

Great potential for biosensor-based technology in diagnosing seed-borne pathogens

**Jameel Akhtar*, Pardeep Kumar, Raj Kiran, Meena Shekhar, Sadhna and
Sunil Chandra Dubey**

ICAR-National Bureau of Plant Genetic Resources, New Delhi-110 012

Protein-based immunological and DNA-based polymerase chain reaction methods have been developed for diagnosis of plant pathogens but their utility is limited for certain reasons. Biosensor-based methods are now available as a potential alternative, which are capable to quickly and accurately identify pathogens. Although biosensor-based methods are widely used in different areas such as food-borne microorganisms, Escherichia, Staphylococcus, Salmonella and Listeria, adulteration of foods, estimation of blood sugar and cholesterol, determination of pregnancy in women, etc., but for assessing the health of seeds and plants, it is not in vogue. It is clear from other field's examples that biosensor-based techniques are able to quickly and accurately identify plant pathogens and can also play a vital role in providing disease-free quality seeds to researchers and farmers. Considering the efforts made in science, increasing expectation level of experimentation and progress, it seems that biosensor-based technologies will prove to be successful in future in the field of plant protection, mainly in disease diagnosis.

Keywords: Biosensor, Diagnosis, Fungi, Seed-borne pathogens

DISEASE-free and quality seeds are considered as the most important unit for the success of crop-based agricultural research as well as farming. Therefore, it is mandatory to ensure the health of seeds through seed health test. Conventional methods of plant pathology that are largely used to identify seed-borne pathogens include visual examination, washing test, NaOH seed soaking, embryo counting, blotter test, culturing and plating, isozyme analysis, grow out test, etc. These techniques/methods of pathogen detection are considered to be more time consuming and laborious as they have been proven to be insufficient to meet the demands of rapid diagnosis.

At present, along with the demand, we need technologies that are rapid, sensitive and selective. As such, there are many biosensors-based techniques having such

characteristics with success in analysing various components of daily life such as blood sugar and cholesterol assessment, determination of conception in women, adulteration of food, etc., and identifying food-borne microorganisms, *Escherichia*, *Staphylococcus*, *Salmonella* and *Listeria*. But the operation of biosensor-based detection techniques to assess seed and plant health is negligible. The most widely used technologies for health monitoring in plants at present are highly expensive, labour-intensive and time consuming. PCR-based molecular techniques are used to identify plant pathogens that require extensive processing of specimen which takes time. Research findings show that biosensor-based technologies are able to quickly and accurately identify plant pathogens and can also play a vital role in providing disease-free and quality seeds to researchers and farmers.

Biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrode, thermal or optical signals. Any high-quality biosensor must have at least 4 important basic features:

Linearity: Where the linearity is high to detect high substrate concentrations.

Sensitivity: Which is the value of the electrode response to the substrate concentrations.

Selectivity: Least interference in the chemical reaction.

Response Time: At least 95% of the response time required to ensure.

Some significant achievements in the field of biosensor technology during 1980s to 1990s have been made like commercial glucose biosensor, bedside artificial pancreas, blood glucose biosensor, SPR-based

biosensor and currently, various biosensors based on Quantum dot, Nanoparticle, Nanowire, Carbon nanotube etc.

BIOSENSOR TECHNOLOGIES

The biggest advantageous aspects of biosensors are those that are able to rapidly provide users with instant information for both field and laboratory analysis, and biosensor-based equipment can also be easily moved from place to place. Biosensor-based technologies have been proven useful in the identification of various plant disease-causing fungi, bacteria and viruses (Table 1), but are limited. Biosensor-based technologies that operate on different principles is given in Table 2 with a brief explanation of their basic category/type.

Optical biosensor

An optical biosensor is a compact analytical device in which a biological sensory element is integrated or connected to an optical transducer system. Optical biosensors generally rely on an enzyme system that converts catalysts into products that are oxidized or reduced at electrodes that can remain stable with their specific potential. Optical biosensors are a powerful alternative to traditional analytical techniques, particularly due to their high size and sensitivity, as well as their small size and low cost, and the electrodes used in it are biodegradable. In the food industry, various types of optical biosensors have been developed only in the last decade for quick identification of pathogens and toxins/contaminants. Optical biosensors are developed mainly on three principles, fluorescence-based, chemiluminescence-based and SPR-based technologies. With the use of green fluorescent protein (GFP) in fluorescence-based biosensors, identification of a number of fungal pathogens including *Phytophthora palmivora*, *Ustilago maydis*, *Colletotrichum lindemuthianum*, *Aspergillus nidulans* and *Cochliobolus heterostrophus* have become possible. The identification of a fungus, *Brettanomyces bruxellensis* also from extremely low amount of DNA (12.5

Table 1. Biosensors based on various principles to identify pathogens

Pathogen	Biosensor type	Detection limit
Optical Biosensor		
<i>Escherichia coli</i>	Fluorescence labelled antibody (FLA)	1×10 ³ cfu/ml
Potato virus X	Lateral Flow Immunoassay (LFIA)	2 ng/ml
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	LFIA	1×10 ⁵ cfu/ml
Chilli vein-banding mottle virus	FLA	1 ng/ml
Watermelon silver mottle virus	FLA	20.5 ng/ml
Melon yellow spot virus	FLA	35.3 ng/ml
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	FLA	1×10 ³ cfu/ml
<i>Acidovorax avenae</i> subsp. <i>citrulli</i>	FLA -DNA	6×10 ⁵ cfu/ml
<i>Botrytis cinerea</i>	FL-DNA Microarray	1 fM
<i>A. avenae</i> subsp. <i>citrulli</i>	SPR	0.48 nM
<i>Cymbidium</i> mosaic virus	SPR	48 pg/ml
<i>Odontoglossum</i> ringspot virus	SPR	42 pg/ml
<i>Pseudomonas syringae</i>	Flocculation - DNA	0.5 ng/μl
<i>P. syringae</i>	Flocculation - DNA	15 ng/ml
Banana bunchy top virus	F-DNA	0.13 nM
<i>B. streak</i> virus	Electroluminescence DNA (EL-DNA)	50 fM
<i>B. bunchy</i> top virus	(EL-DNA)	50 fM
Piezoelectric biosensor		
<i>Candida albicans</i>	Quartz crystal microbalance (QCM)	1×10 ⁶ cfu/ml
<i>Escherichia coli</i>	QCM	1×10 ⁶ cfu/ml
<i>Salmonella</i>	QCM	1×10 ⁶ cfu/ml
<i>Staphylococcus epidermis</i>	QCM	1×10 ² cfu/ml
Electrical Impedance		
<i>S. aureus</i>	Bulk acoustic wave impedance (BAWI)	1×10 ⁶ cfu/ml
<i>Proteus vulgaris</i>	BAWI	3×10 ² cfu/ml
<i>Neisseria meningitidis</i>	Potentiometric Immunosensor	
	Light-addressable potentiometric sensor assay (LAPS)	1×10 ³ cfu/ml
<i>Brucella militensis</i>	LAPS	6×10 ³ cfu/ml
<i>Francisella tularensis</i>	LAPS	3×10 ³ cfu/ml
Electrochemical biosensor		
Cucumber mosaic virus	Enzyme linked	0.5 ng/ml
<i>P. stewartii</i> subsp. <i>stewartii</i>	Enzyme linked	7.8×10 ³ cfu/ml
Plum pox virus	Impedance spectroscopy (IS) based	10 pg/ml
Maize chlorotic mottle virus	IS-based	250 ng/ml
<i>P. pox</i> virus	DNA Hybridization (DNAH-based)	12.8 pg/ml
<i>Trichoderma harzianum</i>	DNAH-based	10 ⁻¹⁹ Mol/l
Sugarcane white leaf disease	DNAH-based	4.7 ng/μl

Source: Ivnitski D, Hamid I A, Atanasov P and E Wilkins. 1999. *Biosensors and Bioelectronics* 14: 599-624; Khatera M, Muñiza A E and A Merkoçi. 2017. *Biosensors and Bioelectronics* 93: 72-86.

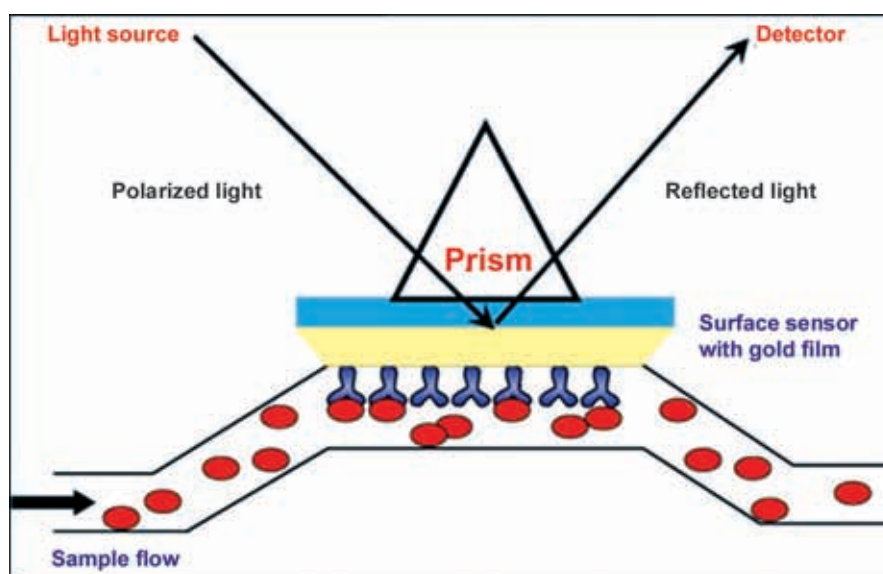


Fig. 1. Mechanism of SPR biosensor.

Source: Ray M, A Ray, S Dash, A Mishra, KG Achary, S Nayaka and S Singh. 2017. *Biosensors and Bioelectronics* 87: 708-723.

Table 2. Biosensors for identification of plant pathogenic fungi.

Biosensor technique	Pathogen	Analysis time	Detection limit
Chemiluminescent DNA optical fibre sensor	<i>Brettanomyces bruxellensis</i>	90 min.	12.5 ng/μl
Luminol chemiluminescent assay	<i>Saccharomyces cerevisiae</i>	10 min.	1.1×10 ³ cfu/ml
	<i>Candida maltose</i>		0.3×10 ³ cfu/ml
	<i>Yarrowia lipolytica</i>		9.0×10 ⁻³ cfu/ml
	<i>Hansenula anomala</i>		0.5×10 ³ cfu/ml
SPR based biosensor	<i>Phakopsora pachyrhizi</i>	90 min.	800 ng/ml
	<i>Fusarium culmorum</i>	-	0.06 pg/μl DNA
Alkane thiolate self-assembled monolayer/gold based impedimetric biosensor	<i>S. cerevisiae</i>	60 min.	1.0×10 ³ cfu/ml
Electrochemical impedance spectroscopy	<i>P. pachyrhizi</i>	8 min.	385 ng/ml
Piezoelectric immunosensor	<i>Candida albicans</i>	30 min.	1×10 ⁶ cfu/ml
Molecularly imprinted polymers-coated QCM Sensor	<i>S. cerevisiae</i>	-	1×10 ⁴ cfu/ml
Micromechanical cantilever array sensor	<i>Aspergillus niger</i>	4 h	1×10 ³ cfu/ml
	<i>S. cerevisiae</i>	-	1×10 ³ cfu/ml
Paper-based immunosensor	<i>P. pachyrhizi</i>	-	2.2 ng/ml
Lateral flow device (LFD)	<i>Phytophthora</i> sp.	3 min.	-
Microfluidic microarray device	<i>Botrytis</i> sp.	3 min.	0.2 ng/μl DNA
FRET-based quantum dot immunosensor	<i>Phytophthora</i> sp.	5 min.	10 ³ cfu/ml
Microfluidic immunosensor with micro-magnetic beads coupled to carbon-based screen-printed electrodes	<i>Botrytis cinerea</i>	40 min.	8 mg/ml

Source: Ray M, A Ray, S Dash, A Mishra, KG Achary, S Nayaka and S Singh. 2017. *Biosensors and Bioelectronics* 87: 708-723.

ng/μl) via a chemiluminescence-based biosensor indicates that these biosensors are the most sensitive.

Piezoelectric biosensor

This biosensor is a device that is also able to detect very small amount of analytes according to the linear relationship between the collected material and its frequency response. Therefore, piezoelectric biosensor is

an effective alternative to optical sensors. Surface plasmon resonance spectroscopy (SPR) and interferometry are successful examples of this, which are one of the excellent methods to determine contamination.

Surface plasmon resonance biosensors

It is a powerful, label-free technique for real-time monitoring of

non-covalent molecular interactions in a non-invasive style. As a label-free test method, SPR does not require any tags, dyes or special reagents, such as enzyme-substrate mixtures to obtain visible or fluorescence signals and its mechanism is shown in Fig. 1.

Quartz crystal microbalance biosensors

This biosensors sensor consist of a thin quartz crystal disc with electrodes plated on it. It is use for plant disease detection where a quartz disc is coated with pathogen-specific antibodies/ nucleic acids/ receptors/ small molecules, etc. depending on analyte to be detected. These methods include bulk acoustic wave (BAW), quartz crystal resonance sensors (QCRS), and thickness shear mode (TCM), which are both qualitative as well as quantitative.

Electronic nose

The identification of pathogens is ascertained via detection of organic volatile compounds (VOCs) through this device. To understand the mechanism of this device, Figure 2 is shown. Currently, some commercial products of electronic nose, such as Cyranose 320 and Bloodhound ST214 are available overseas and their details are given in Table 3.

Looking at Table 3, it appears that all biosensors which are commercially available in the international market to identify plant diseases worldwide are either antibody based or DNA based. It indicates that both DNA and antibody have been established as good biosensing receptors in

Table 3. Commercial products of biosensors/kits for identification of plant pathogens

Manufacturer	Product	Principle	Pathogen
BIOREBA	AgriStrip	Lateral Flow Immuno-chromatography	<i>Spongospora subterranean</i>
Pocket Diagnostics	Pocket Diagnostics kits	Lateral Flow Assay	<i>Phytophthora</i>
Neogen Corporation	Alert test kits	Lateral Flow Assay	<i>Pythium, Phytophthora, Rhizoctonia</i>
Agdia	ImmunoStrip	Lateral Flow Immuno-chromatography	<i>Pythium, Phytophthora</i>
La Chandra Bioscience	Pocket Diagnostic test kits	Lateral Flow Technology	<i>Pythium, Phytophthora, Rhizoctonia solani, Botrytis</i>
Cepheid	SmartCycler	Real Time PCR	<i>Aspergillus, Phakospora</i> sp.
Fera	Portable PCR	ERT PCR	<i>Phytophthora ramorum</i>
Ahram Biosystems	Palm PCR	ERT PCR	<i>Aspergillus</i>
Electronic Sensor Technology	zNose 4200, 4300, 7100	E-nose	<i>Oidium neolycopersici</i>
Sensigent	Cyranose 320	E-nose	<i>Botrytis cinerea, Colletotrichum gloeosporioides, Alternaria</i> sp.
Scensive Technologies Ltd.	Bloodhound ST214	E-nose	<i>Oidium neolycopersici</i>
Texas Instrument	Spreeta™ Biosensor	SPR	<i>Fusarium culmorum</i>

