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4. Role of Biosensors-Based Detection in Plant Protection

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Abstract:

Plant pathogen detection is recognizing microscopic organisms such as bacteria, viruses, and fungi in quick reaction settings at nurseries, natural landscapes and micropropagation stage in infected plant tissue. Early detection provides opportunity to farmer to take proper measurement and save the crops from complete failure. For plant protection or disease control, simultaneous detection of all the present phytopathogenic microbes with quick and high accuracy is of great importance in all areas related agriculture and environmental safety. In this regard, biosensors technology in plant disease detection in broad-spectrum has advantage like lessening the investigation time and sensitivity through automation and integrating multiple processes in a single piece of equipment. The use of different types of biosensors based on colorimeter, electrochemical signal, lights emissions and nanomaterials for pioneering and sensitive biosensing systems for the recognition of pathogens is also shown. The untamed potential of various biosensors with some limitations for plant disease detection has been briefly reviewed in this article.

Keywords:

Biosensors, Microbes, Nanomaterial, Food safety, Environment, Signal processing.

4.1 Introduction:

Biosensors are devices that detect and measure biological responses or analytes, such as specific proteins, enzymes, antibodies, or DNA, and convert them into measurable signals. They are widely used in various fields, including medical diagnostics, environmental monitoring, food safety, and biotechnology research.

4.1.1 Biosensors typically consist of three main components:

A. **Biological recognition element:** This is a biomolecule that interacts selectively with the target analyte. It can be an enzyme, antibody, DNA/RNA, or whole cells. The biological recognition element provides the specificity of the biosensor by binding to the target analyte.
B. **Transducer:** The transducer converts the biochemical signal generated by the interaction between the biological recognition element and the analyte into a

measurable signal. The transducer can be optical, electrochemical, piezoelectric, or thermal, depending on the type of biosensor.

C. **Signal processing system:** This component amplifies, analyses and displays the signal generated by the transducer, allowing for quantitative measurement and analysis of the target analyte.

4.1.2 Biosensors in plant protection with examples:

Biosensors play an important role in plant protection by enabling rapid and sensitive detection of pathogens, pests, and environmental factors that can affect plant health. Here are a few examples of biosensors used in plant protection:

A. **Pathogen detection biosensors:** Biosensors can be designed to detect specific plant pathogens, such as bacteria, fungi, and viruses. For example, DNA-based biosensors can use specific DNA probes to identify the presence of pathogen DNA in plant samples. These biosensors can aid in early detection and monitoring of diseases, allowing for timely interventions to prevent or control the spread of pathogens.
B. **Pest monitoring biosensors:** Biosensors can also be used to monitor insect pests that can damage crops. Some biosensors utilize pheromones or volatile organic compounds emitted by pests to attract and trap them. These biosensors can help in monitoring pest populations, identifying infestation hotspots, and implementing targeted pest control measures.
C. **Environmental biosensors:** Biosensors can be employed to monitor environmental factors that impact plant health, such as soil quality, nutrient levels, and water quality. For instance, biosensors can measure the concentration of specific ions or nutrients in soil or water samples, providing valuable information for optimizing fertilization practices and ensuring proper irrigation.
D. **Toxin detection biosensors:** Certain plant pathogens produce toxins that can harm plants. Biosensors can be designed to detect these toxins, enabling early identification and mitigation of toxin-mediated damage. For example, biosensors can be developed to detect mycotoxins produced by fungi, which can contaminate crops and pose health risks.
E. **Plant stress biosensors:** Biosensors can be utilized to monitor plant stress factors such as drought, salinity, or temperature fluctuations. These biosensors can measure specific physiological or biochemical responses in plants, such as changes in leaf water potential or the accumulation of stress-related proteins or metabolites. By monitoring plant stress levels, appropriate measures can be taken to mitigate the impact and optimize plant growth.
F. **Pesticide residue biosensors:** Biosensors can be used to detect and quantify pesticide residues on plants. These biosensors can help farmers ensure that pesticide application is within safe limits and avoid potential harm to human health and the environment. Various biosensing platforms, such as electrochemical and optical biosensors, have been developed to detect specific pesticides or pesticide classes in plant samples.

These examples highlight the diverse applications of biosensors in plant protection, helping farmers and researchers detect and respond to threats effectively.

Biosensors offer the advantage of rapid and sensitive detection, allowing for timely interventions and more precise plant protection strategies.

4.2 Diagnostic Methods for Plant Pathogens:

Monitoring plant health and implementing an effective integrated disease management (IDM) strategy depend on the early detection of plant pathogens. Differentiating between causative species is crucial because numerous fungal infections alter plants in ways that are similar to one another during disease development. Vulnerable crops frequently experience more obvious signs, such as morphological and color changes, particular necrotic patches, and even the loss of the plant's stem or leaves. However, understanding latent infection with no obvious signs is also essential to ensure fully informed care. (Oerke, 2020).

Visual crop inspection, which requires a skilled grower or pathologist, is the oldest traditional method that is still widely employed for disease and potentially pathogen diagnosis. By the time a visual diagnosis is made, the pathogen will probably have established itself in host populations. The development of earlier pathogen detection techniques with higher sensitivity, accuracy, and identification speed has therefore received considerable attention. Enzyme-linked immunosorbent assays (ELISA), polymerase chain reactions (PCR), and loop mediated isothermal amplification (LAMP) tests have been the three main types of molecular assays used up to this point, all of which are protein- or nucleic acid-based technologies. These widely used methods do have some drawbacks, such as lengthy diagnostic times, difficult sample preparation steps, carrying the sample from the field to specialized laboratories, and the requirement for trained professionals, despite improvements in sensitivity and specificity to particular target pathogens.

As a result, the need for an in-field diagnostic procedure that is more rapid reliable, especially sensitive, and precise has increased. Such "point-of-care technology" might be created by utilizing the primary properties of the electrochemistry or optical. With a quick reaction time, low-cost on-site trials, and no need for interpreting data skills from the user, this approach improves certain aspects of bioassays.

4.3 Biosensor Technologies for Plant Pathogen Detection:

In several research disciplines, such as monitoring the environment, the detection of airborne diseases, the real-time detection of blood-related components and pathogens, and the detection of pesticide residues in foods and beverages, biosensors have emerged as innovative detection techniques. (Liu *et al.*, 2018).

4.3.1 Affinity Biosensors:

Compared to the non-specific nanoparticle-based biosensors, inclusion of a bio-recognition element can greatly increase the specificity of the sensor. Consequently, other types of biosensors have been developed and among them affinity biosensors are popular. In affinity biosensors, the sensing is achieved based on the reaction of the bio-recognition element and the target analyte **(**Sadanandom and Napier (2010)**)**. Affinity biosensors can be developed using antibody and DNA as recognition elements.

A. Electrochemical Biosensors:

An electrochemical biosensor consists of two core components: a molecular recognition layer and an electrochemical transducer, which converts biological data into an electrical signal that can be displayed (Ronkainen *et al*., 2010).

This type of biosensor may detect target pathogens in a variety of environments, including air, water, and seeds on platforms such as greenhouses, in-field, and in postharvest storage vessels (Fang and Ramasamy, 2015). In the meantime, the primary premise of DNA-based biosensors is hybridization or hydrogen bonding between a target DNA sequence and a DNA probe sequence immobilized on a sensing platform. A DNA target sequence and a DNA probe sequence that is mounted on a sensing platform establish a hydrogen bond, which is the fundamental working principle of DNA-based biosensors. A DNA probe is a piece of DNA that has a nucleotide sequence unique to an important chromosomal region. Despite the fact that DNA-based biosensors can measure the quantity of pathogens down to a single cell, DNA degrades quickly in the environment, lowering its sensitivity. Therefore, techniques to increase the sensitivity of this class of biosensor have included the development of nano-structured materials with excellent chemical or electrical properties to enrich the target sequences and to amplify the observed signal. These have primarily included gold, silver, or cadmium sulfide nanoparticles with well-developed biological and chemical characteristics.

These serve as substrates for DNA attachment on the sensor surface, boosting the amount of immobilized DNA and acting as signal amplifiers, enhancing accuracy, sensitivity, and speed of diagnosis. The detection of a particular electroactive indicator or the identification of a signal produced by the most electroactive DNA base serves to characterize the hybridization process between the target DNA sequence and the DNA probe. (Asal *et al*., 2018)

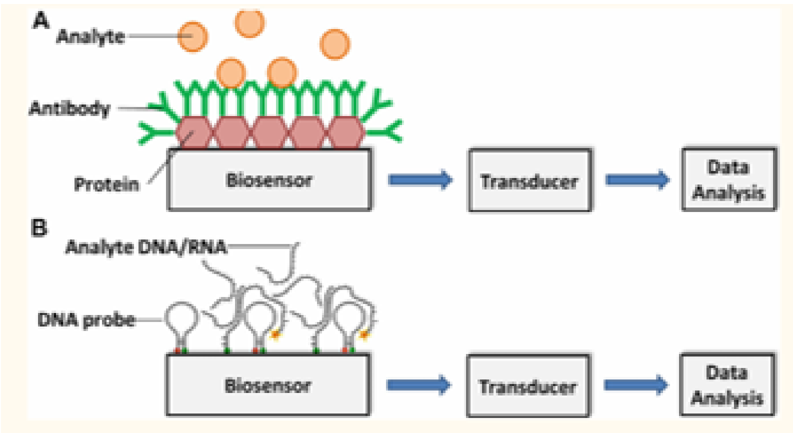

Figure 4.1: Schematic representation of an (A) antibody-based and (B) DNA/RNA-based biosensor for analyte detection. Adapted with permission from Fang and Ramasamy (Fang and Ramasamy, 2015).

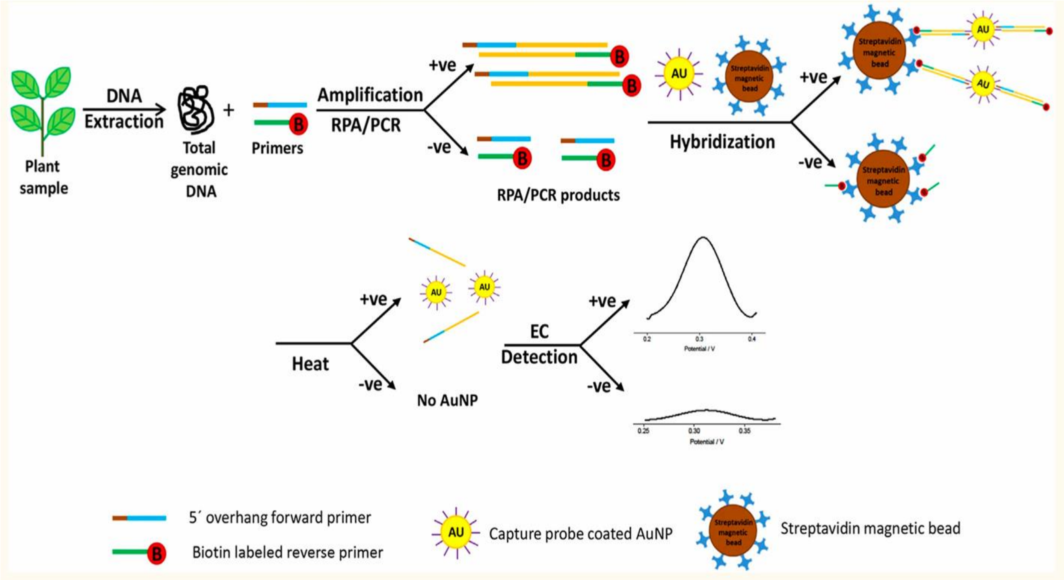

Figure 4.2: Schematic explanation of the DNA based electrochemical bioassay for plant pathogen detection. Adapted with permission from (Lau *et al.*, 2017). EC stands for electrochemical detection and AuNP for gold nanoparticles.

B. Bacteriophage-Based Biosensors:

Bacteriophage is a virus, composed of protein capsid that encapsulates a DNA or RNA genome. It is also emerging as a promising alternative for pathogen detection due to its high sensitivity, selectivity, low cost and higher thermostability [108–110]. Upon the interaction between the bacteriophage and the target analyte, the impedance of charge transfer reactions at the interface changes which is used as a signal for detection. The advantages of using bacteriophage as the recognition element for biosensors are its high selectivity and low cost of the phage. Furthermore, compared to the antibody-based sensor, bacteriophage-based sensors are more thermostable which allows the detection in different temperature ranges and longer shelf life. Bacteriophage-based biosensors are also capable of differentiating the live and dead bacterial pathogens which decreases the false positive signals during measurement. Apart from that, bacteriophage-based sensor can only be fabricated for detection of bacteria rather than fungi and viruses which severely limits its application for the majority of crops that are affected by fungal pathogens.

C. Optical Biosensors:

Optical biosensors measure the interaction between a target analyte and ligand using a light source, an optical transmission medium, an immobilized biorecognition element and a signal detection system. Ultimately, change in amplitude, phase, and frequency of the given light in response to physicochemical conversion (change) generated by the biorecognition process is measured (Ray *et al.*, 2017). Among optical biosensors developed for plant pathogen detection, colorimetric biosensors, fluorescence-based assays-, and surface plasmon resonance-based biosensors are the most common.

Colorimetric biosensors are probably the widest spread tools that allow the user immediate detection of pathogenic microorganisms in the small number of samples just within 10–15 min via a color change. This type of sensors is widely available in the market.

Fundamental principle of fluorescence-based immunoassays relies on the target molecules or antibodies, which are labelled with fluorophores or fluorochrome molecules, producing light during the biological recognition process.

Surface plasmon resonance-based biosensors are predominantly used in optical biosensing techniques with the advantages of label-free, real-time and highly accurate detection (Homola, 2008; Sina *et al*., 2014). The devices contain a sensor chip that is a surface constructed of a metal, such as gold, within two layers comprised of glass and a liquid. The analyte flows over the surface of the chip entering through the bottom or liquid layer and binds with the immobilized ligand that illuminates a light signal that is detectable at a specific angle. The generated signal is then observed with a surface plasmon resonance sensorgram (Damborsky *et al*., 2016).

4.3.2 Biosensor Platforms Based on Nanomaterials:

Nanoparticles display fascinating electronic and optical properties and can be synthesized using different types of materials for electronics and sensing applications. For biosensing application, the limit of detection and the overall performance of a biosensor can be greatly improved by using nanomaterials for their construction. The popularity of nanomaterials for sensor development could be attributed to the friendly platform it provides for the assembly of bio-recognition element, the high surface area, high electronic conductivity and plasmonic properties of nanomaterials that enhance the limit of detection. Various types of nanostructures have been evaluated as platforms for the immobilization of a bio-recognition element to construct a biosensor. The immobilization of the biorecognition element, such as DNA, antibody and enzyme, can be achieved using various approaches including biomolecule adsorption, covalent attachment, encapsulation or a sophisticated combination of these methods. The nanomaterials used for biosensor construction include metal and metal oxide nanoparticles, quantum dots, carbon nanomaterials such as carbon nanotubes and graphene as well as polymeric nanomaterials.

Quantum dots (QD) have also been used for biosensor construction for disease detection (Frasco and Chaniotakis (2009)). Due to their unique and advantageous optical properties, they have been used for disease detection using fluorescence resonance energy transfer (FRET) mechanism (Algar *et al*. (2018)), which describes energy transfer between two light-reactive molecules.

4.4 Conclusion:

Traditional and conventional diagnostic instruments are quickly being replaced by nano biosensing technologies and gadgets. These accessible, quick, highly sensitive, and specialized technologies for plant pathogen detection in the field will soon find widespread use with further optimization for usage in a variety of situations. Utilizing them will probably significantly reduce the frequency and quantity of chemical applications to crops both before and after harvest, as well as the costs associated with on-farm production and the loss of quality and yield due to disease. Multiplexing will be the focus of future research to improve these nano biosensors and allow for the simultaneous detection and surveillance of numerous disease-causing bacteria.

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