SEMINAR REPORT

Biosensors for plant pathogen detection

By

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CERTIFICATE

This is to certify that the seminar report entitled **"Biosensors for plant pathogen detection"** has been solely prepared by **Aswathi Prabhakaran (2018-11-002)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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DECLARATION

I, Aswathi Prabhakaran (2018-11-002) declare that the seminar entitled **"Biosensors for plant pathogen detection"** has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

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CERTIFICATE

This is to certify that the seminar report entitled **"Biosensors for plant pathogen detection"** is a record of seminar presented by **Aswathi Prabhakaran (2018-11-002)** on 21th November, 2019 and is submitted for the partial requirement of the course MBB 591.

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1. Introduction

Crop production for consumption and animal feed constitute a financially very important area worth billions of Euro. It is estimated that 10% of crops are lost to plant disease worldwide each year, which can lead to considerable financial losses for farmers, but also to social concerns in underdeveloped countries where some areas are entirely dependent upon specific crops (Strange and Scott, 2005). Therefore, detailed knowledge of emerging disease outbreaks is of paramount importance, so that control measures, such as crop spraying, can be initiated.

Plants display different symptoms on leaves, stems and fruits due to plant disease infections (López *et al*., 2003; Al-Hiary *et al*., 2011). These symptoms are particularly useful for visual observation as a conventional first step for plant disease diagnosis but it fails in detecting the presence of pathogen in early infection stages when plant infections are symptomless.

Early detection of plant pathogens plays an important role in plant health monitoring. It allows to manage disease infections in greenhouse systems and in the field during different stages of plant disease development and also to minimize the risk of the spread of disease infections. Many strategies have been widely used for diagnosing plant diseases including DNA-based methods such as Polymerase Chain Reaction (PCR) and immunological techniques such as Enzyme-Linked Immunosorbent Assay (ELISA), for the detection of pathogen nucleic acid and protein extracted from infected plant materials, as direct laboratory based techniques in addition to visual inspection of plant symptoms in the field (López *et al*., 2003). Immunoassay technology using monoclonal antibodies offers a high specificity for plant virus detection, being ideal for testing large scale plant samples and for the on-site detection of plant pathogens, as done with tissue print ELISA and LF devices. In contrast, nucleic acid based methods are more accurate and specific enough to detect single target pathogen within a mixture containing more than one analyte and highly effective for detection of multiple targets (Khater *et al*., 2017).

In spite of these advantages, molecular detection methods have some limitations in detecting pathogens at low titres in materials such as seeds and or at early infection stages. Furthermore, false negative results can be produced from cross contamination with PCR reagents which completely block amplification of target DNA, while false positive results can be generated by cross-amplification of PCR-generated fragments of non-target DNA. Another limitation is related to the disability to apply PCR for plant pathogen detection in the field (Louws *et al*., 1999; Schaad and Frederick, 2002; López *et al*., 2003; Martinelli *et al*., 2015).

To overcome such limitations, there is a need for developing cost effective and portable biosensors for rapid and early detection of plant diseases with high sensitivity and specificity.

2. Biosensors

Molecular recognition is a very important step for every bio-sensor device. It can be referred to as the centre of bio-sensing (Madufor *et al*., 2018). A biosensor can be defined as a compact analytical device or unit incorporating a biological or biologically derived sensitive 'recognition' element integrated or associated with a physiochemical transducer (Turner *et al*., 1987). Biorecognition element is a sensing material and may include enzymes, antibodies, microorganisms, tissues, organelles, DNA, and RNA.

Fig. 1: Biosensor

3. Components of biosensors

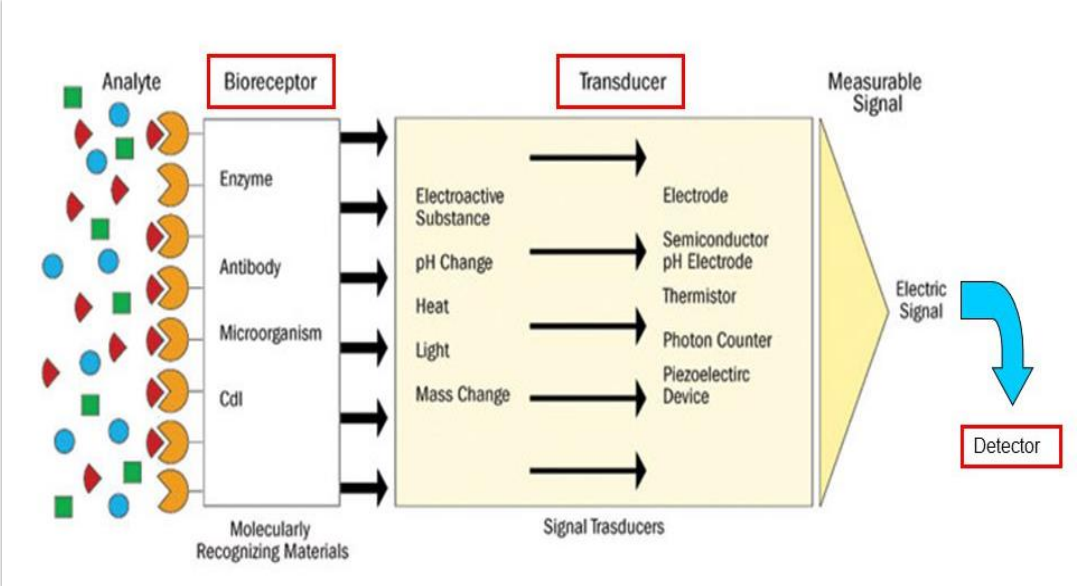

Fig. 2: A simple representation of a biosensor

Biosensors are analytical devices which combine a biological recognition ligand with physical or chemical signaling devices (transducers) (Miso *et al*., 2013) and a detector. A biorecognition element specifically identifies & interacts with an analyte and changes in its physicochemical properties (optical, thermal, electrical and thermodynamic) are converted to signal by a transducer. Sometimes the analyte is converted to a product which could be associated with the release of heat, gas (oxygen), electrons or hydrogen ions. The recorded biomolecular interactions are transformed into digital signals which are interpreted by a computer-aided readout, thereby providing the user with a representation of the interaction that occurs between the bound (ligand) and free (analyte) entities (Byrne *et al*., 2009).

4. Classification of biosensors

The biosensors can be classified based on the type of bio-recognition element or the transducing method used. It can be grouped as enzyme sensors, immunosensors, nucleic acid sensors, and whole-cell sensors based on the recognition element, and into optical, electrochemical and piezoelectric biosensors based on the transducing method used (Arora, 2013; Srinivasan *et al*., 2015), as shown in the figure 3 (Chen *et al*., 2018).

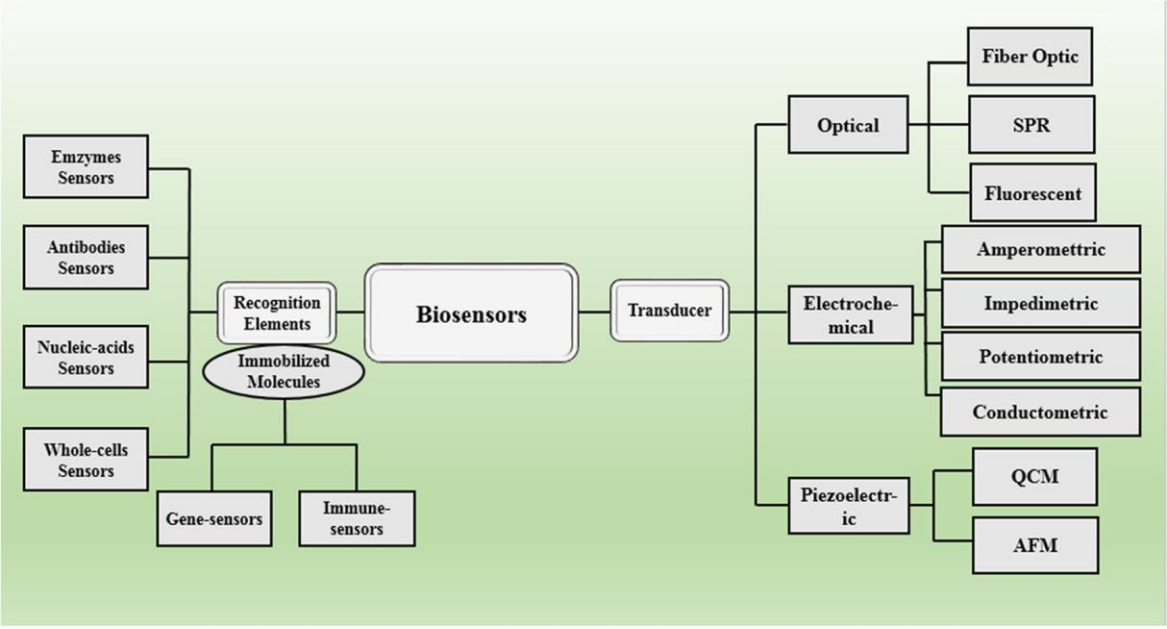

Fig. 3: The different categories of biosensors

Recognition element based classification

1. Enzymatic sensors

Biosensors that work based on the relationship between an enzyme and its substrate are referred to as enzymatic biosensors. This type of biosensors work on two main mechanisms depending on target analyte; substrate detection and enzyme inhibition. Substrate detection mechanisms are based on the conversion of the substrate by an enzyme incorporated in the biosensor. On the other hand, the working principle of inhibition based enzymatic biosensors (IBEBs) lies in the ability of the target analyte to reduce enzymatic activity (Asal *et al*., 2018).

2. DNA-Based Biosensor

The principle of these widely used sensors lies in the hybridization process through spontaneous hydrogen bonding between the target DNA and its complementary strand.

3. Antibody-Based Biosensor

The working principle is based on detecting, processing and displaying the signal caused by the formation of an antibody-antigen (Ab-Ag) complex.

4. Whole-cell based biosensors

Cells consist of naturally evolved receptor, ion-channels, and enzymes that can be used as targets for biological or biologically active analytes. Thus, whole cell-based biosensors are able to measure functional information and the effects of the analyte on the physiological function of living cells.

Transducer based classification

1. Optical biosensors

An optical biosensor transduction process induces change in phase amplitude or frequency of input light in response to the physical or chemical changes during the biorecognition process.

2. Electrochemical biosensors

An electrochemical biosensor consist of an electrochemical transducer coupled with a biological recognition element (biochemical receptor), which functions to convert a binding biomolecule into an electrical signal.

3. Piezoelectric biosensors

Piezoelectric biosensors operate on the principle that a change in mass, resulting from the biomolecular interaction between two entities can be determined.

5. Biosensors in plant pathogen detection

Among the different types of biosensors based on the recognition element used, the antibody-based and DNA based biosensors are most commonly used in plant pathogen detection.

5.1. Antibody based biosensors

These are called as immunosensors. In these sensors antibodies are immobilized through covalent interactions by introducing functional groups such as carboxyl, amino, aldehyde, or sulfhydryl. The principle lies in detecting, processing and displaying the signal caused by the formation of an antibody-antigen (Ab-Ag) complex among the different immunoassay binding configuration used, commonly used one is the sandwich structure formation (Asal *et al*., 2018). These allow sensitive and rapid qualitative and quantitative analysis of pathogens.

Zhao *et al*., (2014) developed a dual amplified electrochemical immunoassay for highly sensitive detection of *Pantoea stewartii* subsp. *stewartii (PSS)*, utilizing the favorable conductivity and large specific surface area of gold nanoparticles (AuNPs) and the excellent catalytic ability of the enzyme horse radish peroxidase (HRP). With a sandwich enzyme-based immunoassay format, the limit of detection (LOD) for *PSS* detection was down to 7.8×10^3 cfu/mL, which increased the detection sensitivity by 20-fold compared with conventional ELISA. The biocompatible recognition and amplified signal provides a useful way to fabricate nanomaterials-driven electrochemical immunosensors for the highly sensitive and multiple plant pathogenic bacteria detection, which were critical on the way for achieving on-site monitoring of maize. Firstly the capture antibody (C-Ab) was immobilized on the glassy carbon electrode surface, then the sample containing the pathogen was added, this resulted in the formation of Ab-Ag complex. The detection antibody (D-Ab) labelled with HRP was added using AuNPs as carriers for the signal amplification and the enzyme was responsible for the development of a signal depicted as the voltametric curve.

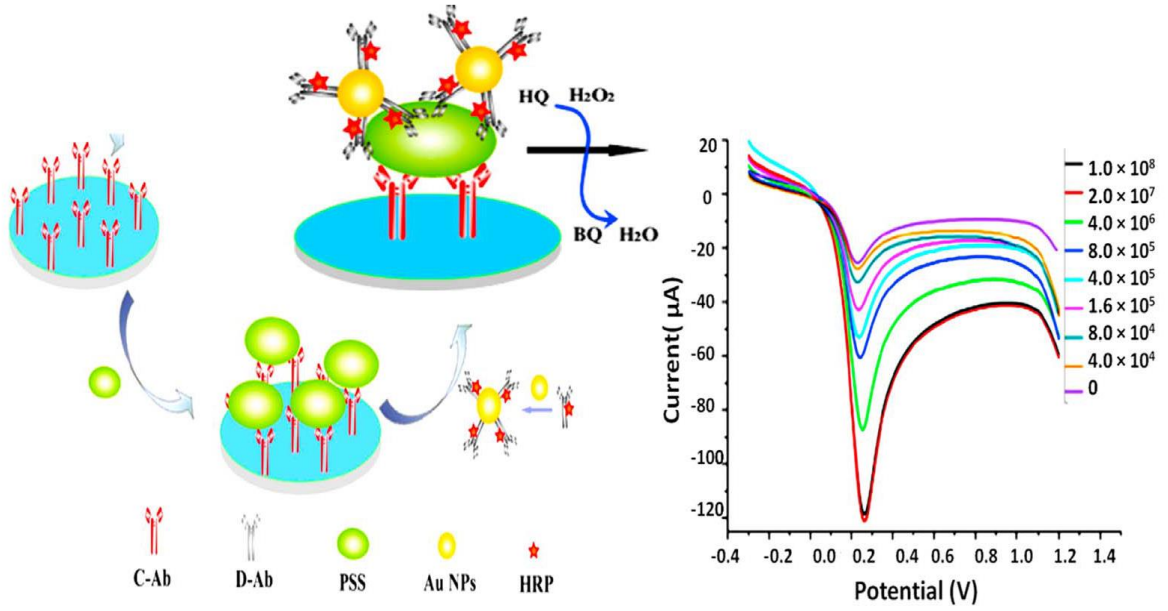

Fig. 4: Example of electrochemical enzyme-linked immunoassay (ECEIA) sensor applied for *Pantoea stewartii* subsp. *stewartii* (PSS)

5.1.1 Nanobiosensors

Biosensors based on nanomaterials are known as nanobiosensors. The nanomaterials used for biosensor construction include metal and metal oxide nanoparticles, quantum dots, magnetic nanoparticles, carbon nanomaterials such as carbon nanotubes and graphene as well as polymeric nanomaterials. Gold nanoparticles are widely used nanomaterials due to their high electroactivity and electronic conductivity (Cao et al., 2011)

Efforts were made in order to develop nanobiosensors in the Centre for Plant Biotechnology and Molecular Biology (CPBMB), College of Horticulture and Centre for Electronics and Materials(C-MET), Thrissur during the academic year 2014-2016.

Saurav (2016) developed an antibody based nanobiosensor for quick detection of Banana bract mosaic virus (BBrMV). Goldnanorods (GNRs) were fabricated through seed-mediated procedure. In order to detect the analyte (BBrMV), surface of a GNRs activated with complete replacement with alkalithiol molecule for covalent attachment of an antibody. Due to addition of BBrMV antigen to antibody labeled GNRs solution, colour of the solution changed from red to black and notable peak shift of (7- 25) nm was observed in peak of GNRs in UV-Vis Spectra. Antigen concentration up to 0.25 mg/ml and above showed stability in the peak shift and colour change in infected sample compared to control sample. In healthy sample no colour changes were observed and only minimum peak shift was there. The accuracy and

sensitiveness of a nanobiosensor, was done by comparing the results of the different serological techniques (ELISA, DIBA) with that of the fabricated solution based nanobiosensor and it was found that nanobiosensor could detect the viral protein at a very low concentration (2-0.02) mg/ml, whereas in the case of other techniques the detection was possible up to 0.12 mg/ml of antigen concentration.

Vinusri (2016) developed a solution phase lateral surface plasmon resonance (LSPR) biosensor using gold nanorods (GNRs) for detection of Banana bunchy top virus (BBTV). GNRs were synthesized using seed mediated growth method characterized using UV-Vis spectrophotometry and Transmission Electron Microscopy. Functionalization of GNRs with BBTV specific antibody were undertaken by conjugating the antibody with GNRs to make a GNR probe. The BBTV antigen isolated from the BBTV infected samples of banana were allowed to interact with GNR probe. A colour change was noticed due to interaction of GNR probe with antigen. In the infected samples, colour change from pinkish red to pale grey was evident, while no such colour change was noticed in healthy samples. Due to the colour change, the developed solution phase sensor as such can be used for field level applications. The efficacy of the developed solution phase LSPR based GNR biosensor was done by comparing with ELISA which showed that, the former could detect a low concentration of antigen *i.e*., 20 ppm while the latter could detect it only at 80 ppm concentration.

5.2 DNA based biosensors

The principle of these widely used sensors lies in the hybridization process through spontaneous hydrogen bonding between the target DNA and its complementary strand, which is utilized by immobilizing the single-stranded DNA (ssDNA) onto a suitable surface. The hybridization event is generally detected by two different methods; (i) the detection of certain electroactive indicator (labeling) (ii) the detection of signal produced by the most electroactive base of DNA (Asal *et al*., 2018).

Wei *et al.*, 2014, successfully constructed a low-cost paper based gene sensor that makes use of hybridization-mediated target capture based on a miniaturized lateral flow platform and gold nanoparticle colorimetric probes. The captured colorimetric probes on the test line and control line of the gene sensor produced characteristic red bands, enabling visual detection of the amplified products within minutes without the need for sophisticated instruments or the multiple incubation and washing steps performed in most other assays. The sensor was used successfully for the identification of banana bunchy top virus (BBTV) and the detection limit was 0.13 aM of gene segment, which was found to be 10 times higher than that of electrophoresis and provides confirmation of the amplified products.

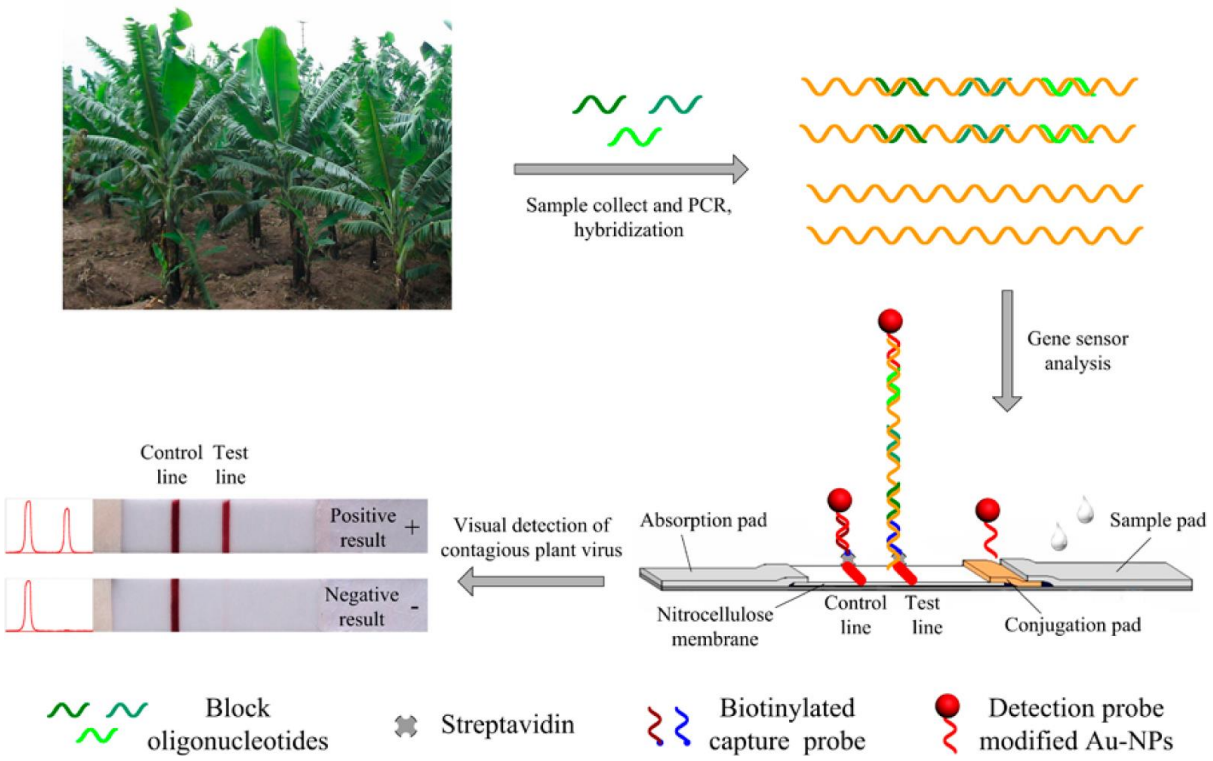

Fig. 5: Operating principle of the paper-based gene sensor for identification of contagious plant virus

5.3 Optical biosensors

In optical biosensor, the transduction process induces change in the phase, amplitude, polarization, or frequency of the input light in response to the physical or chemical change produced by the biorecognition process. The main components of an optical biosensor are a light source, optical transmission medium, immobilized biological recognition element

(enzymes, antibodies, microbes) and optical detection system. Some of the advantages offered by an optical biosensor are selectivity, specificity, remote sensing, real-time measurements and compact design. Optical biosensors can be broadly classified into fluorescence-based biosensors and surface plasmon resonance (SPR)-based biosensors (Ray *et al*., 2017).

5.3.1 Fluorescence- based biosensor

In fluorescence-based detection, either the target molecules or biorecognition molecules are labelled with fluorescent tags or fluorochrome molecules that produce light during the bio-recognition event (Fig. 6). Fluorescence requires an external light source (short-wavelength light) to initiate electronic transitions in an atom or molecule, which then produces luminescence (longer wavelength light). The intensity of the fluorescence indicates the presence of the target molecules and the interaction strength between target and bio-recognition molecules. Optical biosensors based on fluorescence detection use the combination of a fluorescent bioreceptor associated with an optical transducer. Fluorescent biosensors are obtained by immobilizing whole cells on the surface of a sensor layer. This bioactive layer is usually placed in front of the tip of optical fibers to generate a fluorescent signal. The optical fibers send the excitation radiation to the fluorescent bioelement and convey the fluorescence radiation using a fluorimeter. Fluorescent materials and green fluorescent protein have been extensively used in the construction of the fluorescent biosensor (Ray *et al*., 2017).

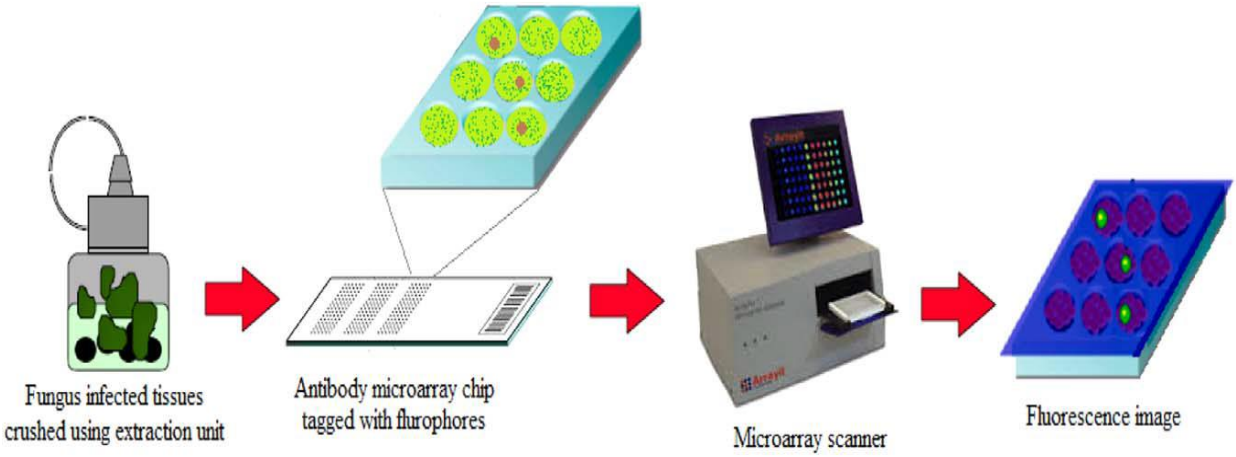

Fig. 6. Overview of fluorescence detection.

Antibodies are tagged with suitable fluorophores and immobilized over the microarray chip. The infected tissues are crushed using a bottle and ball extraction unit and stained with fluorescent dye. The mixture is then dispersed over the antibody microarray chip which leads to the formation of a monolayer of fungal antigens in the microchambers. Fungal infection is detected using a microarray scanner with a fibre optic diode laser system which excites the bound fluorescent linked antibody. The target infection is analyzed quantitatively where the amount of fluorescence exhibited gives the level of infection in the plant.

5.3.2 Surface plasmon resonance (SPR) sensors

The surface plasmon resonance (SPR) detection principle is utilized in many optical biosensor systems and offers real time monitoring of molecular interactions. The technique allows the measurement of a change in the effective refractive index on the surface without a requirement of labeling molecules and also provides information on the interaction of the biomolecules on the surface in real time (Fig. 7). Direct label-free detection of pathogens is also possible using this method (Skottrup *et al.*, 2007).

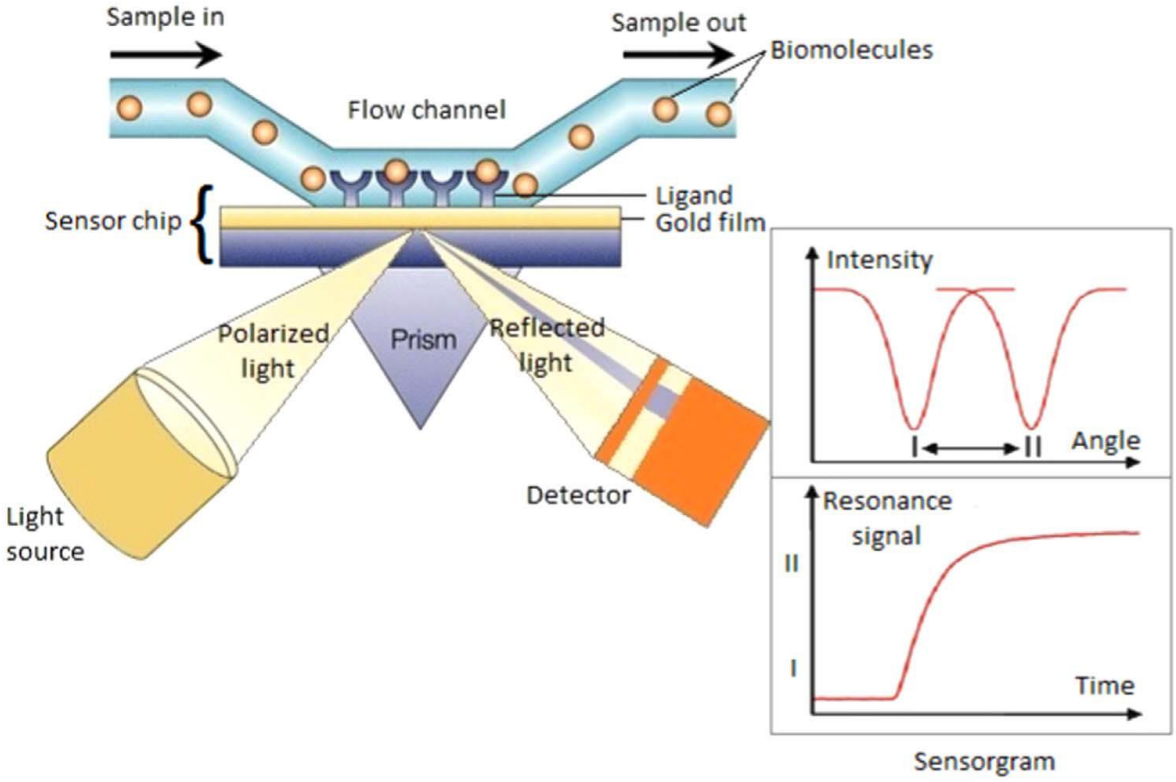

Fig. 7. Overview of surface plasmon resonance biosensor

The sensor chip is composed of a glass surface coated with a thin layer of gold that provide the physical conditions necessary for the SPR reaction. The surface of the chip is immobilized with one interacting molecule (ligand) while the other (analyte) is delivered to the surface through a microfluidic system. Polarized light is incident on the reverse side of this chip, propagating an electron charge density wave phenomenon that arises on the surface of the metallic film. This takes the form of an evanescent wave that extends beyond the sensor surface and detects mass changes on the surface. Binding of analyte to the immobilized ligand is followed by SPR, leading to detection of mass concentrations at the sensor surface. As molecules bind to and dissociate from the sensor chip surface, the resulting changes in the resonance signal create a sensorgram which is measured by a detection unit.

Luna-Moreno *et al.* (2019) developed a highly sensitive SPR immunosensor to detect *Pseudocercospora fijiensis* in real samples of leaf extracts in early stages of the disease. A polyclonal antibody (anti-HF1), produced against HF1 (cell wall protein of *P. fijiensis*) was covalently immobilized on a gold-coated chip via a mixed self-assembled monolayer (SAM) of alkanethiols. A solution of ethanolamine was added inorder to deactivate the remaining unreacted carbodiamide esters. Then injected a solution of HF1, which caused the binding of anti-HF1 and HF1. This resulted in the development of signals observed as a change in the intensity of the light measured by the photodetector which were directly proportional to the concentration of the analyte in the samples.

5.4 Electrochemical biosensors

With the advantages of simple structure, high sensitivity, low cost, and rapid response, electrochemical biosensors express characters of biosensors perfectly and are considered as the most promising technology which is appropriate for microorganisms being tested in real time. Electrochemical sensors usually consist of a working electrode, a counter electrode, and a reference electrode. The reaction on the electrode surface is collected and converted to electrochemical signals which are proportional to analyte concentration present in the sample (Grieshaber *et al*., 2008). Based on the observed parameters such as current, impedance, conductance, and potential, electrochemical biosensors can be classified into amperometric, impedimetric, conductometric, and potentiometric (Caygill *et al*., 2010).

5.4.1 Amperometric biosensors

The principle of the amperometric biosensor is to convert molecular reactions on the surface of electrodes into a detectable current signal and perform further analysis (Tawil *et al.*, 2014)

5.4.2 Imperometric biosensors

Impedance biosensors can detect and/or quantify analyte by recording the change of impedance value caused by the biomolecule reaction on the electrode surface. The basic principle of Electrochemical Impedence Spectroscopy (EIS) is to add small amplitude sine wave perturbations to an electrochemical system in a wide frequency range. And then, the detector can measure the responding signals as a function of frequencies (Lu *et al*., 2013)

5.4.3 Potentiometric biosensors

Conventional potentiometric biosensors are composed of an ion-selective electrode (pH, ammonium, chloride, and so on) or a gas-sensing electrode (pCO2 and pNH3) coated with an immobilized microbe layer (Lei *et al*., 2006). Using a high impedance voltmeter, potentiometric biosensors usually measure electrical potential difference or electromotive force (EMF) between two electrodes when near zero current (Ansari *et al*., 2010). The changes of pH, ionic, or redox at the surface can be converted to corresponding electrical signals by a transformer proportional.

5.4.4 Conductometric biosensors

The conductometric biosensor is an analytical device that can interpret specific biological recognition reaction as electrical conductance. Compared with the other types of biosensor transducers, conductometric biosensors were produced through inexpensive thin film standard technology and there is no reference electrode needed (Nicole *et al*., 2008).

Khater *et al*. (2019) designed a label-free impedimetric biosensor for the detection of nucleic acid of *Citrus tristeza virus*. The sensing platform based on a screen-printed carbon electrode (SPCE) was modified by electrodeposited gold nanoparticles (AuNPs), which allowed to efficiently immobilize thiolated ssDNA probes as well to enhance the electrode conductivity. Hybridization of target ssDNA with that of the probe ssDNA resulted in an

electrochemical change which is recorded as change of impedence value by Electrochemical impedance spectroscopy.

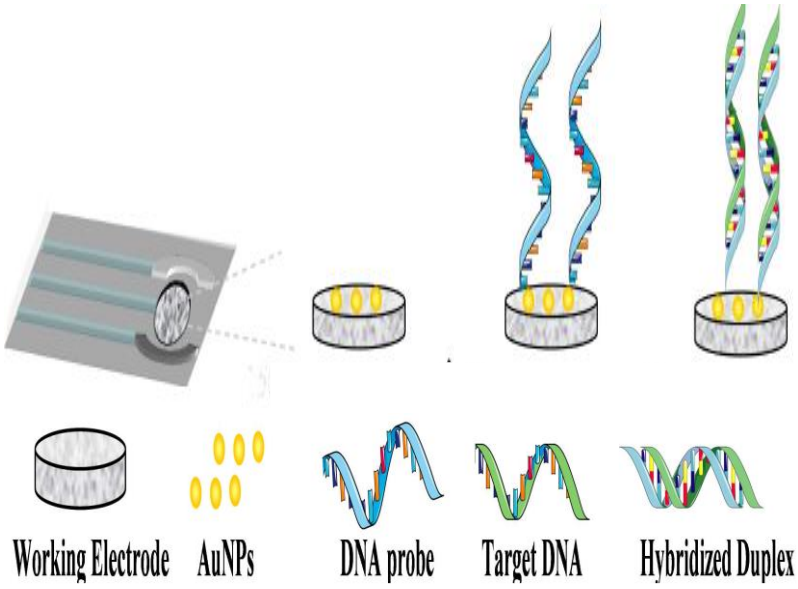

Fig. 8: Scheme of the developed DNA hybridization sensor based on AuNP-modified SPCE employing label-free impedance for the detection of CTV-related nucleic acid

5.5 Piezoelectric biosensors

Piezoelectric biosensors operate on the principle that a change in mass, resulting from the biomolecular interaction between two entities (eg., an antibody and its respective antigenic determinant) can be determined (Ngeh-Ngwainbi *et al*., 1990).

5.5.1 Quartz crystal microbalances (QCM) biosensors

QCM biosensors are used for plant disease detection where a quartz crystal disc is coated with pathogen-specific antibodies. QCM sensors consist of a thin quartz disc with electrodes plated on it. When an oscillating electric field is applied across the disc, an acoustic wave with a certain resonance frequency is induced via a piezoelectric effect (Webster *et al*., 2004). The disc is coated with a sensing layer (antibodies, nucleic acids, receptors, small molecules, etc.), depending on the analyte to be detected. The change in mass, which occurs when analyte accumulates on the surface of the disc, causes a change in resonance frequency. The resonance frequency change can then be directly proportional to biomolecular interactions.

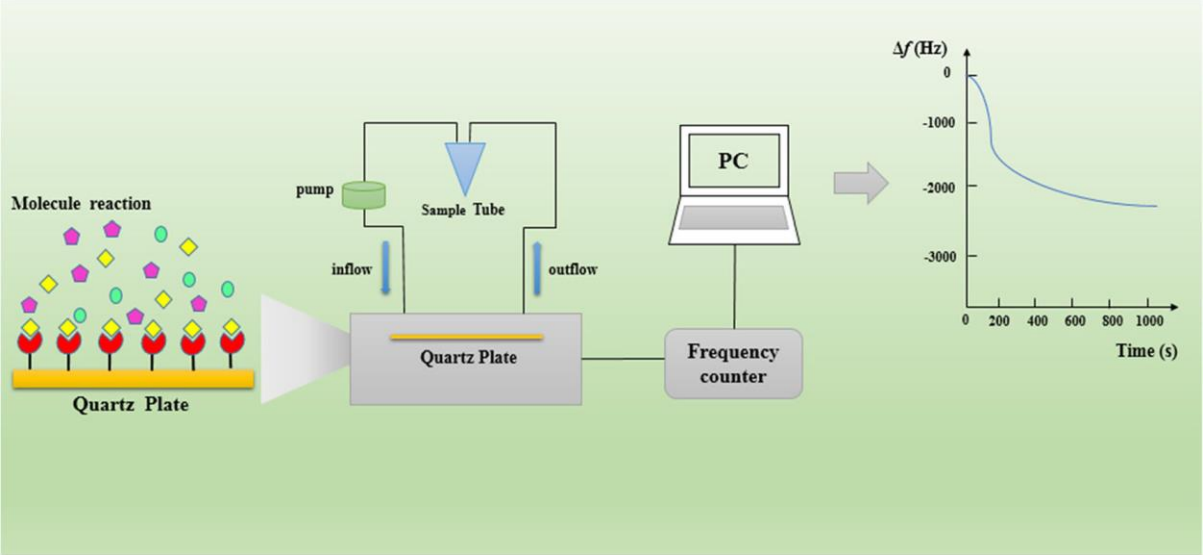

Fig. 9: The schematic representation of QCM

A QCM immunosensor was developed by Eun *et al.* (2002) for the detection of both C*ymbidium mosaic potexvirus* (CymMV) and O*dontoglossum ringspot tobamovirus* (ORSV) by pre-coating the QCMs with virus-specific antibodies. Upon binding of virions in either purified form or crude sap of infected orchids with the immobilised virus antibodies, the increase in mass at the QCM surface resulted in a reduction in the frequency of resonance oscillation in a manner dependent upon the amount of virus bound. The QCM was able to detect as low as 1 ng each of the two orchid viruses. This immunoassay was shown to be specific, sensitive, rapid and economical, thus providing a viable alternative to virus detection methods.

6. Case study

Specific and Sensitive Isothermal Electrochemical Biosensor for Plant Pathogen DNA Detection with Colloidal Gold Nanoparticles as Probes

Lau *et al.* (2017) described a rapid and highly sensitive diagnostic method coupling recombinase polymerase reaction (RPA) with AuNPs as electrochemical probes to detect the presence of plant pathogen DNA by differential pulse voltammetry (DPV) on disposable screen printed carbon electrodes (SPCE). Simultaneously, the performance of their assay was compared with conventional PCR and gel electrophoresis. *Pseudomonas syringae* was used as a model system in this study because it infects wide variety of crops and is an economically important plant pathogen. The flow diagram showing the main steps in their assay for plant pathogen DNA detection is illustrated in Fig. 10. They conducted a specificity study in order to identify a particular pathogen from other species to avoid false positive results. A *P. syringae*-specific assay was challenged with *P. syringae* and two unrelated pathogens: *Botrytis cinerea* and *Fusarium oxysporum* f.sp. *conglutinans* (Fig. 11). *P. syringae* samples produced a strong DPV signal while no signal was detected from *B. cinerea* and *F. oxysporum*. Electrochemical results were verified using gel electrophoresis where the expected 144 bp band was seen only in the *P. syringae* sample but not the controls. In order to identify a suitable amplification method, they compared the sensitivity of RPA with PCR (Fig. 12) using same amount of *P. syringae* genomic DNA and primers. It was found that RPA (15 copies) was 100 times more sensitive than PCR (1500 copies) based on gel electrophoresis. The detection limit of the electrochemical assay in detection amplified DNA was determined by titrating RPA products and measuring the corresponding DPV signal (Fig. 13). The data indicated that the electrochemical assay (214 pM) was 100 times more sensitive than gel electrophoresis (21,400 pM). Together, these data suggested that the combination of RPA with an electrochemical readout could potentially result in a rapid, sensitive and convenient DNA detection platform.

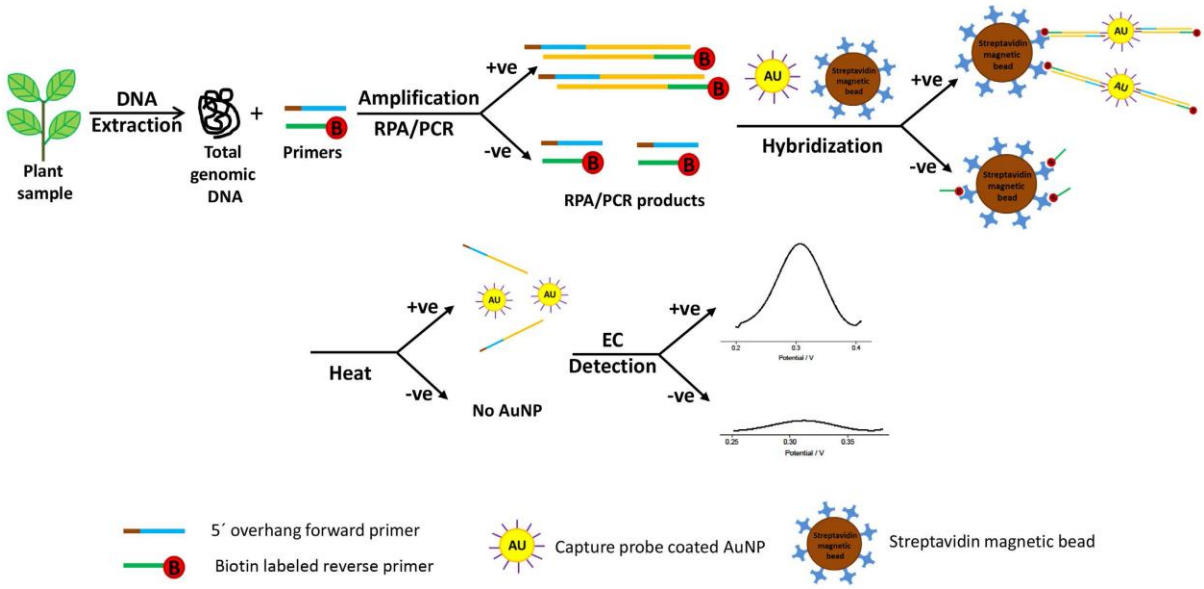

Fig. 10. Schematic illustration of the electrochemical bioassay for plant pathogen DNA detection.

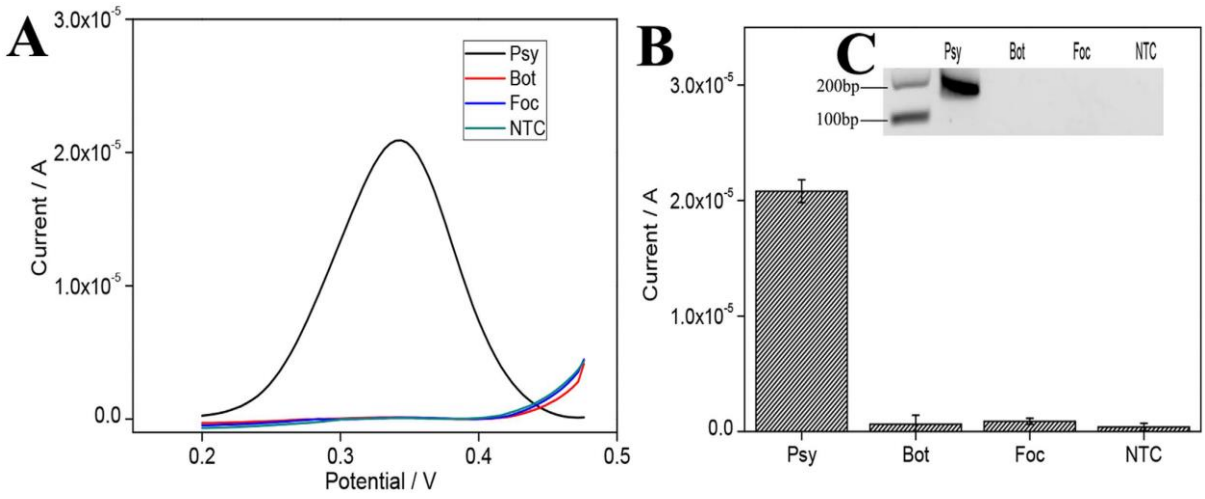

Fig. 11: Specificity study for plant pathogen DNA detection. (A) DPV curve and (B) Current-response to *P. syringae* (Psy), *Botrytis cinerea* (Bot) and *Fusarium oxysporum* f.sp. *conglutinans* (Foc) as well as a no template control (NTC). Error bars represent • } SD, n = 3. (C) Electrophoresis gel image of RPA products.

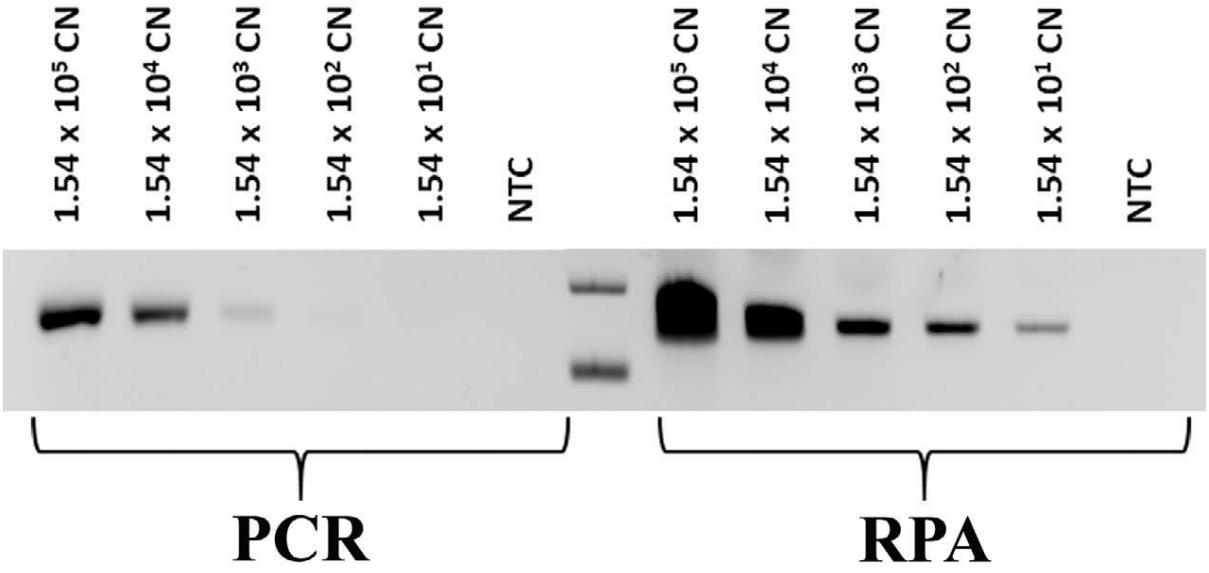

Fig. 12: Electrophoresis gel image for the sensitivity comparison between RPA and PCR over a range of gDNA inputs.

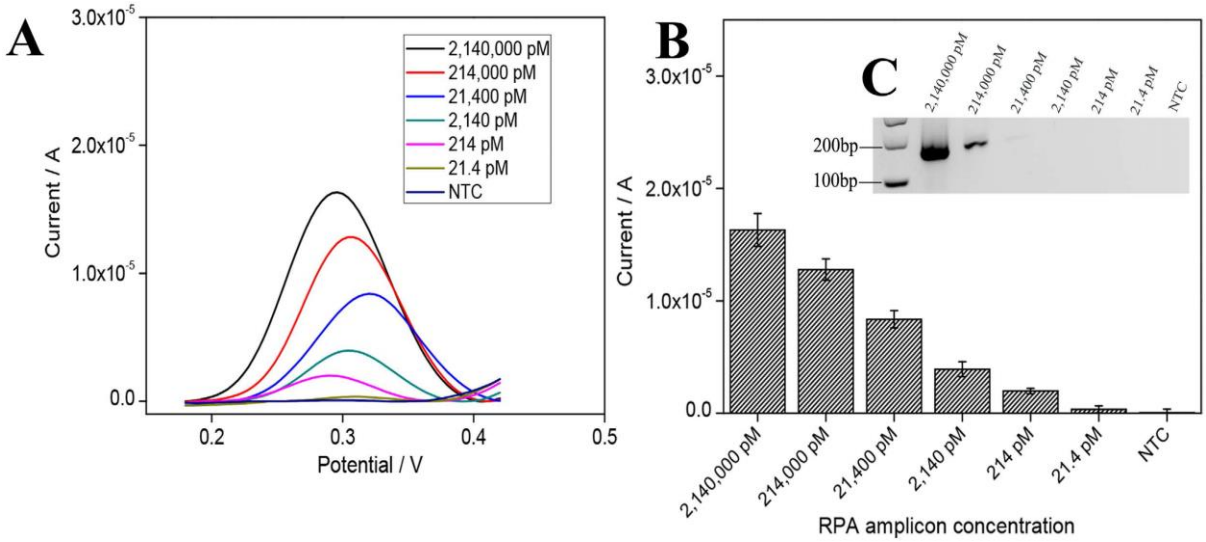

Fig. 13: Sensitivity study for plant pathogen DNA detection via RPA/electrochemistry. (A) DPV curve and (B) Current-response to different amounts of amplification products, the error bars represent • } SD, n = 3. C) Electrophoresis gel image of the RPA products.

7. Commercially available biosensor devices

These days, on-site detection is gaining importance for plant disease diagnosis. The need for on-site detection has led to the production of rapid and sensitive test devices and kits which can be use in-field for plant disease detection, even by the growers themselves. Efforts are in progress for development of on-site devices with better performance for plant pathology. The criteria for selection of a proper device include time, sensitivity, cost and ease of use (Ray *et al*., 2017).

Spreeta^{TM} sensors (Fig. 14) are coin-sized SPR-based analysis systems consisting of a gold surface for ligand immobilization, a source for optical interrogation and a diode array, which measures the SPR angle (Soelberg *et al*., 2005). The standard Spreeta^{TM} version has three parallel channels available, thus enabling reference analyses to be performed. The system can be operated by a mini-pump that delivers sample to the sensor surface and using this portable lightweight system (Spreeta^{TM}, pump and lap-top computer) pathogen detection can be performed on-site in many different settings. Indeed the Spreeta^{TM} systems have been used for *Campylobacter*, *E. coli* and *L. monocytogenes* analysis in laboratory settings (Nanduri *et al*., 2007; Waswa et *al*., 2007; Wei *et al*., 2007). Pricing for the disposable today is $30 and a hand-held reader might cost about $250 (ten times less expensive than a biocore) (Madou, 2002).

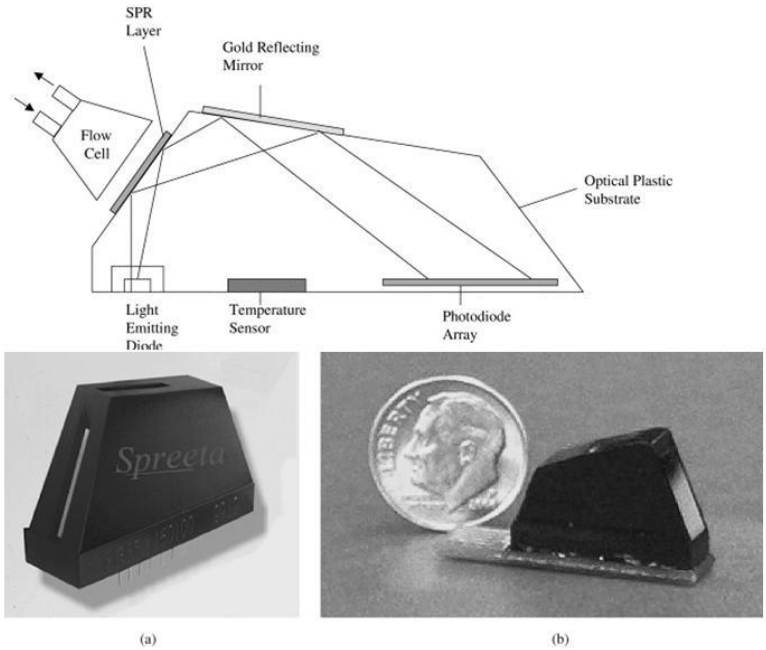

Fig.19: Spreeta^{TM} sensor. Top image shows an optical diagram of the sensor (in cross-section). (a)The original integrated sensor. (b) smaller version of the sensor with less sensitivity but more amiable to system integration.

8. Summary

A biosensor is a sensing device comprising a bioreceptor and a transducer. A bio-recognition element specifically identifies and interacts with an analyte, and the changes in its physicochemical properties (optical, thermal, electrical, or thermodynamic properties) are usually converted into a signal by a transducer. Biosensors can be grouped into enzyme, antibody, nucleic acid and whole-cell sensors based on the recognition element used, and again into optical, electrochemical, and piezoelectric sensors based on the transducer. Among the different types of biosensors based on the recognition element, antibody-based (immunosensor) and DNA-based biosensors are most commonly used for pathogen detection. Biosensors are specific and sensitive as it is useful for detecting very minute load of pathogen even before visual symptoms appear on the infected plant and hence can go a long way in preventing crop loss.

9. Conclusion

- Conventional diagnostic techniques are time consuming, are related to special equipment and require skill to operate the equipment.
- To overcome these difficulties, recent advances in micro and nanotechnologies have enabled for developing biosensors for determination of pathogen infections in plants
- Over the long term, the use of nanotechnology with additional efforts will help significantly to develop high sensitive and selective biosensors for real-time monitoring of plant pathogens in the field conditions

10. Discussion

1. Which among the biorecognition element based biosensor is more sensitive?

Ans. The DNA based biosensors are more sensitive than the antibody based biosensors due to the nucleic acid amplification techniques, which allows to detect plant pathogen before appearance of disease symptoms.

2. Are there any commercially available biosensors.

Ans. Yes there are many optical biosensors that use SPR technology which are commercially Available such as Spreeta™ biosensor (Texas Instrument), BioCore™ biosensor instrument (GE Health Care, USA), Nippon Laser and IBISiSPR (IBIS Technologies B.V. Electronics and Sierra Sensors, Germany).

3. Is there any difference between the dipstick apparatus and biosensor? Or are they the same.

Ans. Yes they are the same. Some of the biosensor devices uses the principle of lateral flow immunoassay same as that of lateral flow devices or dipstick apparatus. The paper based gene sensor mentioned here is an example of a biosensor which uses the similar principle.

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Department of Plant Biotechnology

MBB 591: Master's Seminar

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Admission No : 2018-11-002 Date : 21-11-2019

Major Advisor : Dr. M. R. Shylaja Time : 10:45 am

Biosensors for plant pathogen detection

Abstract

Plant diseases are one of the main factors limiting crop productivity and they account for huge economic losses to farmers. The continual increase in agricultural losses has led to a tremendous interest in the development of pathogen detecting devices for early identification of diseases. Current techniques to detect plant pathogens include immunological techniques such as Enzyme-Linked Immunosorbent Assays (ELISA) and DNA-based techniques such as Polymerase Chain Reaction (PCR). However, these methodologies are time-consuming, expensive, and often require complex instruments with skilled personnel. Consequently, there is a need for developing portable and cost effective biosensors for early and fast detection of plant diseases with high sensitivity, and specificity.

A biosensor can be defined as a compact analytical device or unit incorporating a biological or biologically derived sensitive 'recognition' element integrated or associated with a physicochemical transducer (Turner *et al*., 1987). A bio-recognition element specifically identifies and interacts with an analyte, and the changes in its physicochemical properties (optical, thermal, electrical, or thermodynamic) are usually converted into a signal by a transducer.

Biosensors can be grouped into enzyme, antibody, nucleic acid and whole-cell sensors based on the recognition element used, and again into optical, electrochemical, and piezoelectric sensors based on the transducer (Chen *et al*., 2018). Among the different types of biosensors based on the recognition element, antibody-based (immunosensor) and DNA-based biosensors are most commonly used for pathogen detection.

Immunosensors were developed for the detection of *Pseudocercospora fijiensis* infecting banana (Luna-Moreno *et al*., 2019). Eun *et al.* (2002) reported the development of piezoelectric immunosensors for detecting *Cymbidium mosaic potexvirus* (CymMV) and *Odontoglossum ringspot tobamovirus* (ORSV) in orchid.

DNA-based biosensors are highly sensitive in detecting plant pathogens. If the plant is infected, the pathogen specific DNA hybridizes with the probe DNA immobilized on a sensor. The hybridization is detected by the transducer and a signal is generated. Probe based biosensors have been developed for the diagnostics of *Banana bunchy top virus* (Wei *et al*., 2015). Lau *et al*. (2017) described a rapid and highly sensitive diagnostic method coupling recombinase polymerase amplification (RPA) with gold nanoparticles (AuNPs) as electrochemical probes to detect the presence of *Pseudomonas syringae* in *Arabidopsis thaliana*. They compared electrochemical assay with conventional PCR and gel electrophoresis and found that it was suitable to detect pathogens with high efficiency, specificity and sensitivity.

Thus, biosensors are useful for detecting very minute load of pathogen even before visual symptoms appear on the infected plant and hence can go a long way in preventing crop losses.

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