilized on the electrode's surface to amplify signals and improve the specificity and sensitivity of analyte and biomarker detection. This functionalization of the working electrode is instrumental in enhancing the recognition and quantification of various substances, making it a pivotal component.
2) **Reference electrode:** The potential is applied to the working electrode concerning the reference electrode. Reference electrodes are constructed using various materials, including silver, silver chloride ($AgCl_2$), and saturated calomel. They serve as a stable reference point for measuring the potential at the working electrode, ensuring accurate and consistent electrochemical measurements.
3) **Counter electrode:** It is accustomed to complete the electrical circuit.

Applying a negative potential to the working electrode results in electrons flowing from the working electrode into the solution, leading to the reduction of the analyte. Conversely, when a positive potential is applied, the reverse reaction occurs, and the analyte is oxidized. Cyclic voltammetry, a specific type of potentiodynamic electrochemical measurement, interprets these signals. It involves cycling the potential applied to the working electrode back and forth, allowing for the study of the redox behaviour and electrochemical properties of analytes.

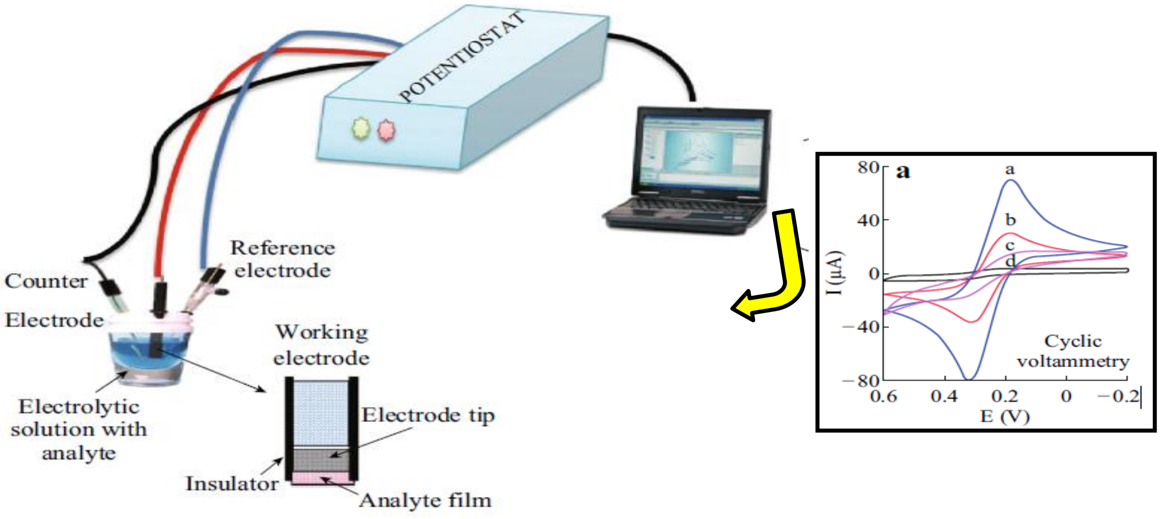

Figure 1. Principle of electrochemical-based nanosensor

To gain a detailed understanding of the mechanism, consider the example illustrated in Figure 2. In this particular case, gold nanoparticles (AuNPs) are coupled with DNA probes. To attach the DNA probes to the AuNPs, a mixture of 100 µL of tris(2-carboxyethyl) phosphine (TCEP) and the DNA probe was prepared. This mixture was created by combining 50 µL of 10 mM TCEP with 50 µL of 100 µM DNA probe and incubating it at room temperature for 2

h. For amplifying DNA targets, recombinase polymerase amplification (RPA) is employed. The resulting amplicon, which includes a 10-nucleotide barcode sequence, hybridizes with AuNPs tagged with the probe. To isolate AuNPs/DNA/biotin products, streptavidin magnetic beads are utilized. Subsequent to this, a washing step is performed to remove any excess reagents. To ensure that magnetic beads do not interfere with the electrochemical signal, the Magnetic beads/AuNPs/DNA-biotin products undergo a heat treatment at 95°C. This denatures the double-stranded DNA amplicons, releasing the AuNPs into the solution. The electrochemical reduction of Au(III) to Au(0) is quantified using differential pulse voltammetry. The amount of AuNPs released is directly proportional to the level of target DNA amplification, signifying a successful RPA/PCR amplification and, consequently, indicating the presence of the pathogens (Lau et al., 2017). Some recently developed electrochemical nanosensors (ECNs) for plant pathogen detection are given in Table 1.

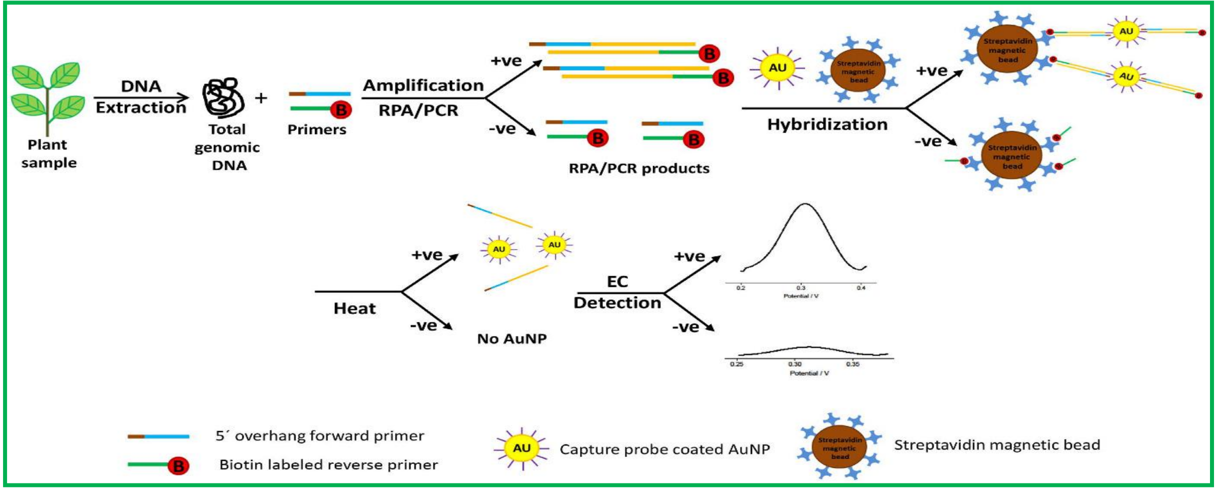

Figure 2 Electrochemical bioassay for plant pathogen DNA detection (Adapted from Lau et al., 2017)

Table 1 ECN-based nanosensors reported for detection of plant pathogens

Nanomaterials	Target pathogens	Target	Test Host	Detection limit	sensitivity	References
Colloidal AuNPs	*Pseudomonas syringae* DC3000	DNA	*A. thalliana*	------	10^4 times more sensitive than PCR/gel electrophoresis	Lau et al., 2017
SnO_2 and TiO_2 NPs	*Phytophthora cactorum*	P-ethyl guaiacol	Strawberry	35-62 nM	High	Fang *et al.*, 2014
Chitosan-mutiwalled carbon nanotubes nanocomposite	*Cauliflower mosaic virus*	DNA		8.5×10^{-14} M	High	Wang *et al.*, 2011

Piezoelectric-based nanosensors

Piezoelectric-based nanosensors (PZN) converts the mechanical vibration into an electric signal and vice versa. It is based on the determination and monitoring of change in mass due to bio-molecular interaction (Fig. 2 (A)). These sensors use the piezoelectric effect to measure shifts in resonance frequency when target molecules bind to receptors on their surface. It has the potential to convert biological mechanical energy, acoustic/ultrasonic vibration energy, and biofluid hydraulic energy into electric signals and facilitate real-time monitoring. The most common material in PZN is quartz crystal microbalance (Fig. 2 (B)). A thin layer of NPs is coated on Quartz crystal which enable the formation of hydrophobic/ covalent bond with biomolecules (Kwak *et al.*,2017)**.** Exploiting this phenomenon several PZN-based nanosensors have been developed (Table 2).

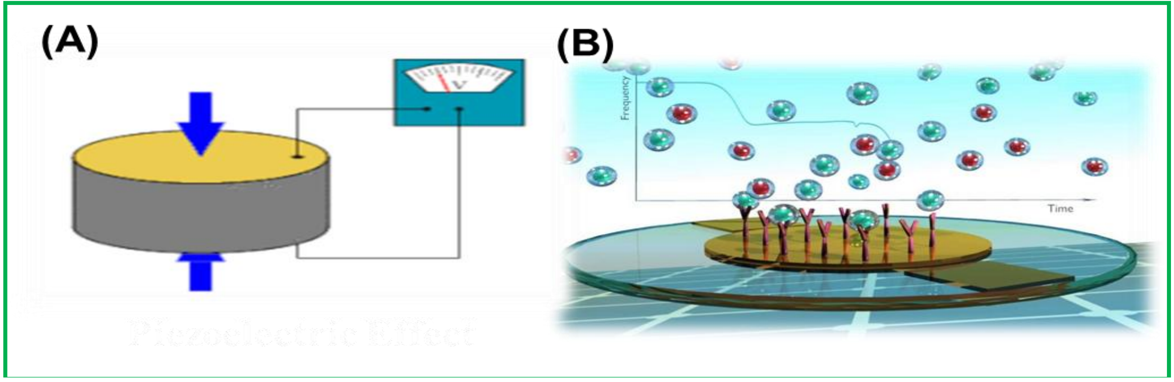

Figure 3: A) Piezoelectric Effect; B) Quartz Crystal microbalance

Mechanism

Within PZN-based nanosensors, Quartz Crystal Microbalance (QCM) surfaces are meticulously layered with AuNPs, which function as electrodes, significantly enhancing the creation of hydrophobic or covalent bonds with biomolecules like specific DNA probes or other selective receptors. Guided by the principle that frequency and mass share an inverse relationship, any interaction between an analyte- such as viruses or DNA molecules from pathogens and the receptor results in a decline in frequency. This decrease arises from the uptick in mass following the binding of the selective receptor to the analyte (pathogens), thereby conclusively confirming the presence of a pathogen (Fig. 4).

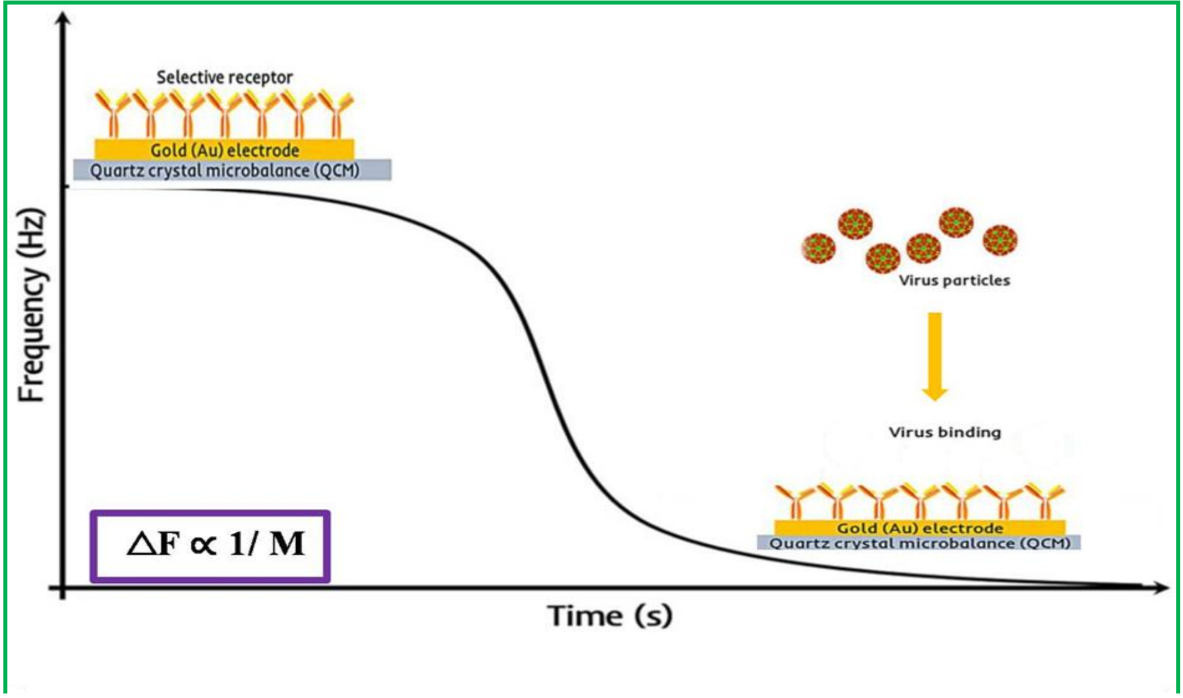

Figure 4 Working of Piezoelectric Nanosensor (AuNPs-Quartz Crystal Microbalance)

Table 2 PZN-based nanosensor reported for the detection of plant pathogens and human disease-causing bacteria

Nanomaterials	Target pathogens	Target	Test host	Detection limit	sensitivity	References
Au-NPs coated QCM crystal	*Maize chlorotic mottle virus*	DNA	Maize	250 ng ML^{-1}	Highly sensitive than ELISA	Huang *et al.*,2017
QCM crystal	*Cymbidium mosaic potextvirus* (CymMV), *Odontoglossum ringspot tobamovirus* (ORSV)	DNA	Orchid	1 ng	Highly sensitive than ELISA	Eun *et al*., 2002
Quartz crystal	*Campylobacter jejuni*	DNA		1.30 log CFU ml^{-1}	High	Masdor, 2019)
Quartz crystal	*Staphylococcus aureus*	DNA		7.41 log CFU ml^{-1}	High	Noi et al. 2019)

Conclusion

In conclusion, nanosensors for plant pathogen detection represent a promising innovation that has the potential to revolutionize plant disease management. Nanosensor entails binding of target species or strains and eventual transformation into detectable signals. Nanosensors facilitate quick, precise, and early detection of disease-inciting agents and hence ensuring quality food production. It offers the benefit of high specificity and sensitivity for plant pathogen detection. Nanosensor has the advantage of portability and is user-friendly for pathogen detection in field condition. As the technology continues to advance and becomes more cost-effective, it is likely to play an increasingly crucial role in safeguarding global food security and sustainable agriculture.

Future prospect

An integrated disease management system including a nanosensors system would be beneficial. Retrieval nanosystems can be developed for specific sampling from soil, air, and plant systems. Moreover, the development of rapid and reliable nano methods for the detection of mycotoxin and toxigenic fungi would be of great help in the medical field. Improvement of existing and development of rapid and reliable nanodevices should be done for better detection of pesticide residues in foods and feeds.

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