



# Utilization of Biosensors in the Identification of Bacterial Diseases in Maize

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Luis Germán López-Valdez, Braulio Edgar Herrera-Cabrera, Rafael Salgado-Garciglia, Gonzalo Guillermo Lucho-Constantino, Fabiola Zaragoza Martínez, Jorge Montiel-Montoya, José Lorenzo Laureano, Luz María Basurto González, César Reyes, and Hebert Jair Barrales-Cureño

## Abstract

Nanotechnology is an emerging technological and scientific breakthrough that can transform agricultural sectors by providing novel tools for the molecular

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L. G. López-Valdez

Laboratorio de Productos Naturales, Área de Química, AP74 Oficina de correos Chapingo, Universidad Autónoma Chapingo, Texcoco, Estado de México, Mexico

B. E. Herrera-Cabrera

Colegio de Postgraduados, Campus Puebla, Santiago Momoxpan, Puebla, Mexico

R. Salgado-Garciglia

Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B-3, Ciudad Universitaria, Morelia, Michoacán, Mexico

G. G. Lucho-Constantino

Instituto Tecnológico de Jesús Carranza, Jesús Carranza, Veracruz, Mexico

F. Z. Martínez

Centro de Investigación y Estudios Avanzados del IPN, Gustavo A. Madero, Ciudad de México, Mexico

J. Montiel-Montoya

Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional Unidad Sinaloa, Guasave, Sinaloa, Mexico

J. L. Laureano · C. Reyes

Universidad Intercultural del Estado de Puebla, Puebla, Mexico

L. M. B. González · H. J. Barrales-Cureño (✉)

Instituto Tecnológico de Estudios Superiores de Zamora, Ingeniería en Innovación Agrícola Sustentable, Zamora, Michoacán, Mexico

e-mail: [hebert.bc@zamora.tecnm.mx](mailto:hebert.bc@zamora.tecnm.mx); [hebert.jair@uiep.edu.mx](mailto:hebert.jair@uiep.edu.mx)

detection of biotic and abiotic stress, and the rapid detection of phytopathogenic diseases. In plants, it has the potential to enhance their capacity to absorb water and nutrients from the soil. Furthermore, nanobiotechnology improves our understanding of crop biology, yields, and nutritional values. The various applications of nanotechnology in agriculture are (1) energy storage, production and conversion (photovoltaic modules); (2) increased agricultural productivity (nanoporous zeolites for prolonged and efficient release of fertilizers); (3) capsules for the specific release of pesticides; (4) the use of biosensors for monitoring the soil quality and plant vitality; (5) pest and phytopathogen detection biosensors; and (6) pesticide biosensors. Nanosensors and intelligent delivery systems based on nano-products are used in the agricultural sector to combat crop pathogens. This nanotechnology seeks to minimize nutrient losses in fertilization and improve crop productivity by optimizing the use of water and nutrients. Nanotechnology provides a wide range of opportunities to produce agro-products based on nanomaterials such as fertilizers, pesticides, herbicides, and nanosensors. These will make it possible to increase the food yield sustainably, reduce the environmental impact and detect infections in plants. This chapter talks about how nanotechnology can be used in plant pathology and how nanomaterials can be used to make biosensors that can detect the main bacterial diseases in maize.

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**Keywords**

Biosensors · Nanobiotechnology · Nanomaterials · Nanoparticles · Nanosensors

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## 14.1 Introduction

*Zea mays* is the third most widely cultivated cereals grain in the world, serving as livestock feed, biofuel, human food, and a raw material in the industry. Its commercial impact exceeds US\$50 billion. A biosensor is an integrated receptor-transducer device structured by a biological recognition element (cell, tissue, receptor, nucleic acid, enzyme, ribozyme, or antibody, among others), or nanomaterials (nanoparticles and nanocomposites), intelligent materials or biomimetic compounds (aptamers, polymers of intrinsic microporosity, and nucleic acid probes), which is associated with a detection mechanism and interpretation of the variation of optical, physico-chemical, and electrical properties, among others, obtained from the interaction between the analyte and the analytical device (Volkov 2000; Turner and Newman 1998). The type of recognition element determines the transducer system, and the physicochemical characteristics of the analyte are determinants for the choice of biological and biometric materials. Biosensors present an analytical approach of greater speed, simplicity, and low economic cost. DNA biosensors based on nucleic acid recognition have applications such as in electrophoresis analysis of amplified DNA. The applications of DNA-based biosensor analysis extend to the field of food control, process control of raw materials, and traceability in industrial processing plants, and in the field of food control, not only for raw materials but also for process

control and traceability in industrial processing plants (Minunni et al. 2005; Mannelli et al. 2003; Bogani et al. 2008). Label-free piezoelectric DNA biosensors present adequate specificity and high sensitivity, allowing rapid and real-time control of DNA hybridisation (Lucarelli et al. 2008; Wu et al. 2007; Sun et al. 2006). Biosensors are designed to detect analytically important molecules such as toxic compounds or pathogens in order to provide reliable, rapid and accurate information about the analyte of interest. Biosensors take part in the important growth of analytical tools useful in the detection of hazardous biological and chemical compounds for health care, food safety and environmental monitoring (Luong et al. 2008; Mascini 2008; Amine et al. 2006). Plant pathogens reduce crop productivity and cause a decrease in food for human and animal consumption. Currently, many methods have been developed to detect crop-dependent phytopathogens of biochemical and molecular types, but they lack speed, reliability, specificity and accuracy, being not suitable for the in situ analysis system. Therefore, there is great interest in developing biosensor systems for early and accurate detection of phytopathogens (bacteria, fungi, and viruses) (Wijesuriya & Rechnitz 1993; Dyussebayev et al. 2021; Ammar 2018).

Climate change and population growth alter agricultural production. Crop engineering is increasingly necessary. Nanoparticle-based biosensors are new tools to advance agricultural practices. As these nanoparticle-based biosensors enter and travel through biofluidic complexes within plants, biomolecules, including proteins, metabolites, lipids and carbohydrates, adsorb onto the surfaces of the nanoparticles, forming a coating known as a “bio-crown”. On the other hand, screen-printed carbon electrodes are adapted to different biorecognition elements, including enzymes, antibodies, and aptamers, often with other modifiers, such as mediators and nanoparticles, to produce electrochemical biosensors for a variety of analytes of importance in agri-food safety. Emphasis is placed on biosensor fabrication strategies and device performance characteristics. In addition to biosensors for a range of analytes in different agri-food matrices, there are also those with potential in agri-food safety (Smart et al. 2020; Voke et al. 2021). Of importance is the high specificity and sensitivity to be able to detect physiological and pathogenic molecules, which offers a useful opportunity in the treatment of plant pathogenic disease with early diagnosis. There is also the optical-based biosensor in which a fibre-optic cable is used in the different investigations. Bacteriophages are ubiquitous viruses found wherever bacteria exist. It is estimated that there are more than 1031 bacteriophages on the planet, more than all other organisms on the earth, including bacteria. In recent years, biosensors have been widely recognized as having several potential applications in the food industry (Nasrullah 2021).

Nano-inspired biosensors have acquired a vital role in improving the quality of life through various botanical and environmental applications worldwide. Several nano-inspired biosensors have been reported, ranging from detection of plant infections (fungal, viral, and bacterial), abiotic stress, metabolic content, phytohormones, miRNAs, and genetically modified (GM) plants to transcriptional and genetically encoded biosensors in a very short time. For in vitro and in vivo measurements, with the existing tools and technologies (such as molecularly

imprinted polymers, microfluidics, plasmonic nanosensors, surface-enhanced Raman scattering (SERS), fluorescence, chemiluminescence, quartz crystal microbalance, and advanced electrochemical measurements), together with customizable nanomaterials or nanocomposites, a potential niche has recently been discovered and is being exploited to make nano-inspired plant-based biosensors. Although the research based on plant-based biosensors has gained momentum very recently, few research results are available (Kumar and Arora 2020). There are new emerging biosensor technologies such as isothermal amplification, nanomaterial detection, paper-based techniques, robotics, and lab-on-a-chip analytical devices. However, these constitute a novelty in research and development of approaches for the early diagnosis of pathogens in sustainable agriculture (Ali et al. 2021).

Both bacterial and fungal diseases can be diagnosed with biosensors because of their potential capacity, real-time detection, and advantages, among other analytical techniques. For example, mycotoxins, which are naturally occurring toxic secondary metabolites produced by fungi, can be determined. Biosensors are effective and efficient for the accurate detection of these toxic molecules in food, combining a biochemical recognition element with a physical transducer (Shrivastava and Sharma 2021).

Plant diseases minimize crop productivity. Another very dangerous plant disease is bacterial stalk rot in maize, which disrupts the flow of nutrients from the primary and secondary roots to other parts of the plant, infecting the inner tissue of the stalk until it rots completely. The disease has been reported to attack maize crops in Asia and Europe. Molecular identification results indicated that this disease is caused by the bacterium *Dickeya zeae* (Patandjengi et al. 2021). The pathogen needs to be identified both in the field and in greenhouse. Current technologies, such as quantitative polymerase chain reaction (Q-PCR), are time-consuming and lack high sensitivity. They require large amounts of target tissue and several assays to accurately identify different plant pathogens. Biosensors are low-cost methods to improve the accuracy and speed of plant-pathogen diagnosis. However, nanotechnology, nanoparticles, and quantum dots (QDs) are essential tools for the rapid detection of a given biomarker with extreme precision. Biosensors, QDs, nanostructured platforms, nanoimaging, and nanopore DNA sequencing tools have the potential to increase the sensitivity, specificity, and speed of pathogen detection, facilitate high-throughput analysis, and be used for high-quality monitoring and crop protection. In addition, nanodiagnostic kits can easily and quickly detect potentially serious and dangerous plant pathogens, allowing experts to assist farmers in the prevention of epidemic diseases (Khiyami et al. 2014; Prasad et al. 2014).

Other biotechnological advances developed are quorum quenching (QQ), which is a technique to control quorum-mediated bacterial pathogens by interfering with population sensing systems, catalysing degradative enzymes, modifying signals, and inhibiting signal synthesis. In many Gram-negative pathogenic bacteria, chemically conserved signalling molecules called *N*-acyl homoserine lactones (AHLs) are studied. AHLs modulate virulence factors in several plant pathogenic bacteria, including *Dickeya zeae*. *Dickeya zeae* is a bacterium that causes plant rot in maize, causing economic crop losses. Zhang et al. (2021) isolated an

AHL-degrading bacterial strain W-7 from samples of *Pseudomonas nitroreducens*. Strain W-7 revealed a superior ability to degrade *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), when it completely degraded 0.2 mmol/L OdDHL in 48 h. By GC-MS, *N*-cyclohexyl-propanamide was identified as the main intermediate metabolite during AHL biodegradation (Zhang et al. 2021).

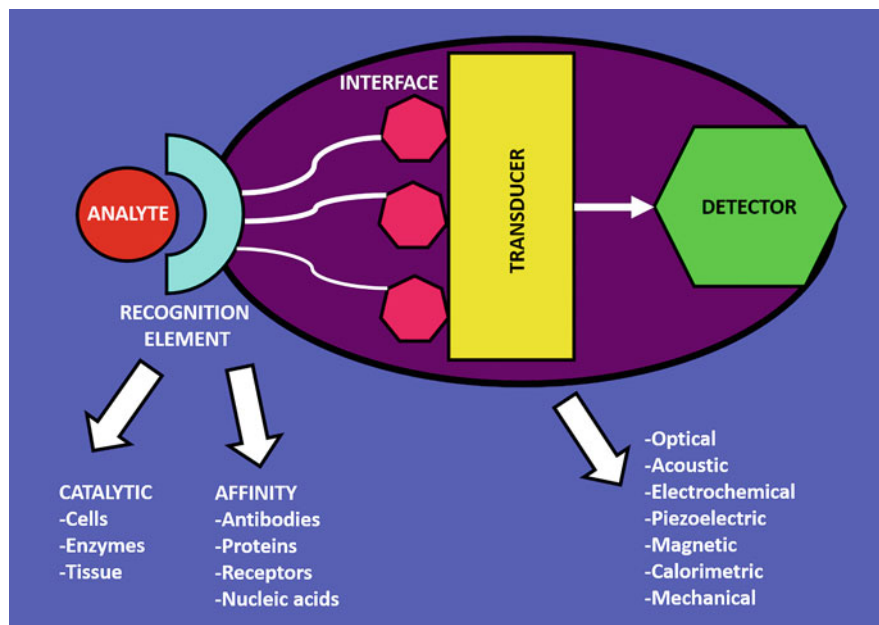
Food safety and security must be ensured for plant pathogenic microorganisms to become a threat to global food consumption. Also, nanomaterials have chemical and physical properties, which are used for high-throughput, non-invasive detection, and as diagnostic techniques for various plant pathogens. The sensitivity and selectivity are currently improved due to the use of engineered nanomaterials corresponding to molecular and sequencing techniques. This is a biotechnological alternative needed for rapid, in situ diagnostics of diseased plants and long-term monitoring of plant health conditions (Li et al. 2020).

Aflatoxin is a carcinogen secreted by fungi and is found dangerously in some food samples. Many detection methods have been developed to determine traces of aflatoxin. Dyussembayev et al. (2021) developed a specific, cost-effective, and simple colorimetric competitive assay method to detect aflatoxin B1 based on the interaction of gelatin-functionalized gold nanoparticles in a specific enzymatic reaction. The results obtained showed that through this approach aflatoxin could be detected in a linear range of 10–140  $\text{pg mL}^{-1}$ , with a detection limit of 4  $\text{pg mL}^{-1}$ . The assay on real saffron samples showed a recovery rate of 92.4–95.3%. The analysis should be efficient and highly sensitive in testing to achieve the best detection of pathogens in food as the limit of detection by analyzing the highest amount of volume. Xu et al. (2019) developed a flow-through immunoelectrochemical biosensor to identify two types of bacteria (*E. coli* O157: H7 and *Salmonella*) in food. The electrode was formed with a porous, antibody-coated graphite felt electrode that served as a solid support coated with biorecognition elements for capturing target pathogens as a signal transducer, and large volumes of the aqueous sample can be rapidly exposed to the solid support through gravity flow (Xu et al. 2019). Therefore, this chapter addresses the applications of nanobiotechnology in plant pathology, as well as biosensor platforms based on nanomaterials to detect the main bacterial diseases in maize.

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## 14.2 Biosensors

A biosensor is a device that measures biological or chemical reaction that detects, records, transmits, and provides specific quantitative or semi-quantitative analytical information from its environment using a specific biological recognition element with a physiological change/process in a biological system, providing specific biochemical interactions or reactions, or uses biological materials to monitor the presence of various chemicals in a substance. According to the International Union of Pure and Applied Chemistry (IUPAC) definition, a biosensor is an analytical device used for sensitive and selective biomarkers for the detection of chemical compounds, usually by optical, thermal, or electrical type signals (McNaught and



**Fig. 14.1** Components of a biosensor

Wilkinson 1997). In most successful biosensors, the principle underlying the determination of a chemical or biological molecule is the specific interaction of that analyte molecule with the biological material present in the biosensor probe device. Figure 14.1 describes the elements of a biosensor.

### 14.3 Mechanism of Biosensors

Biosensors are devices that combine a bioreceptor, and a suitable transducer, which measures the effect produced by the interaction between the substrate and the bioreceptor and transforms it into an electrical signal. Bioreceptors such as tissues, cells, nucleic acids, artificial binding proteins, monoclonal and polyclonal antibodies, as well as enzymes, among others, bind to a specific compound using higher-order structural elements. Depending on the transduction mechanism, biosensors can be classified as electrochemical, piezoelectric, thermal, optical, etc. The overall reaction/interaction of the bioreceptor and analyte is transduced into a signal that is easily quantifiable by the transducer. The biological recognition element is usually in close contact with a transducer, using an additional element located between the recognition element and the transducer corresponding to an interface composed of hybrid, inorganic or organic materials, with the objective of

improving the functionality of the device, either by providing greater stability or by amplifying the signal (Eggins 2002; Bănică 2012; Thévenot et al. 2001).

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## 14.4 Biosensor Types

### 14.4.1 Enzymatic Biosensors

The development of new biosensors has been investigated in a variety of biological materials and transduction methods, such as enzymes immobilized as biological material and electrochemical transducers (Volkov et al. 1998; Volkov and Mwesigwa 2001). One of the alternative applications of enzymatic biosensors is to inspect different pollutants present in the environment in an automated, efficient, fast, and economical way. Oxidative enzymes, such as polyphenol oxidases (laccases and tyrosinases) and peroxidases, are interesting, highly functional and versatile enzymes used as analyte recognition elements in biosensors. With these biosensors, contaminants can be detected, as recognition elements mediate the use of oxidative enzymes and detection of contaminants such as toxic compounds and environmental pollutants: pharmaceuticals, heavy metals, phenols, and pesticides (Patel 2002; Rebollar-Pérez et al. 2020).

The generation of electrochemical sensing and biosensors based on the modification of the working electrode is a suitable tool for quality assurance in the food industry (Table 14.1). Petrlova et al. (2007) reported that the process could be used to determine an avidin-modified carbon paste electrode to determine concentrations up to 3 pm in solution and 170 nM in a corn seed extract.

### 14.4.2 Chemical Biosensors

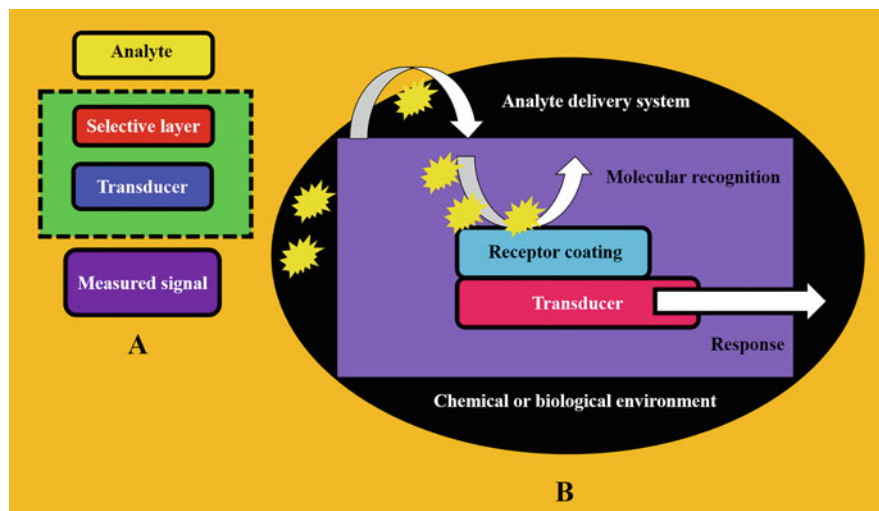
In 1924, Palmer studied the coherence of contact-free thin filaments induced by electromagnetic waves in the presence of different gases and the correlation between the observed responses and the heat of gas absorption. This was one of the first chemical sensors ever recorded (Datskos et al. 2005). A chemical sensor is defined as a physical transducer (transducer of physical quantities into suitable output signals) and a chemically selective layer so that measurable output signals can be produced in response to a chemical stimulus (Datskos et al. 2005; Liawruangrath et al. 2001). In the design of a chemical sensor, molecule-selective coatings can be used, which means that these coatings can be chemically functionalized with compounds that recognize or interact with other chemical molecules of interest for detection or monitoring, such as sensors used for the detection of polluting particles in the environment or in water, to cite some examples. Another relevant aspect of these sensors are the different transduction modes; basically, these can be thermal, mass, electrochemical, and optical (Fig. 14.2).

Chemical sensors have been actively used within the MEMS (microelectromechanical systems) family, especially the simple structures called microcantilevers that have proven to be very useful as transducers of physical,

**Table 14.1** Biosensors applied to evaluate food quality

Analyte	Matrix	Recognition enzyme	Transduction system	References
Glucose	Grape juice, wine, juice, honey, milk, and yogurt	Glucose oxidase	Amperometric	Centonze et al. (1997), Ángeles and Cañazares (2004)
Fructose	Juice, honey, milk, gelatin, and artificial edulcorants	Fructose dehydrogenase, D-fructose 5-dehydrogenase	Amperometric	Bassi et al. (1998), Palmisano et al. (2000)
Lactose	Milk	$\beta$ -galactosidase	Amperometric	Marconi et al. (1996), Palmisano et al. (2000)
Lactate	Cider and wine	Transaminase and lactate dehydrogenase	Amperometric	Silber et al. (1994), Ramanathan et al. (2001)
Lactulose	Milk	Fructose dehydrogenase and $\beta$ -galactosidase	Amperometric	Sekine and Hall (1998)
L-amino acids	Milk and fruit juices	D-amino acid oxidase	Amperometric	Sarkar et al. (1999)
L-glutamate	Soya sauce and condiments	L-glutamate oxidase	Amperometric	Kwong et al. (2000)
L-lysine	Milk, pasta and fermentation samples	Lysine oxidase	Amperometric	Kelly et al. (2000), Olschewski et al. (2000)
L-malate	Wine, cider and juices	Dehydrogenated malate, others	Amperometric	Miertus et al. (1998)
Ethanol	Beer, wine and other alcoholic drinks	Alcohol oxidase, alcohol dehydrogenase, NaDH oxidase	Amperometric	Katrlik et al. (1998)
Glycerol	Wine	Glycerophosphate oxidase and glycerol kinase	Amperometric	Niculescu et al. (2003)
Catechol	Beer	Polyphenol oxidase	Amperometric	Eggs et al. (1997)
Cholesterol	Butter, lard, and egg	Cholesterol oxidase and peroxidase	Amperometric	Akyilmaz and Dinckaya (2000)
Citric acid	Juice and athletic drinks	Citrate lyase	Amperometric	Prodromidis et al. (1997)
Lecithin	Egg yolk, flour, and soya sauce	Phospholipase D and choline oxidase	Electrochemical	Mello and Kubota (2002)





**Fig. 14.2** (a) Schematic representation of a chemical or biological sensor with an output signal in response to the presence of an analyte source or chemical compound of interest. (b) Chemical sensor with a receiver layer that provides a selective response to chemical or biological molecules

biological or chemical stimuli into measurable signals. Sensors based on cantilevers involve measurements of their deflection, resonance frequency, and damping characteristics.

### 14.4.3 Biological Sensors

A biological sensor has an operating principle similar to that of a chemical sensor, but in this case, specific interactions can occur between biomolecules of the functionalized device, with the biomolecules of interest for detection, such as antibody-antigen, enzyme-substrate (biomolecule) interactions, and DNA strand recognition; even microorganism-culture medium or culture medium interactions can occur to carry out the biodetection of the recognition of the biomolecule of interest (Capobianco et al. 2021). These interactions result in the variation of one or more physico-chemical properties (pH, electron transfer, heat transfer, change of potential, mass variations, and variation of optical properties, among others) that are finally detected by the transducer. This system transforms the response of the recognition element into an electrical signal indicative of the presence of the analyte under study proportional to its concentration in the sample or to the growth of the micro-organism (Velasco-García and Mottram 2003). Biosensors can be classified in four different ways (Gonzalez et al. 2005) according to Table 14.2.

In practice, the choice of biological material depends on the characteristics of the compound to be analyzed, and the choice of the transducer is conditioned by the type of element to be recognized, as this determines what variation in physicochemical properties will occur as a consequence of the interaction (Datskos et al. 2005).

**Table 14.2** Biosensors classification

Type of interaction	Characteristic
Between the recognition element and the analyte	Biocatalytic, bioaffinity
Method used to detect such interaction	Direct and indirect
Nature of the recognition element	Enzyme, organelle, tissue or whole cell, biological receptor, antibody, nucleic acids, PNA (peptide nucleic acid), aptamers (single-stranded nucleic acids or chemical antibodies)
Transduction system	Electrochemical, optical, piezoelectric, thermometric, nanomechanical

#### 14.4.4 Mass Biosensors

A mass biosensor is a device capable of detecting the magnitude of mass and transforming this detection into an electrical variable: resistance, capacitance, voltage, current and frequency, among others. Currently, there are systems capable of detecting mass variations in picograms up to sensitivities of 0.18 ag/cm<sup>2</sup>, in commercial devices, at high frequencies (10–15 MHz) (Qsense 2011). Probably the most widely used biosensor with this function is the quartz microbalance (QCM, Quartz Crystal Microbalance or QMB, Quartz microbalance), which achieves an absolute mass resolution of 0.9 ng<sup>2</sup>/cm<sup>2</sup>. Quartz balances are used in chemical reaction monitoring, biomedical biosensors, metal deposition monitoring and environmental control. These systems sometimes allow electrochemical measurements in liquid, known as EQCM (Electrochemical Quartz Crystal Microbalance).

The quartz microbalance works by applying an external electrical potential to a quartz disc with two metal electrodes (usually gold), producing an acoustic wave that propagates through the crystal. This wave encounters a minimum impedance when the thickness of the system is a multiple of half the wavelength of the acoustic wave. The quartz crystal disc must be cut with a specific orientation with respect to the crystalline axes. The deposition of thin layers on the crystal surface decreases the frequency proportionally to the mass of the deposited layer. By detecting the variation in frequency, the deposited mass can be determined (O'Sullivan and Guilbault 1999).

Zhang et al. proposed a system based on a comb microoscillator using parametric resonance amplification with picogram resolution in the air (Zhang and Turner 2005). Ekinici, on the other hand, presents a resonant nano-bridge with magnetic detection that allows absolute mass resolutions in the order of the atogram (Ekinici et al. 2004). This bridge is placed in a perpendicular magnetic field to excite the resonance, and together with the alternating current passing through it, an electromotive force is generated, which is detected through a network analyzer, and the mass changes are known. Devices capable of detecting 7 zeptograms have been designed, taking measurements in ultra-high vacuum and at temperatures below 7 K. Other results from biosensors based on piezoelectric resonant

membranes for biochemical detection indicate that resolutions close to 300 femtograms/Hz can be achieved (Nicu et al. 2005).

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## 14.5 Biosensors to Detect Pathogens

Among the biological sensing components that can be used by optical biosensors are aptamers, which are single strands of DNA or RNA containing include aptamers with a three-dimensional structure, capable of recognizing specific molecules by binding to them (IBIAN 2020; Tombelli et al. 2009). There are several advantages of using aptamers, such as their high affinity and specificity, as they can be synthesized in a customized way (IBIAN 2020), their thermal and chemical stability, their low cost, and, in general, their numerous applications (Song et al. 2012). In plants, optical biosensors have been used for detecting pathogens of agricultural or epidemiological importance, as well as for detecting the presence of substances of interest, including allergens, toxins, and heavy metals (Sadanandom 2010; Michelini et al. 2008). Particularly, aptamer-based biosensors promise to be an ideal technique for the detection of commercially important metabolites, displacing traditional detection methods that can be time-consuming and resource-intensive to perform (Sadanandom 2010; Amini and Saify 2017). An important application of optical biosensors in plant biology is the assessment of the physiological state of a plant according to the content of secondary metabolites present in a given tissue (Coppedè et al. 2017). Secondary metabolites are compounds that play an important role in the interaction of plants with the environment, as their synthesis constitutes a physiological defence response against biotic stress conditions (insect attacks, infections, etc.) or abiotic stress conditions (droughts, extreme temperatures, etc.) (Pagare 2015; Zimdahl 1999; Kumar and Kumar 2018).

### 14.5.1 Biosensor Applications in *Zea mays*

Goron and Raizada (2016), studied more than 1500 maize seedling leaf extracts, which were treated with different N rates under uptake/assimilation systems. In situ imaging allowed demonstrated in all leaves sampled those multifactorial interactions allow Gln accumulation at the position within each leaf. In situ imaging localized Gln in leaf veins for the first time. These authors reported to GlnLux biosensor, which can measure relative Gln levels inexpensively with tiny amounts of tissue.

Liu et al. (2020) designed an electrochemical DNA biosensor based on nitrogen-dropped graphene nanosheets and gold nanoparticle nanocomposites for event-specific detection of the transgenic maize MIR162. This biosensor exhibits high reproducibility of fabrication, high selectivity, and good stability. The response choice they chose to monitor the target DNA hybridization event was methylene blue differential pulse voltammetry. Under optimal conditions, the peak current increased linearly with the logarithm of the DNA concentration in the range of  $1.0 \times 10^{-14}$  to  $1.0 \times 10^{-8}$  M, and the detection limit was  $2.52 \times 10^{-15}$  M. The

biosensor was effectively applied to detect MIR162 in real samples, demonstrating its potential as an effective and efficient tool for transgenic crop identification analysis.

Zeng et al. (2013) reported a biosensor based on Surface Plasmon resonance (SPR) to detect maize chlorotic mottle virus (MCMV). The effects of coupling reaction time and antibody concentration on detection sensitivity indicated that the developed SPR biosensor showed highly specific recognition for both purified MCMV and crude extracts from real-world samples.

Fumonisin is a natural toxin produced by fungi species of the genus *Fusarium*. Fumonisin B1, B2 and B3 (also called FB1, FB2) are found in foods and were discovered in 1988. Fumonisin has health effects on livestock and other animals, contributing to health problems such as cancer or birth defects. The fungi *F. verticillioides*, *F. proliferatum* and *F. fujikuroi* are species that emerge in warm climates and tropical zones, and are the main contaminants of corn. An evanescent wave fiber-optic biosensor, which was competitive for fumonisin B1 and non-competitive for aflatoxin B1 was developed by Maragos and Thompson (1999).

#### 14.5.1.1 Bacterial Detection Biosensors in Maize

Aflatoxin B1 (AFB1) is mycotoxin, carcinogenic, nephrotoxic, and hepatotoxic in humans and animals. Mycotoxins infect maize. Zearalenone is a mycotoxin considered as a xenoestrogen, similar to natural estrogens because it binds to estrogen receptors leading to various reproductive diseases, especially hormone imbalance. ZEN has toxic carcinogenic effects on human health. Valuable electrochemical detection assays based on nanomaterials included several immunodetection studies for the highly sensitive determination of several ZEN families (Sohrabi et al. 2022; Shahi et al. 2021).

Wang et al. (2021) developed an immunochromatographic assay with polystyrene microspheres to detect AFB1 mycotoxin sensitively and quantitatively. The reliability of the microspheres was confirmed with Liquid Chromatography-Tandem Mass Spectrometry.

A wide range of specific biosensors for mycotoxins and bacterial toxins are available for environmental and food control (Guilbault et al. 1993; Carter et al. 1994; Delehanty and Ligler 2002; Palleschi et al. 1997; Tran and Pandey 1992). Boiarski et al. (1996) developed an integrated optical biosensor to analyze aflatoxin B in maize plants to analyze ricin and saxitoxin, based on the impedance of an ultrathin platinum film with an immobilized layer of antibodies against staphylococcal enterotoxin B. On the other hand, Kumar et al. (1994) designed an evanescent wave immunosensors detecting botulinum with ultra-low detection limits while Ogert et al. (1992) obtained a highly specific reaction fiber-optic based biosensor that uses the evanescent wave of a conical optical of a sensitive and rapid immunosensor type to detect *Clostridium botulinum* toxin A by means of a rhodamine label at concentrations of 5 ng/mL.

The technique lateral flow immunoassays are based on gold colloidal nanoparticles for the detection of various plant pathogens, such as potato virus X (Drygin et al. 2012), *Fusarium* species (Xu et al. 2019), and *P. stewartii* subsp. *stewartii* (Pss) bacteria in maize was also detected (Zhang et al. 2014; Feng et al.

**Table 14.3** Biosensors developed for the detection of plant pathogens in *Zea mays*

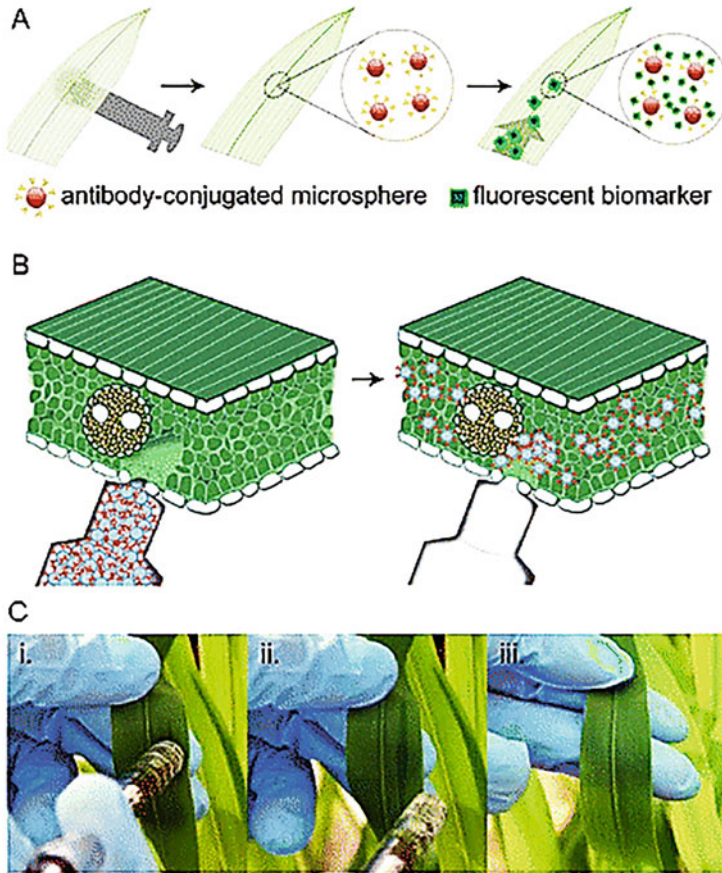
Biosensor type	Bio-recognition element	Technique	Pathogen	Detection limit	References
Optical	Antibody	Lateral flow immunoassay	<i>Pantoea stewartia</i> sbsp. <i>stewartii</i>	538 pg/mL	Feng et al. (2015)
Optical	Antibody	Lateral flow immunoassay	<i>Pantoea stewartia</i> sbsp. <i>stewartii</i>	5.38 pg/mL	Zhang et al. (2014)
Electrochemical	DNA	Quartz crystal microbalance-based detection	Maize chlorotic mottle virus	$2.5 \times 10^5$ pg/mL	Huang et al. (2014)

2015). The causal agent of late blight in potatoes and tomatoes was detected by a combined lateral flow biosensor (Zhan et al. 2018) and integrated asymmetric PCR, mediated by a universal primer (Table 14.3).

Wen et al. (2015) generated a new low-cost and easy-to-use real-time technology with the objective of detecting biotic stress in the field; this system consisted of a lateral flow detection biosensor integrated into a corn leaf, while microspheres conjugated with analyte-specific and concentration-specific capture antibodies are non-invasively injected. In order to achieve infiltration and immobilization in the corn leaf, the size of the microspheres was optimized. In addition, a fluorescent biomarker, fluorescein, is detected in a living corn plant.

Syringe agroinfiltration is a system for introducing genes into host plants using *Agrobacterium* (Chen et al. 2013). It has been successful in several plant species (Wroblewski et al. 2005) because it uses simple equipment. The method consists of filling a needleless syringe with a solution containing *Agrobacterium* and injecting it manually. The tip is positioned on the dorsal side of an intact leaf. A temporary color change from light green to dark green indicates infiltration of *Agrobacterium* into the leaf (Annamalai et al. 2006). Wen et al. (2015) complemented this biosensor technology using a live corn leaf as a lateral flow “test strip”, but injecting and immobilizing antibody-conjugated microspheres in the leaf interstitium (Fig. 14.3).

Detection and identification of plant pathogens are essential to improve crop yields by PCR or ELISA assay, which are time-consuming and destructive to the sample. Raman spectroscopy (RS) is a non-invasive and non-destructive analytical technique to know the chemical structure of the sample. Faber and Kourousky (2018) studied that Raman spectrometer, in combination with chemometric analysis in a stand-alone, portable and sample-independent manner, could distinguish between healthy and diseased maize (*Z. mays*) kernels, as well as in other crops, between different diseases, with 100% accuracy (Faber and Kourousky 2018). Faber and Kourousky (2018) demonstrated that RS can be used to detect and identify plant pathogens in intact maize kernels. These researchers obtained Raman spectra of



**Fig. 14.3** One-step lateral flow detection method of plant-pathogen markers in live maize leaves. Detection of a fluorescent biomarker using antibody-conjugated microspheres (**a**) Detection of non-fluorescent biomarkers by incorporation of stimuli-sensitive colorimetric vesicles, (**b**) Schematic of microsphere infiltration into leaf tissue (left) before infiltration and (right) after infiltration, (**c**) Infiltration into a maize (*Zea mays*) leaf: (i) infiltration with a needleless syringe, (ii) immediately after infiltration, when the injected buffer solution is visible and (iii) 10 min after infiltration, when the injected buffer has evaporated without leaving visible marks. (Source: Wen et al. (2015))

individual maize kernels using a Rigaku Progeny ResQ portable spectrometer equipped with a 1064 nm Nd:YAG laser. These spectra show the average spectra of a healthy corn and the corn infected by the plant pathogenic fungi *Diplodia* spp., *Fusarium* spp., *A. niger*, and *Aspergillus flavus*.

Biosensors are bacterial cells containing a reporter gene (fluorescence marker), such as a green fluorescent protein (GFP) expression cassette (Sorensen et al. 2009). There are a limited number of reporter genes. With this method, using epifluorescent and confocal microscopy, bacterial colonization and activity are detected at the

single-cell level in rhizosphere microsites. Götz et al. (2006) and Germaine et al. (2004) successfully introduced GFP-tagged plasmids to monitor rhizosphere colonization of endophytic bacterial strains as *Pseudomonas putida* PRD16 and *Enterobacter cowanii* strain PRF116. Weyens et al. (2012) investigated the ability and colonization of plant growth promotion by endophytic *P. putida* strain W619 with GFP-tag insertion, without growth promotion. High background fluorescence limits the performance and detection of biosensors as a function of sample preparation and handling.

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## 14.6 Nanosensors

The origin of nanotechnology goes back to research by the American physicist Richard Phillips Feynman, winner of the Nobel Prize in Physics. Important events for the foundation of nanotechnology lie in the 1982 invention of the scanning tunneling microscope by Swiss Gerd Binnig and German Heinrich Rohrer, which made it possible to observe objects on a nanometer scale. In September 2003, the application of nanotechnology in agriculture and the food industry was discussed for the first time at the United States Department of Agriculture (USDA) (Weiss et al. 2006; Alam et al. 2016; Agrawal and Rathore 2014). Nano-sensors are devices that can treat and detect a fungal or bacterial infection, nutrient deficiency, or any other phytosanitation problem, long before phenotypic symptoms appear in plants (Fraceto et al. 2016; Rai et al. 2012). The application of nanotechnology in agriculture and the food industry receives a lot of attention nowadays. Investments in nanotechnology for food and agriculture are increasing due to its potential benefits, which range from improved quality and safety of agricultural inputs to better processing and higher nutritional value of agricultural inputs (Dasgupta et al. 2015). Agricultural scientists face a wide range of challenges such as stagnant crop yields, climate change, multi-nutrient deficiencies, low macro- and micro-nutrient use efficiency, reduced availability of arable land, declining soil organic matter, and a shortage of water and labor for the field (Shiva, 2016). Recent research on the use of nanotechnology in plants shows that the incorporation of synthetic nanoparticles can increase photosynthesis and transform leaves into biochemical sensors. The single-walled carbon nanotubes (SWNTs) coated with single-stranded DNA infiltrate the lipid envelope of extracted plant chloroplasts and assemble with photosynthetic proteins. The same occurred when SWNTs were released into the living leaves of *Arabidopsis thaliana* through the stomata. The researchers demonstrated that photosynthetic activity was three times higher in SWNT-containing chloroplasts than in controls due to increased light capture by the photosynthetic molecules. The use of nanotechnology allows the development of potential techniques for disease management in crops. Nanoparticles can be used in the preparation of new formulations such as insecticides, fungicides, insect repellents, and pheromones, which is made possible thanks to the new properties of these materials, such as their reactivity, quantum effects, and electrical conductivity.

## 14.7 Nanobiosensors

These biosensors have a huge impact on precision agriculture methods. Nanotechnology allows monitoring to be done in real time where biosensors are linked to GPS systems. These biosensors monitor the soil conditions and crop phenological status over large areas of land (Nair et al. 2010). Some commercial biosensors use plant redox enzymes. For example, superoxide dismutase is used to assess antioxidant activity and tyrosinase (monophenol monooxygenase) to monitor phenolic contamination. The enzyme laccase is used to monitor the presence of flavonoids in foods. Some biosensors such as electronic noses are used to analyze volatile organic compounds from diseased and healthy plants in crops such as potatoes and tomatoes.

The work of Pérez and Rubiales (2009) highlighted that nanotechnology is opening new potential applications for agriculture, which are already being explored. These authors also point out the potential of nanotechnology to develop nanodevices and nano-transporters to be used as smart systems to target specific chemical emission sites in plants.

Nanometer gold with sizes from 5 to 25 nm is used to deliver and incorporate DNA into plant cells, while 30 nm iron oxide was used in nano-sensors to detect pesticides at very small concentrations. These functions aid the development of precision agriculture, minimizing contamination and allowing maximizing sustainable agricultural practices (Malsch et al. 2015; Subramanian et al. 2015). N toxicity can be attributed to the following two actions: (1) chemical toxicity based on the release of toxic ions; (2) stress or stimuli caused by surface area, particle size, and/or shape. NPs oxide solubility has been confirmed to significantly affect plant response.

In the studies of Zhang et al. (2014), the phytotoxicity of ZnO NPs on the germination of maize (*Zea mays L.*) and cucumber (*Cucumis sativus L.*) seeds was investigated. Regarding root elongation, all seedlings were affected when exposed to a concentration of 1000 mg L<sup>-1</sup>. On their side, research by El-Temsah and Joner (2012) determined the phytotoxic potential of iron (Fe) NPs, using three types of particle sizes in the range of 1–20 nm, in the seed germination of barley and flax species.

Researchers at Iowa State University have used 3 nm-sized mesoporous silica (MSN) NPs as carriers and for the delivery of DNA and chemicals inside isolated plant cells. The MSN NPs are chemically coated and serve as gene containers that are then applied to the plants. This coating causes the plant to take up the particles through the cell walls and membranes where they are inserted, activating the biological genes in a precise and controlled manner without causing any toxic side effects afterwards. This technique has been successfully applied to introduce NPs into pumpkins and DNA into tobacco and corn plants (Corredor et al. 2009).

Silver can be integrated into inert materials, such as zeolite, silicate, and clay. Silver zeolite (Ag-zeolite) is produced by replacing Na ions in the zeolite with Ag ions; it is one of the most widely used antimicrobial agents, as it is a broad-spectrum antimicrobial agent that kills bacteria, yeasts, and mycelia, but not the spores of heat-resistant bacteria. Ag-zeolite incorporated into chitosan film shows strong antimicrobial activity against Gram-positive and -negative bacteria. Nanocomposites such



as silver silicate have been produced using a flame spray pyrolysis process and incorporated into polystyrene. This complex showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. A green synthetic approach for the preparation of antimicrobial silver nanoparticles has been suggested, by using carbohydrates from sucrose, or waxy and soluble corn starch (*Zea mays* L.).

Carbohydrates act as reducing agents and as a template for the realization of silver nanoparticles with excellent antibacterial activity.

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## 14.8 Carbon Nanotubes

In the agri-food sector, water intake, crop growth rates, and uptake of essential nutrients are enhanced by the use of multi-walled carbon nanotubes (Scrinis and Lyons 2007). One of the functions of carbon nanotubes is the promotion of plant growth without any inhibitory, toxic or adverse effects on plants (Srilatha 2011). Rameshaiah et al. (2015) have reported that a concentration of 50  $\mu\text{g mL}^{-1}$  with multi-walled carbon nanotubes increased the root and shoot length, and improved the seed germination time and growth of crops such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), groundnut (*Arachis hypogaea* L.), and garlic (*Allium sativum* L.).

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## 14.9 Conclusions

Corn (*Zea mays* L.) is a crop of great importance that is exposed to factors such as the presence of disease-causing phytopathogens, which limit the maximum expression of its productive potential. Nanosensors can prevent the spread of diseases between crops by non-destructively detecting the presence of plant pathogens before symptoms appear.

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