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# Current progress in plant pathogen detection enabled by nanomaterials-based (bio)sensors

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ABSTRACT

The identification and quantification of plant pathogens in the early stages of infection play an important role to ensure food security and decrease crop loss. Over the past years, advances in nanomaterials research have allowed the development of novel plant disease (bio)sensors with high sensitivity and specificity. In this review, we address the use of different 0D, 1D, 2D and 3D nanomaterials for designing varied plant disease (bio)sensors. Specifically, the appealing features of nanomaterials, including high surface area/volume ratio, tunable physical-chemical properties and capability to incorporate biomolecules, are discussed, while illustrative examples on how they can be applied to improve the performance of electrical, electrochemical, optical, gravimetric and thermal sensors are presented. Finally, future trends, challenges and opportunities on the use of such nanomaterial-based (bio)sensors for on-site and expedite plant pathogen detection are also presented.

## 1. Introduction

The need to feed the world's growing population is putting pressure on agriculture activities in order to increase productivity combined to food safety. In this direction, invasive plant pathogens, such as viruses, fungi, and bacteria, are usually unwanted impactful agents that can potentially cause diverse plant diseases and decrease crop productivity. For instance, according to FAO [1], plant diseases cost around $220 billion for the global economy. Therefore, plant pathogens and/or their effects on the plants should be early identified/diagnosed for further assertive actions and less costly measures. Currently, many plant pathogens are usually identified by naked eyes based on leaf aspects and visual condition of a plant [2–4]. Such detection in many cases occurs when the plant disease is already in an advanced stage, making it difficult to be remediated and cured [5]. However, depending on the type of plantation and consequent infection, early detection is required and preferred for further agrochemical remediation [6,7]. When pathogenic detection is evaluated by instrumentation, some well-known techniques are employed, such as polymerase chain reaction (PCR) [8], enzyme-linked immunosorbent assay (ELISA) [9], and other classic methods like colony counting, fluorescence *in situ* hybridization (FISH) [10] and flow cytometric detection (FCM) immunology-based method [11,12]. Nevertheless, these techniques are time consuming, expensive and require trained operators [13] , which can be an obstacle for achieving fast diagnoses required for some crops. Besides, on-site individualized plant monitoring is still a challenge to be overcome.

Nanotechnology has been shown to be an important tool for plant's pathogen identification and quantification. For instance (bio)sensors with superior performance in terms of sensitivity, selectivity and limit of detection, summed up to the possibility of miniaturizing devices for on-site detection can be achieved using varied nanostructures [14]. For example, sensor conductivity can be enhanced by using graphene derivatives [15] or carbon nanotubes [16]. A substantial increase in surface-to-volume ratio can be obtained through the use of electrospun nanofibers [17] or metallic nanoparticles [18], with adjustable surface functionality, enabling binding sites for bio-specific immobilization [19–21]. Tuning the sensor selectivity is possible by using molecularly imprinted polymer (MIP) which interacts exclusively with the target analyte, avoiding interferences [22]. Those examples are illustrative of the various approaches enabled by nanotechnology that can be applied in the design of (bio)sensors with enhanced properties.

Herein we survey on recent results on nanomaterial-based (bio)

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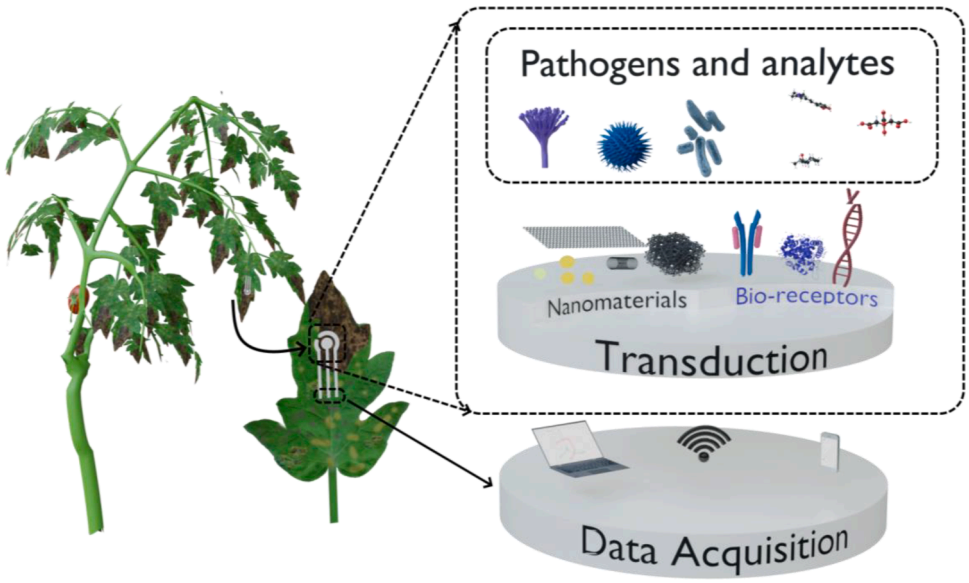



**Fig. 1.** Schematic illustration of plant disease detection enabled by nanomaterial-based sensors. In this example, a sick tomato plant infected by a pathogen agent like fungi, virus, or bacteria that can be monitored by nanomaterial-based sensors operating under varied transduction mechanisms through the interaction of nanomaterials and bio-recognition elements with the target analytes.

**Table 1**
Examples of plants and cultures and their respective pathogens.

| Culture | Virus | Fungi | Bacteria | Symptoms |
|---|---|---|---|---|
| Almond | | | *X. arboricola pv. Pruni* [27] | Leaf, stem, and trunk injuries. Defoliation and fruit drop |
| Apple plants | | | *Erwinia amylovora*/ *Pseudomonas syringae* pv. *syringae* [28] | Storage disease in apple with evident moisture formation on the fruit<br>Wilt and blackening twigs, flowers, and leaves<br>Leaf necrosis and systemic vascular wilt |
| Citrus plants | *Citrus Tristeza* [29–31] | | *Candidatus Liberibacter* [32] | Decline of plants and yellowing of leaves<br>Yellowing of shoots, leaf spot, decrease in size, and deformity of the fruit |
| Cucumber plant | | *Oidium neolycopersici* [33] | *Pseudomonas syringae pv. Lachrymans* | Chlorosis and white powdery lesions on the leaves. Rapid aging and reduction in the size and quality of the fruit<br>Leaves with water-soaked lesions. Necrosis and reduction of photosynthetic capacity |
| Brassica | | | *X. campestris* [34] | Leaf necrosis with V-shaped lesions and blackened vascular bundles |
| Ginseng | | *Alternaria panax Whetz* [35] | | Reddish to dark brown elongated lesions |
| Maize plants | | *Arbuscular mycorrhizal* [36] | | Change in root mass, length, or architecture. |
| Orchids | Cymbidium mosaic/ Odontoglossum ringspot [37] | | | Leaves and flowers with necrotic chlorotic stains, growth inhibition<br>Color break in flowers and spots yellowing on leaves |
| Pear plants | | | *Erwinia amylovora* [38] | Wilt and blackening twigs, flowers, and leaves |
| Pepper plant | | *Oidium neolycopersici* [33] | | Chlorosis and white powdery lesions on the leaves.<br>Rapid aging and reduction in the size and quality of the fruit. |
| Plantsap samples | | | *Xanthomonas axonopodis* [20] | Fruit stains, leaf falls and fruit tree decline |
| Potato | *Leafroll virus* [39] | *Phytophthora infestans* [40,41] | | Tuber is stunted and erect. Rigid, curled leaves. Leaves like brownish-purple oily patches. Leaves with grayish white mycelium rings and spores |
| Scots pine | | *Mycorrhizal colonization* [42] | | Change in root mass, length, or architecture |
| Stone fruit trees | *Plum Pox* [43] | | | Leaves with stains or chlorotic rings, unblocking of veins.<br>Deformed fruits |
| Strawberry | | *Rhizopus* sp. *and Aspergillus* sp. *Section Nigri* [44] */P. cactorum* [14] | | Grayish color for Rhizopus and black appearance at Aspergillus infected fruits/Leaf size reduction and decreased productivity |
| Tabaco | Tobacco mosaic virus [45] | | | Leaf with chlorine or mosaic with white to light green color |
| | | Yellow leaf curl virus [41] | | |
| Tomato crops | Yellow leaf curl virus [46] | *Oidium lycopersicum* [47]/ *Phytophthora infestans* [21] | | Infected leaves are small, yellow in color and curve upwards.<br>Leaves, petioles, and stems have lions superficial with white powdery. Desiccation, necrosis, and defoliation<br>Leaves like brownish-purple oily patches. Leaves with grayish white mycelium rings and spores. |

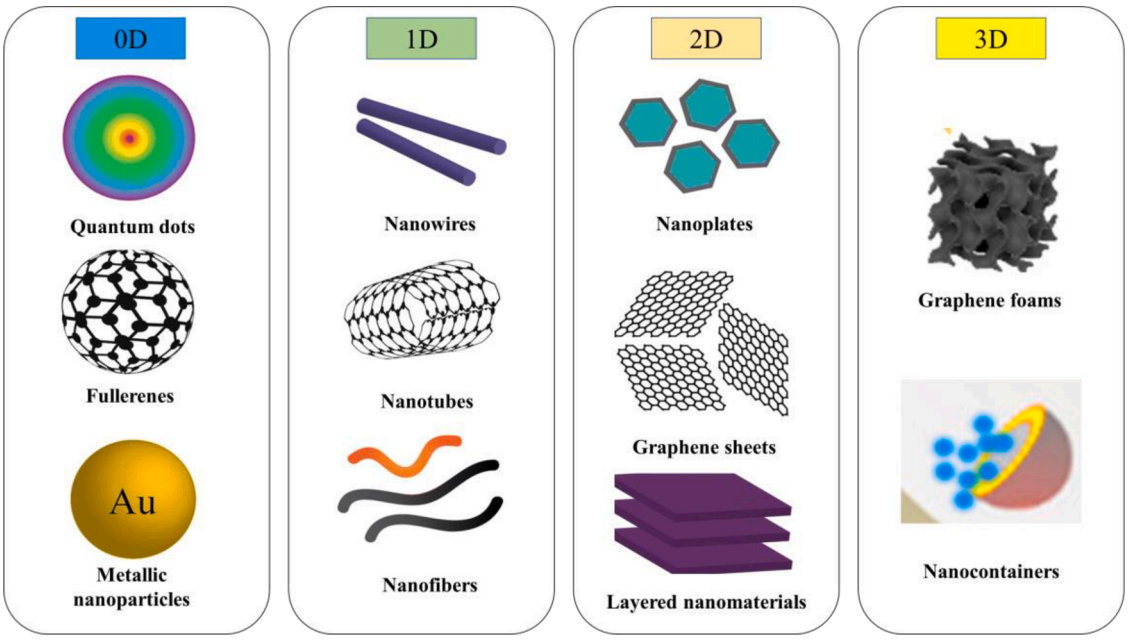



**Fig. 2.** Schematic illustration of zero (0D), one (1D), two (2D), and three (3D) dimensional nanostructures. Representations of 0D, 1D, and 2D were reprinted with permission from reference [55]. Copyright 2018 Springer Nature. The illustration of graphene foam structure (3D) was reprinted with permission from reference [56]. Copyright 2021 Elsevier. The illustration of the nanocontainer structure was reprinted with permission from reference [54]. Copyright 2019 American Chemical Society.

sensors for plant pathogen detection. We provide insights on how the use of functionalized (bio)sensors operating under varied transduction mechanisms, combined with nanomaterials-based structures, can achieve specific interactions towards varied analytes associated to plant diseases, as illustrated in **Fig. 1**. Such nanomaterial-based devices arise as a promising alternative to the classic and more expansive methods of pathogens identification. Despite some recent publications regarding the use of nanotechnology towards plant smart sensing [23], wearable sensors [24] and real time analytes focused on strategic sanitary areas [25], this review brings a recent survey on nanomaterials contribution to enable sensing of pathogens in plants and crops, covering varied transduction mechanisms. Specifically, in this review we first provide information on varied nanomaterials (0D, 1D, 2D, and 3D) and biorecognition elements applied in sensors. Then, we move to biorecognition transducers, and transduction mechanisms. Finally, challenges on plant sensor manufacturing as well as on other trends [26] are also discussed.

As mentioned before, plant pathogens can cause adverse effects on varied plants and crops, representing a threat to crop productivity. Some of common plant pathogens, including fungi, bacteria and virus, as well as their symptoms, are illustrated in Table 1.

## 2. Nanomaterials and biorecognition elements

### 2.1. Nanomaterials for sensing applications

Nanomaterials have attracted significant interest for sensing applications due to their outstanding features, including high surface-to-volume ratio, possibility to modulate their shape, size, arrangement, and compositions, as well as versatility in terms of surface modifications with a broad range of molecular ligands, which can play an important role in maximizing the sensor sensitivity and selectivity [48–50]. These features are essential in the design of (bio)sensors for plant pathogen detection, once this process generally requires the quantification of the target analytes at low concentrations and in complex matrices [51]. In addition, the nanomaterials allow rapid response times and enable advances in the design of miniaturized and portable devices, which is highly advantageous for the on-site and remote plant health monitoring, therefore affording the prediction or early diagnosis of plant diseases [24,52]. According to their dimensionality, nanomaterials can be classified into: zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) [53]. Specifically, 0D, 1D and 2D nanostructures exhibit all, two and one dimensions at the nanoscale range, respectively, while 3D nanomaterials are bulk materials that contain features at the nanoscale, as illustrated in **Fig. 2** [54]. In this regard, the following subsections will briefly introduce the principal aspects of the four different general nanomaterial classes and their advantages for (bio)sensing applications. Those nanomaterials, if properly combined with bio-receptors in a rational sensor setup, can bring substantial signal amplification in an analytical signal acquirement and/or avoid signal interference. For example, the use of gold nanoparticles in an electrochemical sensor allowed to efficiently immobilize thiolated ssDNA probes that ensured selectivity as well enhanced the electrode conductivity [30]. In another approach, carbon nanotubes (CNTs) were employed to modify interdigitated electrodes of an electronic nose used to detect different fungal microorganisms (*Aspergillus* sp. section *Nigri* and *Rhizopus* sp.) in strawberries [44]. In order to set a baseline for this discussion, this section aims to show the abundance and classification of the many types of nanomaterials contributing to the development of sensors for monitoring crops regarding plant pathogens.

#### 2.1.1. Zero dimensional nanomaterials (0D)

Zero-dimensional nanomaterials are well-suited for sensing applications due to their large specific surface area, the diversity of composition and surface functionalities, as well as tunable size (1–100 nm) and shape (sphere-, rectangle-, hexagon-, cube-, triangle-, and star-, and branch-like outlines) [50,57]. These features, combined with a broad spectrum of optical and electrical properties, result in promising 0D systems for electronic, electrochemical, and optical sensing modalities. In recent years, 0D structures including carbon [58] and graphene quantum dots [15,59], inorganic quantum dots [60], magnetic nanoparticles [61], metal nanoparticles (*e.g.*, Au, Ag, Ni, Co, Pt, Pd, Cu, Al), [62,63] and semiconductor nanoparticles (*e.g.*, $SnO_2$, ZnO, and $TiO_2$)

[62] have been widely used in a variety of sensing applications, leading to improved sensitivity, detection range, and reaction time.

#### 2.1.2. One dimensional nanomaterial (1D)

One-dimensional nanostructures mainly in the form of nanotubes, nanowires, nanorods, and nanofibers have also been extensively explored in the development of sensing platforms [64,65], Nanotubes, for instance, consists of cylindrical structures exhibiting nanometer-sized diameter and length varying from nanometers to centimeters, which can be inorganic (*e.g.*, Pt, $Co_3O_4$, $Fe_2O_3$, $SnO_2$, and $TiO_2$) [66], organic (*e.g.*, carbon) [16] or composite (*e.g.*, ZnO/carbon) [67]. Among them, CNTs, which are tubular structures of rolled-up sheets of graphene (a two-dimensional honeycomb structure with $sp^2$-hybridized carbon) are one of the most studied 1D nanostructures for sensing applications, particularly in electrochemical biosensing [16]. This interest arises from their outstanding properties that include high conductivity, wide electrochemical window, large specific surface area, and chemical stability [54]. Furthermore, CNTs, both single-walled (SWCNTs) and multiwalled (MWCNTs), containing carboxyl groups are highly useful for the immobilization of biorecognition elements [68,69], via covalent conjugation, thus resulting in highly selective sensors.

Nanowires (NWs) exhibit a non-hollow elongated circular nanostructure with a diameter in the range of a few tens of nanometers and the length usually from micrometers up to millimeters, but longer lengths can also be obtained [70,71]. These nanomaterials can be synthesized with diverse architectures and compositions, including: metallic (*e.g.*, Ag, Au, Pd) [72], metal oxides (*e.g.*, $SnO_2$, $TiO_2$, $WO_3$, ZnO, $Fe_2O_3$ and $In_2O_3$) [73], and conducting polymers (*e.g.*, poly(3, 4-ethylenedioxythiophene):poly-(styrenesulfonate) (PEDOT:PSS)) [74]. Due to their high flexibility, conductivity, and optical activity, NWs have gained increased interest in the design of sensing devices, including optical-chemical [75], electrochemical [76], and electrical [77].

Nanorods are rod-like-shaped nanomaterials and correspond to a shorter form of nanowires exhibiting diameters in the range of a few tens of nanometers while their lengths vary from several tens to a hundred nanometers [78]. Nanorods composed of metal oxides (*e.g.*, ZnO [79], $MoO_3$ [80], tungsten oxide [81]), metals (*e.g.*, Au [82]), and metalloys (*e.g.*, Ag/Au [83]) exhibit outstanding electrical, mechanical, and optical properties, which make them promising building blocks for sensing platforms. In recent years, gold nanorod (AuNR) has emerged as powerful signal elements for colorimetric sensing owing to their localized surface plasmon resonance extinction in the visible range [78]. These sensing platforms are simple, instrument-free, and exhibit visual sensitivity with a naked-eye-detectable readout, and are potentially useful for the detection of various chemical and biological analytes [78, 82,84,85].

Nanofibers represent a remarkable class of nanomaterials for the design of sensing platforms, since they exhibit outstanding features including, mechanical flexibility, high surface area to volume ratio, high porosity, and the possibility of chemical or physical modifications [86, 87]. In the last decade, spinning techniques such as electrospinning, solution blow spinning, centrifugal spinning, and microfluidic spinning have been considered the most promising strategies to prepare nanofibers with diverse compositions (*e.g.*, polymeric, ceramic, metallic, and composites), structures (*e.g.*, uniaxial, core-shell, Janus), and electrical properties (*e.g.*, conducting, semiconducting, and insulating) [17,88, 89]. This great versatility has been explored to construct different sensing platforms (*e.g.*, opto-chemical [90], chemoresistive [91], electrochemical [92], and electronic-tongue [93] sensors that exhibit enhanced properties in terms of sensitivity, selectivity, response time, recovery ability, and detection limit.

#### 2.1.3. Two-dimensional nanomaterials (2D)

Two-dimensional nanomaterials represent a class of sheet-like structures with thicknesses of a single layer or a few atomic layers and lateral dimensions larger than 100 nm, reaching up a few micrometers and even larger [94,95]. In recent years, a great variety of 2D nanostructures including graphene and its derivatives [96], transition metal dichalcogenides (TMDs, *e.g.*, $MoS_2$, and $WS_2$) [97], transition metal oxides (TMOs, *e.g.*, $MoO_3$, $WO_3$, and $MnO_2$) [98], graphitic carbon nitride (g-$C_3N_4$) [99], hexagonal boron nitride (h-BN) [100], and metal carbides and carbonitrides (MXenes) [101,102] have emerged as promising materials for sensing applications owing to their remarkable physical and chemical properties. These nanostructures possess a high surface-to-volume ratio, thus providing a high density of active surface sites to target analytes. In addition, the surface of 2D nanomaterials can be tailored via functionalization or defect engineering to selectively respond to specific analytes with extremely high sensitivity [95]. Another important characteristic of 2D nanostructures is their variety of electronic properties, which can be metallic/semimetallic (*e.g.*, graphene, $VS_2$, $TaS_2$, MXenes [103]), semiconducting (*e.g.*, black phosphorus (BP), $MoS_2$, $WS_2$), and insulating (*e.g.*, h-BN) [104], rendering them appealing candidates for electrical and electrochemical sensing applications. Furthermore, according to their composition and structure, 2D nanomaterials display optical properties including fluorescence quenching or emitting as well as plasmonic behavior, making them promising for the fabrication of optical sensors and bioimaging [104].

#### 2.1.4. Three-dimensional nanomaterials (3D)

Three-dimensional nanomaterials exhibit all three arbitrary dimensions higher than 100 nm and are included in the class of nanomaterials due to their hierarchical architectures comprised by multiple arrangements of nanosized materials such as bundles of nanofibers, nanowires, and nanotubes, dispersions of nanoparticles as well as multinanolayers [105,106]. These 3D hierarchical materials feature high accessible surface areas in combination with the interconnected structure and large porosity, which can lead to enhanced mass transport and the increase of active sites, thus making them promising constructs for sensing applications [107]. Examples of 3D nanomaterials include graphene foam [108], hierarchically structured nanofibers [109], and nanocontainers [110], which have been studied in electrical and electrochemical sensing applications.

### 2.2. Bio-recognition transducers

#### 2.2.1. Bioreceptors

Biosensors are often categorized according to the type of the biorecognition element, which has the primary purpose of providing specificity for a particular analyte [111]. Knowing the advantages and limitations of each biorecognition element in terms of sensing performance is important for the design of biosensors [93]. Generally, the biorecognition element can be classified according to its nature into three categories: natural (*e.g.*, enzymes), semi-synthetic (*e.g.*, aptamers), and synthetic (*e.g.*, MIPs – molecularly imprinted polymers) [94]. In this section, we will focus on the main aspects related to antibodies, nucleic acids, and aptamers, which are the key classes of bioreceptors used in biosensor applications [112–114]. However, it is important to mention that other bio-probes, beyond those described in this review, do exist, and are helpful alternatives in the task of designing a biosensor.

Immunosensors are based on the transduction of a signal generated by the interaction between antibodies (or antibody fragments) and antigens, being considered a promising approach for the development of high-performance affinity-based biosensors for pathogens detection [43, 115,116]. The immunosensor signal can be either generated directly (non-labeled immunosensors) or indirectly (labeled immunosensors) [117]. Label-free or direct immunoassays allow a direct detection of the affinity event by evaluating the physical changes induced by the production of the antibody-antigen (Ab-Ag) complex. In this regard, Berto et al. reported the use of anti-Plum Pox Virus (PPV) polyclonal antibodies for the detection of PPV in plant extracts [43]. The Ab-virus interaction was transduced into an electric signal, which was found to

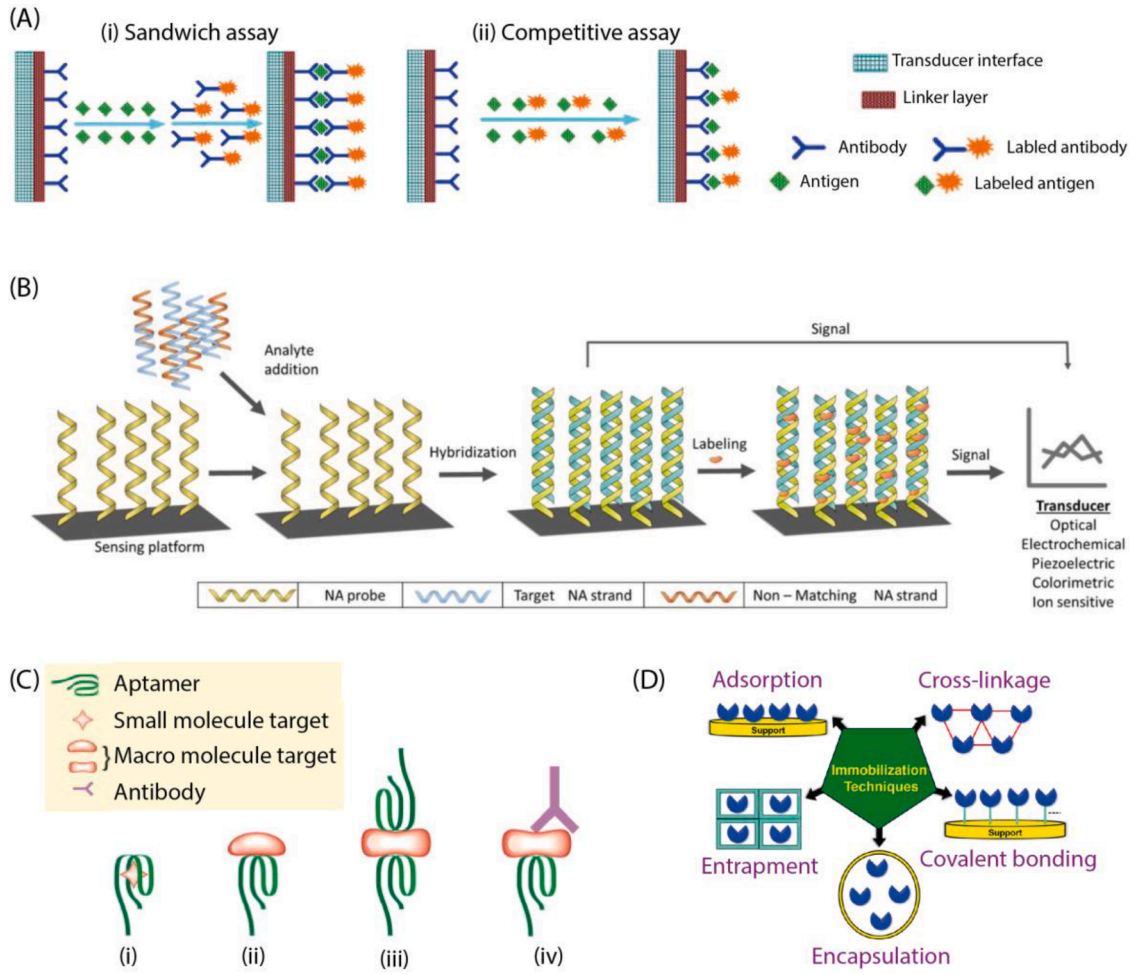



**Fig. 3.** (A) Schematic of labeled (i) sandwich and (ii) competitive immunoassays. Reprinted with permission from [119]. Copyright 2008 Elsevier. (B) General design and working principle of nucleic acid (NA)-based biosensor. Reprinted with permission from [124]. Copyright 2018 MDPI. (C) Aptamer-based assay formats. (i) Small-molecule target buried within the binding pockets of aptamer structures; (ii) single-site format; (iii) dual-site (sandwich) binding format with two aptamers and (iv) sandwich binding format with an aptamer and an antibody. Reprinted with permission from [125]. Copyright 2008 Elsevier. (D) Examples of biomolecule immobilization techniques. Reprinted with permission from [126]. Copyright 2021 MDPI.

be proportional to the PPV levels allowing the specific label free detection of PPV with a limit of detection (LOD) of 180 pg $mL^{-1}$. Labeled immunosensors use signal-generating labels that allow more sensitive and versatile detection modes to detect whether a binding event has occurred. A variety of labels can be used, including enzymes, fluorescent or electrochemiluminescent probes, and nanomaterials [118,119]. Typically, most of the labeled immunosensors are based either on a sandwich or competitive assay according to the molecular size of the analytes [119–121]. In sandwich assays (Fig. 3Ai), typically designed for the detection of high molecular weight molecules, the antigen is "sandwiched" between two antibodies and the signal response is directly proportional to the analyte concentration [121]. In this regard, Zhao et al. reported a dual amplified electrochemical sandwich immunosensor for *Pantoea stewartii* sbusp. *stewartii* (PSS) plant bacterial pathogen detection, using the favorable conductivity and large specific surface area of gold nanoparticles, and the excellent catalytic ability of the horseradish peroxidase enzyme [122]. In competitive-type assays (Fig. 3Aii), preferred for low molecular weight molecules, the analyte competes with labeled analyte for a limited number of antibody binding sites. As the analyte concentration increases, more labeled analyte is displaced, resulting in a decreased signal if the antibody-bound labeled analyte is detected [119]. The use of antibodies as bio-probes may be limited by several drawbacks, including stringent storage conditions, low shelf life, high susceptibility to change in pH, temperature, and ionic concentrations [123].

Several studies addressed plant disease diagnosis and pathogen detection using nucleic acid-based methods to determine the genetic content of pathogen [114,127–129]. Deoxyribonucleic acid (DNA) [18,30], ribonucleic acid (RNA) [130] and peptide nucleic acids (PNAs) [131] have been used for this purpose. The fundamental principle behind nucleic acid-based detection lies in the complementary recognition pattern between the immobilized nucleic acid fragment (probe) and the target sequence [111]. Recognition by nucleic acid receptors is basically an affinity reaction. It is therefore not surprising that the transduction methods in nucleic acid-based sensors are similar to those described for immunosensors: the hybridization event can be detected directly or a transduction tag can be used for indirect detection [132], as depicted in Fig. 3B. As an example, Machini et al. reported the development of a DNA-electrochemical biosensor for the detection of *Xylella fastidiosa* (Xf), a Gram-negative bacterial plant pathogen [133]. Often, the amount of available DNA analyte is too low to allow reliable quantitation by a nucleic acid assay, and therefore DNA amplification strategies are required to enhance the biosensor sensitivity [132,134]. Enzymatic methods to amplify DNA have been available for a long time and the polymerase chain reaction (PCR) methods have been used in the detection wide range of pathogens [112,114,135].

In addition to antibodies and nucleic acids, aptamers have also been employed as as biological recognition elements for pathogen detection

**Table 2**
Plant disease diagnostic using electrical and electrochemical techniques combined to nanomaterials.

| Target/Culture | Nanomaterial | Substrate | Bio-recognition | Detection method | LOD or accuracy | Other figuresof merit/ Minimum value to be detected | Observation | Ref. |
|---|---|---|---|---|---|---|---|---|
| Sec-delivered effector 1 (SDE1)/Citrus | SWCNTs | Gold microelectrodes onto Si/$SiO_2$ wafer | Anti-SDE1polyclonal | FET | LOD: 5 nM | Dynamic range from 3 nM to 2.6 μM / N/A | Specific label-free Candidatus Liberibacter bacteria in citrus through SDE1 biomarker using a novel chemiresistive biosensor at plant tissue extracts. | [5] |
| *Plum Pox Virus* (PPV)/Stone fruit trees | Au and pentacene films | Gold gate electrode | Anti-Plum Pox Virus polyclonal | EGOFET | LOD: 180 pg $mL^{-1}$ | Dynamic range from 5 ng $mL^{-1}$ to 50 μg $mL^{-1}$ / N/A | Label-free, rapid, and specific biosensor for PPV virus in partially purified stone fruit trees plant ex- tracts. | [43] |
| p-Ethylphenol released by Phytophthora/ Strawberries | SWCNTs | Silicon wafer cover with $SiO_2$ | ssDNA | E-nose | 0.13% of P-ethylphenol | Detection of 4-ethyl phenol over a wide range (0.25% to 100%) / N/A | FET immobilized with SWCNTs and ssDNA to detect 4-ethyl phenol for the diagnoses of P. cactorum in strawberries. | [14] |
| VOCs exhaled by Aspergillus and Rhizopus fungi/ Strawberry | N and B-dopped MWCNTs | Interdigitated electroless nickel immersion gold electrodes | – | E-nose | – | N/A | E-nose-based on carbon nanostructures to identify microorganisms fungal infection in strawberries inoculated with *Rhizopus* sp. or with *Aspergillus* sp. section *Nigri*. | [44] |
| VOCs exhaled by *Phytophthora infestans* infection/ Tomato | rGO and AuNPs | Kirigami-based structure with AgNW electrodes | – | Chemiresistive sensor array | > 97% accuracy | N/A | The classification of 13 VOCs was performed. Late blight disease in tomatoes was diagnosed 4 days after inoculation. | [155] |
| Xanthomonas axonopodis /Citrus | AuNPs | GCE | Anti-PthA | FET-SWV | LOD: 0.01 nM | Linear range from 0.03 to 100 nM and recovery levels from 96 to 103% in real samples / N/A | Glassy carbon novel modification immunosensor using GNP, CNT and PB | [20] |
| *Citrus Tristeza Virus* (CTV)/Citrus | AuNPs | SPCE | thiolated ssDNA | EIS | LOD: 100 nM | Recovery levels from 90 to 97% in real samples / N/A | AuNPs label free modified SPCE with covalently bonded thiolated ssDNA as a bio-recognition for CTV-related nucleic acid. | [30] |
| CTV detection/- | AuNPs | SPCE | thiolated primer | EIS | LOD: 1 pg $\mu L^{-1}$ | Good reproducibility (RSD of 8%) / N/A | The AuNPs dispensed heating sources frequently required in solid-phase approach detections. | [156] |
| *p*-ethylguaiacol, volatile compound due to *Phytophthora cactorum* fungus infection/- | $TiO_2$ and $SnO_2$ nanoparticles | SPCE | – | CV and DPV | LOD: 35–62 nmol $L^{-1}$ | LOQ: 106–188 nmol $L^{-1}$ / 20.8 μmol $L^{-1}$ of p-ethylguaiacol | $TiO_2$ and $SnO_2$ enabled the qualitative and quantitative determination of the volatile compound *p*-ethylguaiacol produced by fungus infection of plants and fruits. | [157] |
| *Pantoea stewartia* sbusp. *Stewartia* (PSS) | AuNPs | GCE | HRP | LSV | $7.8 \times 10^3$ cfu $mL^{-1}$ | Recovery levels from 90.6 to 107.5% / N/A | The low LOD was attributed to the synergistic effect of the conductivity and large specific surface area of AuNPs and the catalytic ability of HRP. | [158] |

(*continued on next page*)

**Table 2** (*continued*)

| Target/Culture | Nanomaterial | Substrate | Bio-recognition | Detection method | LOD or accuracy | Other figuresof merit/ Minimum value to be detected | Observation | Ref. |
|---|---|---|---|---|---|---|---|---|
| False smut caused by Ustilaginoidea virens | GO | Paper electrodes | ssDNA | CV and LSV | 10 fmol $L^{-1}$ | Linear range from 10 μmol $L^{-1}$ to 10 fmol $L^{-1}$ / N/A | ssDNA and GO provided selectivity and high sensitivity to the paper-based electrodes towards rice false smut disease. | [159] |
| Groundnut bud necrosis orthotospovirus (GBNV) | GO | ITO | anti-GBNV | DPV | LOD: 5.7 ng $mL^{-1}$ | Sensitivity of 221 ± 1 μA $\mu g^{-1}$ $mL^{-1}$/ N/A | The GO was used to take advantage of its functional groups that enabled the antibody immobilization and to facilitate the electron transfer capability of the biosensor. | [160] |
| Detection of plant pathogen DNA | AuNPs | SPCE | Recombinase polymerase amplification | DPV | 214 pmol $L^{-1}$ | N/A | Combining recombinase polymerase amplification with nanoparticle and electrochemistry was suitable to detect infections before disease symptoms. | [161] |
| *Cucumber mosaic virus*/ Cucumber | PPY nanoribbon | Gold microelectrode | anti-CMV IgG | Chemiresistive microelectrode | LOD 10 ng $ml^{-1}$ | Nano-immunosensor response strongly affected by buffer concentration / N/A | Lael free chemiresistive fabricated by LPNE | [162] |
| *Cucumber mosaic virus*/ Cucumber | GNP | SPCE | | Chronoamperometry | – | N/A | Determination of set potential voltages for cucumber mosaic virus detection using screen printed carbon electrode | [163] |
| *Rice tungro disease*/ *Rice* | GNP | SPCE | anti- RTBV/ RTSV | Cyclic voltammtry | – | N/A | Reviewed immunosensor format using nanomaterial for tungro virus detection | [164] |
| *Citrus Tristeza Virus* (CTV)/Sweet orange trees | AuNPs | Carbon ink 8-WE SPCE | Monoclonal anti-bodies Ab1 and Ab2 | Amperometry | LOD: 0.3 fg $mL^{-1}$ | Linear range from 1.95 to 10.0 × $10^3$ fg $mL^{-1}$ / N/A | Disposable microfluidic immunoarray allied with Immunomagnetic separation towards the detection of the citrus biomarker pathogen. | [31] |

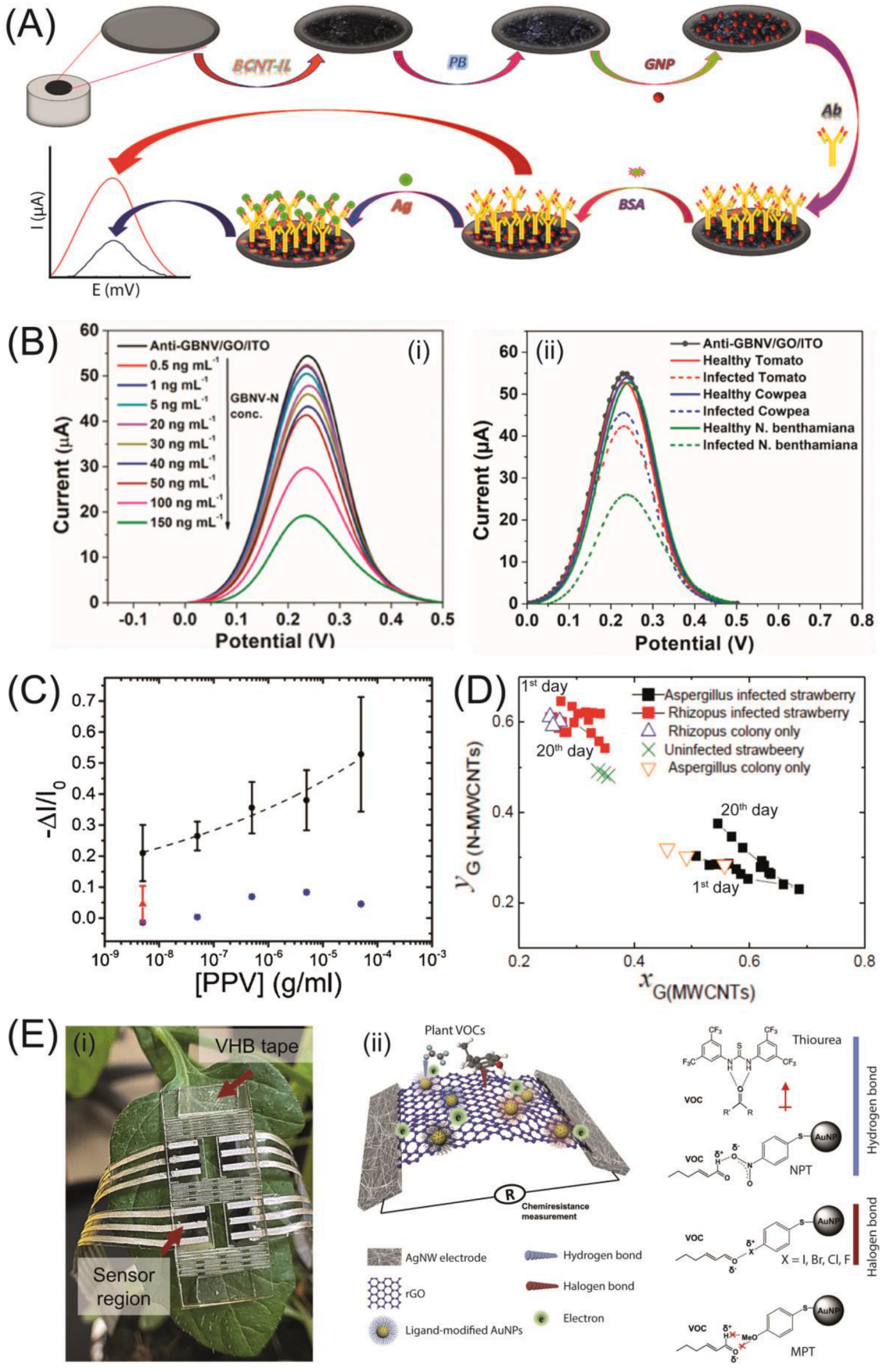
LOD: Limit of Detection; VOC – Volatile organic compounds; FET – Field-effect transistor; EGOFET – Electrolyte-gated organic field-effect transistor; E-nose – Electronic nose; FFT-SWV –Fast Fourier transform square wave voltammetry; EIS – Electrochemical Impedance Spectroscopy; SPCE – Screen-printed carbon electrode; rGO – Reduced graphene oxide; AgNW – Silver nanowire; SWCNTs – Single walled carbon nanotubes; MWCNTs – Multiwalled carbon nanotubes; GCE – Glassy carbon electrode; LSV – Linear sweep voltammetry; GO – Graphene oxide; ITO – Indium-tin oxide; AuNPs – Gold nanoparticles; ssDNA – Single strain deoxyribonucleic acid; CTV – *Citrs tristeza virus*; CV - cyclic voltammetry; DPV - differential pulse voltammetry; 8-WE-SPCE – working electrode screen-printed carbon electrodes; PPY – Polypyrrole; Lithographically patterned nanowire electrodeposition (LPNE); GNP – gold nanoparticle; RTBV – Rice tungro bacilliform virus; RTSV – Rice tungro spherical virus.

[114,136,137]. They are isolated from a large random sequence pool by an *in vitro* screening process called Systemic Evolution of Ligands by Exponential Enrichment (SELEX) [111]. Aptamers are short, single-stranded synthetic nucleic acids or peptides able to recognize target molecules with high affinity and specificity [138,139]. They are similar to antibodies regarding their binding affinity and can adopt different assay configurations to transduce the bio-recognition event (**Fig. 3**C) [125]. However many studies have reported distinct advantages of aptamers over antibodies [140], including chemical and thermal stability, cost and ease of production and chemical modification [123,141]. Lautener et al. developed a aptamer-based biochips for label-free detection of apple stem pitting virus (ASPV) by surface plasmon resonance (SPR) imaging [137].

### 2.2.2. Immobilization strategies

The biomolecule immobilization is an important feature to design biosensors with appropriate performance, in terms of reproducibility, sensitivity, reliability, response time, and operational stability [142]. It is crucial that the biological element retains its structure, sensitivity, and biological activity while simultaneously not decaying or desorbing over the use of biosensor [126]. Several approaches have been used for the immobilization of bio-probes, including adsorption, covalent attachment, encapsulation, and entrapment, as illustrated in **Fig. 3**D [142,143]. The choice of the most appropriate and judicious technique depends on the biomolecule nature, the transducer, and the detection mode [139]. The immobilization efficiency also depends on the physicochemical characteristics of the analytical procedure (such as the pH value and the ionic strength) and matrix surface (such as pore size and shape, as well as hydrophilic and hydrophobic properties) [144].

The most common procedures for immobilization of biocomponents are adsorption and covalent bonding [114,144]. Adsorption is an attractive method due to its simplicity, rapidity and low cost, and




*(caption on next page)*

**Fig. 4.** (A) Schematic representation of the immunosensor fabrication showing the immobilization of the antibody (Ab) onto the gold nanoparticles (GNP). Adapted with permission from ref. [20]. Copyright 2018 Elsevier. (B) DPV curves showing the decrease in current values in relation to the concentration of the GBNV-N protein (i) in the range of 0.5 ng $mL^{-1}$ to 150 ng $mL^{-1}$ and (ii) in comparison between healthy and infected leaf extracts from the three host plants. Reproduced with permission from ref. [160] Copyright 2021 Elsevier. (C) Variation of the current response as a function of PPV concentration for the sensor functionalized with anti-PPV antibodies (black circles), anti-Tumor Necrosis Factor antibodies (blue squares), and the sensor response for a sample free of PPV (red triangle). Adapted with permission from ref. [43]. Copyright 2019 Elsevier. (D) Projection plot obtained using the conductance values for strawberry deterioration due to fungal inoculation. Adapted with permission from ref. [44]. Copyright 2016 MDPI. (E) (i) Photograph of the graphene-based wearable sensor attached to a tomato leaf and (ii) schematic illustration of the sensor and the representation of its interaction with volatile organic compounds through hydrogen and halogen bonds. Adapted with permission from ref [178]. Copyright 2021 Elsevier. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

involves the attachment of the biomolecule to the matrix surface via van der Waals forces, electrostatic forces, hydrophobic interactions, and/or hydrogen bonds [126]. For instance, Wongkaew and Poosittisak reported the successful immobilization of a DNA probe onto a glassy carbon electrode coated with chitosan film for the electrochemical detection of sugar-cane white leaf disease [145]. The ssDNA remained on the electrode surface even after washing, indicating that they were strongly attached via electrostatic interaction between the negatively charged DNA probe and the positive-charged amine terminal group of chitosan. Despite the advantages of the adsorption method, the overall performance of the adsorbed bioreceptor-based sensor is usually low because of leaching and lack of orientation, which might restrict the interaction between the immobilized bio-probe and the analyte [132, 146].

Covalent immobilization procedures offer a way to permanently fix the bioelement to the matrix surface, resulting in high efficiency, specificity, and stability [128]. These methods are based on the reaction between the terminal functional groups of the biomolecule (not essential for its bioactivity) and functional groups on the matrix surface, such as carboxyl (–COOH), amine ($–NH_2$), hydroxyl (–OH) and sulfhydryl (–SH). Functional groups available in the biomolecule mainly originate from the side chain of the amino acid, which include the amino groups from lysine, carboxyl groups from aspartate and glutamate, sulfhydryl groups from cysteine and phenolic hydroxyl groups from tyrosine [144]. Is important to mention that the immobilization reaction should be performed under mild conditions to avoid the denaturation and conformational change of the bioreceptor [147,148]. The covalent attachment of the biomolecule onto the electrode substrate can be performed by a direct reaction or by using cross-linking agents, and in both cases, blocking steps may be necessary to limit non-specific binding [119]. In addition, depending on the nature of the electrode, several protocols can be used to introduce active groups onto the matrix surface [143]. Keeping this in mind, Jarocka and coworkers developed an immunosensor for the detection of *Prunus necrotic ringspot virus* (PNRSV) by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry to modulate the surface chemistry of the electrode [149]. The immunosensor fabrication followed a step-by-step procedure: (i) creation of –COOH groups by oxidation of the electrode surfaces, (ii) covalent immobilization of protein A with EDC/NHS coupling reaction, (iii) immobilization of anti-PNRSV IgG polyclonal antibody and (iv) filling free spaces with BSA. Each step was controlled with cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

## 3. Nanomaterials-based sensors for monitoring crops and plants diseases

Generally, biosensors can be classified according to the transduction mechanism. A transducer is used to convert an input signal into a measurable output signal. In this way, biosensors commonly employ one or more of the following signal transduction mechanisms: mechanical, thermal, magnetic, electrical, chemical, gravimetric, and radiation [150]. The application intended, the sensitivity and selectivity of the sensor, the available materials, the operating conditions, and other given features will affect the choice of the most suitable transduction mechanism [151,152]. Among them, the electrical/electrochemical, optical, and gravimetric techniques are the most frequently used for the development of plant and crop disease sensors and will be discussed in the next subsections.

### 3.1. Electrical and electrochemical

Electrical and electrochemical techniques have been increasingly employed for plant disease monitoring. Among some advantages over conventional methods, one can highlight the simple operational methods used, sensitivity, selectivity towards specific pathogens, and the possibility of developing portable commercial devices enabling *in situ* measurements [114,135,153,154]. Table 2 summarizes some works using nanomaterials in electrical and electrochemical methods to detect plant disease.

The vast majority of plant disease detection by electrochemical methods are based on pathogen recognition through the use of biosensors [12,165]. Specifically, biochemical reactions between the functionalized electrode and the analyte generate electron transfer that can be used to detect and quantify this analyte using amperometric, voltammetric, potentiometric, and impedimetric measurements [135, 166]. Besides the high specificity and sensitivity, electrochemical biosensors can be used to detect the analyte in turbid media and can be miniaturized [12,165]. Freitas et al. detected the capsid protein from the Citrus tristeza virus (CP-CTV) using gold nanoparticles (AuNPs), magnetic beads, antibodies anti-CP-CTV, and horseradish peroxidase enzyme (HRP) [31]. A disposable microfluidic device was constructed to magnetically capture the biomarker in which the monoclonal antibodies specific for the capsid protein of Citrus tristeza virus were immobilized on the AuNPs and the HRP enzyme and the polyclonal capture antibody were conjugated to the magnetic beads. The biosensor presented a wide linear range of 1.95–10.0 x $10^3$ fg $mL^{-1}$ and a low limit of detection (LOD) of 0.3 fg $mL^{-1}$. Selectivity tests were performed with 3 other citrus plants pathogens. The response signals for these pathogens presented the same magnitude of the blank solution and the control (non-infected plants). The device was applied in the detection of CTV in healthy and infected plant samples, which results agreed well with the standard comparative method (ELISA). Furthermore, the estimated cost to produce the sensor was about USD 1.99 per device, lower than the ELISA assay. Zhao and coworkers also used Au NPs and HRP to detect a Gram-negative plant pathogenic bacterium, namely the *Pantoea stewartii* sbusp. *stewartii*-NCPPB 449 (PSS) [158]. The authors used linear sweep stripping voltammetric (LSV) and the modified glassy carbon electrodes (GCE) to achieve a wide linear range ($2.0 \times 10^7$ to $4.0 \times 10^4$ cfu $mL^{-1}$) and a LOD of $7.8 \times 10^3$ cfu $mL^{-1}$, which was 20-fold higher than the conventional ELISA method. Also, the sensor showed good recovery results (ranging from 90.6% to 107.5%) and good selectivity when tested with four other types of plant pathogenic bacteria.

In another work, Haji-Hashemi et al. developed an electrochemical immunosensor to detect PthA protein for citrus canker diagnosis [20]. The immobilization of the anti-PthA antibody was performed onto AuNPs (GNP), as shown in **Fig. 4A**, and fast Fourier transform square wave voltammetry (FFT-SWV) technique was used to detect the antigen. By increasing the PthA concentration, the FFT-SWV peak currents decreased due to the antigen-antibody complex formation. A linear

relationship between the current response and logarithm of the PthA concentration was observed from 0.03 to 100 nM and a LOD of 0.01 nM was obtained. The immunosensor presented reproducibility (relative standard deviation of 3.9%), selectivity (through the analyses of healthy plant sap sample, BSA, and myoglobin), and stability (showing 97% of the original response after 7 days). Furthermore, it was tested against healthy plant sap samples artificially infected. The results obtained with the electrochemical biosensor showed good agreement with those of the PCR method, revealing its potential to be used for the early diagnosis of citrus canker disease.

Fang et al. used $SnO_2$ and $TiO_2$ nanoparticles on screen-printed carbon electrodes (SPCE) to detect *p*-ethylguaiacol using differential pulse voltammetry (DPV) [157]. The analyte is a volatile produced by fruits and plants infected with the *Phytophthora cactorum* fungus. Both sensors presented low LOD: 35 nmol $L^{-1}$ and 62 nmol $L^{-1}$ for the electrodes modified with $TiO_2$ and $SnO_2$, respectively. An interference study performed with 6 compounds showed that the maximal response variation was 6.7%, demonstrating the high selectivity of the sensors. The determination of *p*-ethylguaiacol in real infected samples was evaluated by mimicking the composition of a real fruit volatile signature. The recovery range varied from 91 to 101% and the relative standard deviation values were between 4 and 5% for both electrodes. Chaudhary et al. used a graphene oxide (GO) based electrochemical immunosensor to detect Groundnut bud necrosis orthotospovirus (GBNV), a pathogen responsible for viral epidemics that requires early detection and periodic monitoring to avoid a fast vector transmission [160]. The GO was deposited onto indium-tin oxide (ITO) substrates to provide electrical conductivity due to the $sp^2$ carbon domains and enabled the modification of the sensor with anti-GBNV antibodies. DPV technique was used to detect the GBNV nucleocapsid (GBNV-N) protein in the range of 0.5–150 ng $mL^{-1}$ (**Fig. 4**B(i)) with and a LOD of 5.7 ± 0.7 ng $mL^{-1}$. The reusability of the sensor was tested and revealed that after 3 and 7 cycles the activity decreased by less than 3% and 10%, respectively. Furthermore, leaf extracts from the three host plants (Tomato, Cowpea, and N. benthamiana) infected with GBNV were analyzed. As shown in **Fig. 4**B (ii), the measurement with the infected plants resulted in a decrease in the current signal due to the antigen-antibody interaction.

Impedance spectroscopy technique has shown to be a useful technique for plant disease analyses, which can be applied either by monitoring changes that occur in plant tissues, revealing the physiological state of the organism (indirect method), or by detecting pathogens (direct method) [153,167,168]. The method works by measuring changes in electrical or electrochemical properties as a function of the excitation frequency [153,154]. The use of the techniques to assess plant disease using the indirect method is very usual. Frequently, such methods do not make use of nanomaterials since the analyses are performed submitting only the part of the plant to be analyzed to the impedance measurements [36,169]. However, the use of nanomaterials can be employed to achieve enhanced performance in the detection of pathogens or substances that indicate plant disease. In this direction, Khater et al. used electrochemical impedance spectroscopy (EIS) to detect *citrus tristeza virus* (CTV), responsible for causing the Tristeza disease in citrus and consequently a low fruit production [30]. For this, AuNPs were electrodeposited onto screen-printed carbon electrodes to immobilize thiolated single-stranded DNA (ssDNA) and enhance the electrode conductivity. The impedimetric biosensor was designed to detect the nucleic acid of the CTV by the change in charge transfer resistance values due to the DNA hybridization. The sensor presented a logarithmic relationship between electrical resistance values and CTV-related DNA concentrations in the range of 0.1 to 10 μmol $L^{-1}$ and presented a LOD of 100 nmol $L^{-1}$. The analyses with real samples (leaf extracts) also showed high sensitivity of the biosensor, which was able to detect the target DNA in a concentration of 500 nmol $L^{-1}$ and presented good recovery values (90 – 97%).

Sensors based on field-effect transistors (FETs) have also been used as an analytical technique to detect plant pathogens. The FET sensors identify the analytes through changes in the conductance of the FET channel that occur due to the adsorption of the target molecules [170]. Tran et al. used a FET chemiresistive biosensor to detect citrus greening, or Huanglongbing (HLB), a very important citrus disease [5]. Since no treatment is effective against HLB, disease management relies on minimizing its spread by detecting infected trees in the early stages of the infection and making their remotion. To detect the HLB, the authors developed a sensitive FET biosensor using SWCNTs and selective antibodies against Sec-delivered effector 1 (SDE1), a secreted protein biomarker of the bacterial species *Candidatus* Liberibacter associated with HLB. The detection of the SDE1 HLB protein biomarkers was performed in real plant tissue samples, in which an increase in resistance response was observed with the increase in the SDE1 concentration. The LOD was found to be 5 nM and the FET biosensor showed selectivity towards SDE1 when tested with bovine serum albumin (BSA) even in complex plant tissue matrices. The suggested mechanism was related to the net of positive charges located onto the surface of the antibody-bound SDE1 proteins that induced an n-doping of the p-type SWCNT channel causing the decrease in conductance.

In another work, the Plum Pox Virus (PPV) was detected in plant extracts by using an electrolyte-gated organic field-effect transistor (EGOFET) [43]. The PPV causes a highly infectious disease (Sharka) in stone fruit trees and the damage can only be diminished by early detection and frequent monitoring. To fabricate the device, the source and drain electrodes were made of Au 50 nm thick with a few nm of Cr adhesive layer on a quartz substrate, to yield a roughness lower than 2 nm. Then, a pentacene film of 15 nm was deposited onto the substrates. The polycrystalline Au wire gate electrode was functionalized with Cys-Protein G and PPV antibodies, which endowed selectivity to the device. A current decrease was noticed with increasing concentrations of PPV. Figure 4C shows the selective response of the EGOFET sensors towards PPV, in which no detection is observed when the sensor was functionalized with a different antibody (anti-Tumor Necrosis Factor, TNFα) and in healthy samples. The sensing principle was ascribed to the capacitance decrease upon PPV biorecognition due to the total passivation of the gate electrode, which led to changes in current. The obtained LOD of 180 pg $mL^{-1}$ and a dynamic range from 5 ng $ml^{-1}$ to 50 μg $ml^{-1}$ suggest the use of the proposed immunosensor as a low-cost, portable, and sensitive alternative for Sharka monitoring.

Electronic nose (e-nose) is another approach that has been frequently used for plant disease detection [171–173]. The e-nose is an analytical instrument used for gas analysis that mimics the olfaction biological system. It is composed of a non-specific multisensory array and a data-processing method. The e-nose must present cross-sensitivity, *i.e.*, sensitivity to several components of the analyzed sample, and reproducibility [174,175]. In plant disease detection, e-noses are used as an indirect method to evaluate volatile organic compounds (VOCs) that indicate the plant health status [172,173]. These VOCs can be originated from protective compounds, plant hormones, insect pheromones, digestive or metabolic byproducts, and compounds deriving from cell damage [12,21,135,173,176]. The e-nose may not determine the VOCs analyzed or make their quantification. Instead, when the analyte interacts with the sensor units, there is a change in the electrical response of the sensing material, and a pattern corresponding to the gas composition of the sample is generated. In this way, each sample analyzed generates a fingerprint and can be distinguished from other samples and known pattern samples through statistical tools [172,173]. As advantages of the e-nose in the monitoring of plants' health, one can highlight the operational simplicity, non-invasive, rapid, cost-effective, and bulk sampling analyses [171–173]. Like the EIS, many reports using e-nose do not require the use of nanomaterials or labels to detect diseases in plants. In these cases, they use the plant directly in the measurements. Some examples are the detection of the CTV using leaf samples [29] and basal stem rot (BSR) disease using the trunk of a Besout oil palm [177].

By combining nanomaterials with the e-nose approach, one can achieve enhancement in performance in terms of sensitivity. In this

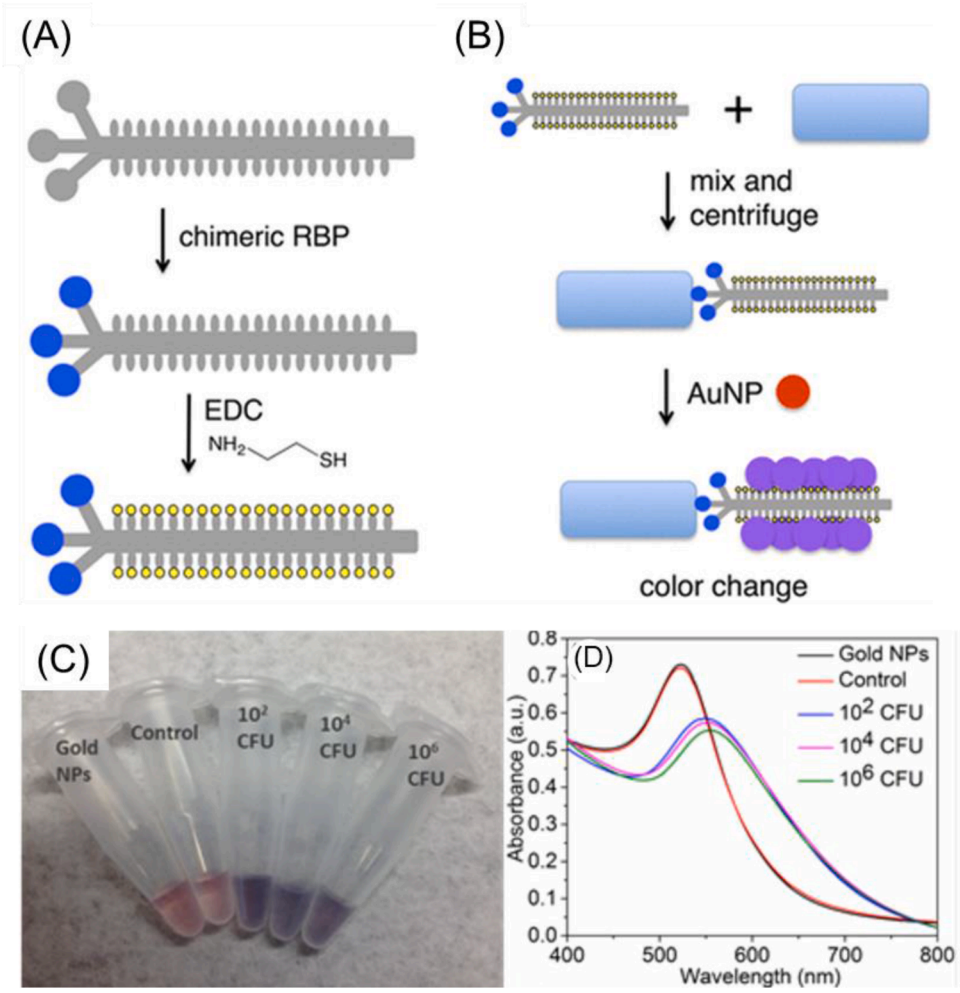



**Fig. 5.** Chimeric Phage Scheme for Pathogen Detection [181] Copyright 2018 American Chemical Society. (A) M13 phage (gray) expresses a foreign receptor binding protein (blue circle). Chimeric phages are thiolated (yellow) via EDC chemistry. (B) Thiolated chimeric phages are added to a bacteria-containing medium (blue rectangle). The device is resuspended in solution with AuNPs (red), whose aggregation in the thiolated phage produces a color change (purple). (C) Colorimetric detection of X. campestris (pv campestris) in water. (D) Quantification of X. campestris (pv campestris) by UV–vis Spectroscopy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

regard, a bioelectronic nose was used by Wang et al. to detect p-ethylphenol, an important plant VOC released by the *Phytophthora cactorum* (*P. cactorum*) in infected strawberries [14]. Seven sensing units were fabricated using SWCNTs immobilized onto a FET and non-covalently functionalized with ssDNA through π–π interaction, and a bare electrode. The functionalization with the ssDNA with different sequences led to distinct resistive responses of the sensing units and assured cross-sensitivity to the e-nose. The system was able to detect 4-ethyl phenol, the compound responsible for the characteristic off-odor of strawberries infected by P. *cactorum*, in concentrations ranging from 0.25% to 100% of saturated vapor. The performance of the sensing unit without the biorecognition element was found to be nearly one order of magnitude lower than one of the sensing units using the ssDNA. The e-nose was tested against ten VOCs at different concentrations released by fungus in infected strawberries. Data were treated with neural network fitting (NNF) and Gaussian process regression (GPR) to build prediction models. The bioelectronic nose was further used to diagnose if strawberries were infected by *P. cactorum*.

Greenshields and co-authors also used an e-nose to detect fungus infection in strawberries [44]. For this end, the authors employed three different carbon nanostructures (CNSs), *i.e.*, MWCNTs doped with nitrogen (N-MWCNTs) and boron (B-MWCNTs), to modify the interdigitated ENIG (Electroless Nickel Immersion Gold) electrodes of the e-nose by drop-casting. The prepared electrodes were exposed for 240 s to VOCs exhaled by the strawberry fruits inoculated with *Rhizopus* and *Aspergillus*. The measurements were also conducted with only the fungal colonies, without the fruits. Variation of the conductance as a function of time was used to calculate the response and the tristimulus methodology was used in data treatment. The yielded projection (Fig. 4D) showed that the e-nose was able to identify strawberries infected with *Aspergillus* from those infected with *Rhizopus* and no infected ones. Moreover, the response of the fungal colony alone was close to the respective infected fruits, revealing the ability of the system in detecting the VOCs produced by the fungus.

Li et al. used a leaf-attachable (Fig. 4E(i)) chemiresistive sensor array to continuously monitor VOCs related to plant disease [178]. The array was composed by reduced graphene oxide (rGO) and AuNPs as sensing materials. Flexible silver nanowire (AgNW) electrodes and a stretchable substrate were used to minimize strain interference. The size of the sensor patch containing the array was 12 mm × 30 mm, with a weight of approximately 0.7 g, and an estimated cost of $1.1 per patch. The choice of the materials allowed a selective detection of oxygen- and nitrogen-containing organic compounds through reversible interactions via hydrogen and halogen bonding, as schematized in Fig. 4E(ii). Moreover, the system was able to differentiate individual plant VOCs. Specifically, 13 plant VOCs at 10 ppm were distinguished with an accuracy of 97.6%. The wearable sensor platform was also used in the early detection of tomato late blight after only 4 days of inoculation. The device proposed by the authors in this work paves the way for the development of miniaturized platforms that enable noninvasive sensing with high sensitivity, real-time, and in-situ measurements, in which the use of nanomaterials will certainly play a fundamental role.

### 3.2. Optical

Optical sensors are devices capable of detecting and measuring

variations in the optical properties of a material and converting these changes into a measurable electronic signal. The techniques most used in optical sensors are colorimetry, fluorescence, surface plasmon resonance (SPR), flow cytometry, lateral flow assay (LFA), chemiluminescence, and bioluminescence [179]. The use of optical sensors offers advantageous features, such as rapid detection, simple operation, low cost when compared to other detection techniques. In this section we explore the different optical sensors that have been applied for the detection of diseases in plants, and some examples are displayed in Table 3.

Colorimetric sensors are devices that enable qualitative and quantitative detection of analytes through color changes, where the optical signal produced may be observed by naked eye or measured by a photodetector [180]. For instance, Li et al. developed a smartphone-based VOC sensing platform for the colorimetric detection of *Phytophthora infestans* in tomatoes [21]. A sensor array was prepared by the modification of nitrocellulose paper with functionalized AuNPs with different chemo-responsive organic dyes. The proposed sensor array demonstrated ability to detect the main volatiles of the plant at 10 ppm in 1 min of reaction. Additionally, early detection of tomato late blight occurred after 2 days of inoculation and with a detection accuracy of $\geq$95%. Only two samples were misdiagnosed by the VOC sensor, one being false positive and the other being false negative. Sensor selectivity was also evaluated by comparing the VOC pattern of *Phytophthora infestans* with those of two other tomato pathogens. The sensor was able to differentiate between pathogens and control with an overall classification accuracy of 95.4%. Therefore, the sensor was efficient in accurately differentiating infected plants from healthy ones. Peng and Chen developed a colorimetric detection device for the phytopathogen *Xanthomonas campestris* [181]. The authors produced the M13 phage to display the receptor-binding protein of a phage that naturally targets the bacteria. The phages obtained enabled the binding of AuNPs that acted on signal amplification, resulting in a visible color change (**Fig. 5**). The pathogens were detected using a colorimetric sensor and UV–vis spectroscopy was used for quantification, yielding a LOD of $10^2$ CFU $mL^{-1}$ and a linear working range from $10^2$ to $10^6$ CFU $mL^{-1}$. The authors further assessed the specificity of detection by identifying 6 different bacteria. No change in SPR peaks was observed in any case. This result demonstrated the selection accuracy, indicating little cross-reactivity within the group of tested Gram-negative microorganisms. Miranda and collaborators developed an immunosensor for the diagnosis of Asian soybean rust in the early stages of the disease [182]. The immunosensor was obtained by immobilization the antigen on a nitrocellulose membrane substrate with a size of 0.5 $cm^2$. Later, fluorescent nanoparticles coated with polyclonal IgG antibodies were used as recognizers. The immunosensor showed a linear range of responses for concentrations from 0.0032 to 3.2 μg $mL^{-1}$ and the LOD was 2.2 ng $mL^{-1}$. The proposed sensor presented a detection range comparable to ELISA and PCR. In addition, field tests demonstrated the sensor was superior in terms of performance to commercial test kits available in the market.

SPR-based sensors are capable of measuring biomolecular interactions in real-time, being useful analytical tools for measuring the adsorption of target analytes onto molecular probes attached to the metal surface [41,183]. Different studies in the literature report the use of SPR to diagnose plant pathologies [41,183,184]. Razmi and colleagues used localized surface plasmon resonance (LSPR) of AuNPs to obtain colorimetric biosensors [46]. The device was used to detect the *Tomato yellow leaf curl virus*. Basically, the authors designed a DNA probe based on the virus genome to complement the coat protein region. Using both infected and uninfected plants, DNA was extracted from chlorotic and curly leaves of infected plants and further mixed with buffer. Afterward, AuNPs were added to the extract. The samples of infected plants showed color change, also confirmed by UV-Vis spectroscopy. The selectivity was evaluated by comparing the detection of a control sample and the beetroot virus (BCTV-Svr), which has 30% similarity with the *Tomato yellow leaf curl virus* genome. The authors observed that the sensor did not present a colorimetric response to control and BCTV-Svr, indicating good selectivity. Biosensor sensitivity was performed using comparative PCR analyses. PCR was able to detect 1.5 ng of DNA extracted from infected plants, while the biosensor showed detection of 5 ng. The linear calibration curve was determined from 0.75 to 200 ng $\mu L^{-1}$ and the LOD was calculated as 5 ng $\mu L^{-1}$ .

The LFA is a technique that uses chromatography together with conventional immunoassay [185]. LFA has gained prominence in diagnosis due to its low cost, ease of use, quick response, transportability, and visual assessment. LFA uses nanoparticles with colorimetric or fluorescent properties to act as optical labels providing visual inspection for the detection of the analyte of interest. Among the nanoparticles used in LFA, AuNPs, colored latex beads, magnetic particles, CNTs, and quantum dots (QDs) stand out [185]. In this direction, Wei and colleagues developed a biosensor to detect the pathogen *Alternaria panax Whetz* that attacks ginseng [186]. The authors used as a strategy the use of nested single-tube PCR LFA. The biosensor was based on colloidal gold nanoparticles-streptavidin (AuNPs-SA with 30 nm), mouse anti-Fam antibody and BSA-Biotin conjugate. The proposed biosensor provided a 100 times higher sensitivity when compared to the conventional PCR technique, and LOD = 0.01 pg $\mu L^{-1}$ of genomic DNA. In addition, selectivity was evaluated in a trial with six other pathogens. The authors found that the biosensor did not cross-react with other non-target samples. And in real samples with multiple soil samples, the biosensor showed 100% selectivity, indicating its suitability for diagnosing plants under attack by the pathogen.

Zhan and colleagues also used the same technique employing the assembly of biosensors based on 15 nm-AuNPs and universal primer to detect the *Phytophthora infestans* pathogen responsible for potato late blight. [185]. The linear range of biosensor response was from 100 pg $\mu L^{-1}$ to 0.1 pg $\mu L^{-1}$ with $R^2 = 0.96$. The biosensor LOD was 0.1 pg $\mu L^{-1}$ of genomic DNA and the selectivity was also proven. The selectivity of the proposed biosensor was tested against 5 species of pathogenic and control fungi. The sensor responded only to the pathogen of interest.

Still on the diagnosis of diseases that affect the potato crop, Panferov and collaborators produced an immunosensor to detect the *leafroll virus* [39]. The immunosensor was based on anti-PLRV antibodies, AuNPs as labels and silver enhancement, yielding a LOD = 0.2 ng $ml^{-1}$. The linear range of studied concentration was from 0.1 to 100 ng $ml^{-1}$. Therefore, the immunosensor was 15 times more sensitive than the conventional technique. The authors concluded that the immunosensor allowed for rapid and accurate control of primary screening. To detect the pathogen that causes bacterial spot disease in stone fruits and almonds, López-Soriano and collaborators used LFA and an immunosensor based on carbon nanoparticles and polyclonal antibodies 2626.1-WC mounted on nitrocellulose strips [27]. The obtained LOD was $10^4$ CFU $mL^{-1}$ and the linear concentration range was from 10 to $10^8$ CFU $mL^{-1}$. Biosensor selectivity was performed using different pathogens from nine countries. The biosensor displayed good selectivity, while cross-reactivity was observed in only four, which contained pathogens outside the interest of the work.

As demonstrated, many optical sensors for plant disease detection are based on AuNPs due to their simple syntheses, easy functionalization and their optical properties that facilitate detection by visual inspection. Therefore, there is still a gap of possibilities to explore other nanomaterials such as metallic nanoparticles and QDs in plant disease sensors.

### 3.3. Gravimetric

Gravimetric transducers rely on the detection of mass changes that occur during the sensing event [187]. Quartz crystal microbalance (QCM) is the most used technique for this approach [118]. This strategy to detect mass variation was reported in 1959 by Günter Sauerbrey, who established a correlation between quartz resonance frequency with the gravimetric amount of a specific target accumulated on the transducer

**Table 3**
Optical sensors using nanomaterials to detect diseases in plants.

| Target /Culture | Nanomaterial | Bio-recognition | Detection method | LOD or accuracy | Other figures of merit | Performance Detection | Observations | Ref |
|---|---|---|---|---|---|---|---|---|
| *P. infestans*/ tomato | AuNPs | (Cys)-capped | C | 0.4 ppm | Linear range from 1.000 to 10.000 sporangia $ml^{-1}$ | Diagnostic specificity of 95% and classification accuracy of 95.4% | VOCs detection by platform integrates the smartphone | [21] |
| *Xanthomonas campestris*/ brassica | AuNPs | – | colorimetric | $10^2$ CFU $mL^{-1}$ | Linear range from $10^2$ to $10^6$ CFU $mL^{-1}$ | Diagnostic specificity of 100% | Application of M13 phage to display target receptor binding protein | [181] |
| *Phakopsora Pachyrhizi*/ Soybean | fluorescent nanoparticles | IgG antibodies | fluorescence | 2.2 ng $mL^{-1}$ | Linear range from 0.0032 to 3.2 μg $mL^{-1}$ | Sensitivity of 2.8 a.u.n $g^{-1}$ mL and detection limit comparable to ELISA and PCR | Use paper as a substrate for immobilization of biomolecules | [182] |
| *Yellow leaf* curl *virus*/ tomato | AuNPs | reverse primer (20-mer) | LSPR | 5 ng $\mu L^{-1}$ | Linear range from 0.75 to 200 ng $\mu L^{-1}$ | Diagnostic specificity 100% and, detection 3 times less efficient than PCR | Use of AuNPs as colorimetric probes first reported | [46] |
| *Alternaria panax Whetz*/ ginseng | AuNPs-SA | Mouse anti-Fam antibody and BSA-Biotin | LFA | 0.01 pg $\mu L^{-1}$ | Linear range from 1 ng $\mu L^{-1}$ to 0.01 pg $\mu L^{-1}$ | Sensitivity 100 times greater than PCR and, Diagnostic specificity 100% | Result 100 times more sensitive than traditional PCR | [186] |
| *Phytophthora infestan*/ potato | AuNPs | streptavidin-biotinylated T and C | LFA | 0.1 pg $\mu L^{-1}$ | Linear range from 100 pg $\mu L^{-1}$ to 0.1 pg $\mu L^{-1}$ | Diagnostic specificity 100% | Detection from infected leaf and stem samples | [185] |
| *Leafroll virus*/ potato | AuNPs and silver | Anti-PLRV antibodies | LFA | 0.2 ng $ml^{-1}$ | Linear range from 0.1 to 100 ng $ml^{-1}$ | 15 times more sensitive than conventional detection technique | Nanoparticles increased the detection sensitivity 15 times | [39] |
| *X. arboricola pv. Pruni*/ stone fruits and almond | carbon nanoparticles | Polyclonal antibodies 2626.1-WC | LFA | $10^4$ CFU $mL^{-1}$ | Linear range from 10 to $10^8$ CFU $mL^{-1}$ | Diagnostic specificity of 100% and diagnostic sensitivity of 96.1% | Obtaining results in 15 min, portability and field application | [27] |

LOD: Limit of Detection; AuNPs: Gold nanoparticles; VOCs: Volatile organic compounds; LSPR: Surface plasmon resonance; LFA: lateral flow assay ELISA: Enzyme linked immuno sorbent assay; PCR: Polymerase chain reaction. Gravimetric.

surface [188]. To be used as a biosensor, the QCM electrode is in general modified with a bio-recognition element, such as antibody, nucleic acid probes or even molecular imprinted polymers [189]. The gravimetric variation that reflects on frequency is a binding response of the bio-element with a specific receptor, and a frequency variation.

Plant pathogens quantification using QCM apparatus based on antibody immobilization [19,190] have been proposed, but improvements enabled by nanotechnology are scarcely reported. One of those few examples was published by Dickert and Mann [22] who explored the presence of nanoparticles to detect tabaco mosaic virus (TMV) using a QCM apparatus. In this work, the TMV was detected by using a biomimetic polymer at the nanoscale, built with the isolated and purified virus as a template during the monomers self-organization synthesis process. The MIP obtained yielded an occupancy of the imprinted templates pits of 90%. The mass variation measured were proportional to the TMV concentration due the nano-structured patterns and trenches of different sizes and shapes that serve as recognition sites during the virus detection. The novel QCM sensor could quantify TMV from 100 ng $mL^{-1}$ to 1 mg $mL^{-1}$ with results were obtained within minutes, directly in the plant sap according to the authors.

VOC's analysis can deliver information on plant health condition, and QCM for VOC sensing is well described on literature [191], but there is a gap when the focus is on plant pathogen detection. Nevertheless, QCM sensing performance can be improved when allied with nanomaterials, as reported by Diltemiz and Ecevit [192]. The authors proposed a formaldehyde QCM sensor with improved detection limit of 41 ppb, brought by the presence of electrospun nanofibers (200 to 250 nm of diameter) containing CuO/ZnO composite. The increased performance was related to the nanofiber features such as rough, porous structures and large surface area that permitted better access of the analyte formaldehyde at the sensor interface.

### 3.4. Thermal

Temperature variations can be measured by several thermometric transducers such as thermocouples, resistance thermometers, thermistors, diodes [193], and thermography [135]. Leaf temperature variations can deliver information regarding stomata activity towards water balance and photosynthesis performance, being correlated to disease detection [7,194–197]. Thermography allied with other imaging techniques allows the acquisition of a specific pathogen signature [198]. Thermal imaging uses long-wave infrared cameras and the equipment is calibrated to deploy temperature readings. The great advantage of this approach is that the image acquirement is not invasive [199], can be easily adapted to real-time monitoring, and can be combined with other imaging and data-mining techniques. However, it is important to mention that the use of thermal responses for plants' pathogenic detection cannot distinguish different pathogens infections with the same thermal patterns [135]. As an alternative to temperature probes based on resistance variations, Azra and collaborators investigated a thermo-responsive nanofiber mat employing electrospinning and electrospraying techniques [200]. The authors demonstrated the possibility of using thermoplastic polyurethane (TPU) nanofibers as a time-temperature indicator (TTI), which are devices able to deliver an

easily measurable time-temperature dependent change, and is commonly reported at perishable foods bioproducts [201]. The device is based on the mats deformation and shows controlled release of liquids upon heating. The liquid release is a consequence of the heat-induced relaxation of a non-equilibrium stretched state of the polymer nanofibers formed during the electrospinning process. The liquid flow from the mat was correlated as a function of temperature, which was possible due the unique nanometric diameters of the fibers that reached from 500 to 800 nm, and the rounded shape pores that the authors claim to be beneficial for the encapsulation of fluids.

Aalthough thermal transduction combined to nanotechnology is not yet widely explored for plant pathogen detection (which gap is believed to occur owing to the large amount of thermal probes and sensors that are in compliance with the needs of greenhouses and plantation in general), this topic can represent an opportunity of research and development in the future.

## 4. Conclusions and perspectives

Nanotechnology has demonstrated a great potential to enhance the development and performance of varied (bio)sensors for plant disease monitoring and management, as discussed throughout this review. Key features of 0D, 1D, 2D and 3D nanomaterials such as high surface area to volume ratio, ability to incorporate bio-recognition molecules, and the possibility of tuning specific properties are fundamental for designing high performance (bio)sensing devices. Bioengineering advances combined with nanostructures represent a step forward towards novel biosensing architectures for plant disease detection development, and should bring innovation and deployable devices towards agriculture growth and sustainability. Besides, wearable plant sensors and strategies to directly print functional circuits (towards freeform fabrication) represent promising topics for the future of on-site and real-time plant monitoring using sensing technologies.

Among the different transduction mechanisms (namely, electrical, electrochemical, optical, gravimetric and thermal ones) covered in this review, more remarkable results have been achieved using electrical and optical (bio)sensors enabled by nanotechnology. However, the transduction mechanisms that do not present major innovations achieved or improved by nanotechnology so far represent opportunities to be addressed and explored in the next years. Besides, considering the future perspectives in the field of plant disease diagnostics, we highlight the interest in developing devices that offer portability, ease of detection, real-time and expedite *in situ* monitoring for large scale and on-field applications. To meet these requirements, the nanomaterial-based sensors need to be tested under real agricultural conditions in which their performance can be affected by weather, plant growth and development. Although there are challenges to overcome, the use and commercialization of such devices are expected to increase in the coming years, in order to meet the current demands for food security and loss reduction of crops and agricultural inputs.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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