



## Advanced strategies of the *in-vivo* plant hormone detection

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### ABSTRACT

*In vivo* plant hormonal detection methods are the strategies executed on living organisms while not taking out the samples from the body. Modern methods like spectroscopy, biosensors, and electrochemical sensors have increased the quality and sensitivity of the detection and decreased the cumbersome, time, and solvent-consuming preparational efforts of traditional methods. Preparational efforts generally consisted of several samplings, extraction, purification, and enrichment steps to analyze plant hormones. Concentrations and levels of plant hormones change concerning biotic and abiotic stresses that plant faces. Spectroscopy, biosensors, and electrochemical sensors detect different plant hormones with specific accuracy and desired results, but their handling and sample preparations are challenging. At the same time, advanced ultrasensitive nondestructive methods are biocompatible and can be used for a lifetime with excellent stretchability performance. In modern science, portable, wearable, and nondestructive measurement is the trend of plant sensors that can make the "Internet of Plants" concept a reality. This review covers the significant aspects of numerous applications, advantages, and disadvantages of spectroscopy, biosensors, electrochemical sensors, and new ultrasensitive nondestructive devices to measure *in vivo* plant hormones. The summary of the advanced ultrasensitive plant devices, their challenges, and future prospects within the fields for *in vivo* plant hormone detections is presented. Furthermore, it can guide researchers to design new experiments using ultrasensitive nondestructive sensing devices for detecting *in vivo* plant hormones.

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## 1. Introduction

Plant hormones are the assembly of compounds, not related to

each other, both structurally and chemically, but they hold a strong impact on plant metabolic activities [1]. Plants' physiochemical performances [2,3], photosynthetic activities, crop growth and development, plant size and yield are ultimately influenced by hormonal activities, which are expected to enhance food production and resolve the world food shortage problem [4]. Plant hormones are commercially important too, viz., medicinal use and agricultural applications, which have made scientists to explore translocation mechanisms, signaling, biosynthesis, transportation, and responses of plant hormones [5]. The main species of hormones include gibberellin (GA) [6], auxin [7], abscisic acid (ABA) [8], salicylic acid (SA) [9], cytokinin (CTK) [10], ethylene [11],

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brassinosteroid (BR) [12], polypeptide hormones, strigolactones (SL) [10] and nitric oxide [1]. Due to their diverse characteristics and presence in trace amounts, the detection methods also need a variety of instrumentations and preparation methods leading scientists to discover numerous analytical techniques.

Many scientists have given plant hormone detection critical importance for many reasons, most importantly to discover new hormones and their metabolites and to trace the existence of specific hormones within a particular cell [13]. The analytical technologies to measure plant hormones and other elements have been classified into the categories of accurate, rapid, and sensitive methods through the development of scientific research [14]. The existing analytical techniques are subjected to conventional detection methods. Bioassays, immunoassays, and chromatographic techniques involve extensive sample preparation steps such as extraction, concentration, and purification [15]. Moreover, these techniques are not good enough to separate the molecules and measure them at the lowest point of their existence within the plant tissues. Modern methods have identified the quality and sensitivity aspects of the detection methods that have switched laboratories to spectroscopy, biosensors, electrochemical sensors, and wearable devices [16,17]. These have significantly improved the detection limits and sample recoveries by allowing selective and sensitive detections at the individual tissue levels [18–20].

Nuclear magnetic resonance spectroscopy (NMR) [21], mass spectrometry (MS) [22], and Raman spectroscopy [23] are the most renowned modern detection methods in spectroscopy. NMR is good at using, but MS with modified techniques can identify and quantify in a simple matrix specifically for detecting trace plant hormones. Surface-enhanced Raman spectroscopy (SERS) with aptamer has elaborated more significant, sensitive, and reproducible results for detecting plant hormones [23,24]. With the detection efficiencies, biosensors can locate the substrates for metabolites and hormonal activities, receptors, and transporters by adopting the in vivo methods of determinations. Biosensors with in-vivo techniques are claymore which provides the temporal and spatial data and identifies missing processes, components, and signaling networks [25,26]. Especially genetically encoded biosensors have the ability to provide cellular and subcellular resolutions and can detect rapid changes in the concentrations and distributions of plant hormones in living cells [27].

Electrochemical detections are most commonly used for the determination of auxin hormone concentrations in different zones of individual plants [28,29]. The quantification level can reach the lowest level of nanogram with the required sample solution volume of 10  $\mu\text{L}$  [30]. Conductive carbon tape electrodes with carbon nanotubes are developed to use in paper-based analytical instruments with a multichannel electrochemical station to quantify the levels of hormones in salinity conditions [31]. Side by side, with the influx of online data availability and immediate mobile communication, the interaction of computers-humans based wearable sensors has been increasing in multi-folds. Various wearable sensors are also a category of human detection technologies and are used in early detection [32]. Most of the plant wearables are made from biosensors, optic sensors, and spectroscopic concepts and are used for monitoring the external factors to simulate the internal changes of the plants.

Living plants interfaced with wearable sensors can detect the internal biochemical processes and changes in the cells that occur due to the outer factors faced by plants [33]. Gas sensors installed on the leaf surfaces can detect simultaneous gas exchange from the stomata. These analyses ultimately provide data on plants' internal conditions and physiology for continuous plant observations [34]. Plant-based sensors can be installed in the field for onsite and live hormone detection within the plants' cells to continuously observe

the biotic and abiotic effects that occur in the plants. Syringe injectable mesh nano-electronic wearable device [35] that monitors mice's in vivo neural activity may record the rapid waves of plant signaling molecules with the millisecond temporal resolution. Cumulatively it can be stated that spectroscopy, biosensors, and electrochemical sensors are used to detect different plant hormones with specific accuracy and desired results, but their handling and sample preparations are challenging to use.

Analytical approaches like wearable devices are advancing and are easier to use and produce live and simultaneous results. There are numerous in vivo and in vitro methods for the measurements of plant hormones (Table 1). In vitro studies are relatively cheap, reliable, and efficient. Procurement is simple but less translatable due to its inherent inability to capture the complex metabolomic and biochemical processes within the cells. In vivo studies can elaborate safety, toxicity, and real-time efficacy with more precise, high accuracy and sensitivity. Both methods possess advantages and disadvantages with their adequate safety, efficiency, and quality of data, but the results of in vivo techniques are more specific and detailed. Moreover, findings of in vitro methods are to be confirmed on live volunteers by in vivo methods [36].

The thorough literature study has raised the main objectives of this review article significantly, indicating 1) the applications of spectroscopy, biosensors, electrochemical sensors, and advanced ultrasensitive nondestructive methods to measure in vivo plant hormones, their advantages and their disadvantages. And 2) finding the research gaps of new ultrasensitive nondestructive sensors to use for in vivo hormone detection. Furthermore, the efficiency, precision, and rapidness of spectroscopy, biosensors, electrochemical sensors, and advanced ultrasensitive methods to detect plant hormones are important, as discussed in this review.

In addition, to discuss the information about the statistics of publications over the years, the web of science has provided to show how the field of plant hormone detection is evolving, but a very limited number of publications on "In-Vivo plant hormone detection are there (Fig. 1). This review on in vivo plant hormonal detection methods is organized as follows: First, the principles, instrumentation, and applications of spectroscopy, biosensors, electrochemical sensors, and new ultrasensitive nondestructive devices to measure in vivo plant hormones. Next, presented the methodology of the manuscript by describing the device setup, sensing mechanism, and sensing performance of plant sensors in a clearer way. Further, we focused on several fundamental studies, illustrating their detection ranges for in vivo plant studies. In addition, we discussed the need for the most advanced, precise, cost-efficient, and long-lasting ultrasensitive plant sensors to be used in the agriculture sector. To round out our discussion, we provided specific examples of portable, sensitive, and nondestructive testing technologies with higher temporal resolution and expressed their future perspectives.

## 2. Plant hormones

Plant biomolecules are responsible for the enormous physiological activities occurring within plants. The major physiological responsibilities include photosynthesis, growth, responses against stresses, reproduction, longevity and senescence [90]. The plant hormones regulate these activities and are the product of secondary metabolism that helps the plants cope with environmental stimuli. The phytohormones are majorly classified into six types, auxin, abscisic acid, cytokinin, ethylene, gibberellic acid, and brassinosteroid. Jasmonate and strigolactones are new additions to this list (Fig. 2).

Auxin is the first discovered growth hormone of plants that simultaneously plays inhibitory and promotor roles on the basis of

**Table 1**  
Comparison of in vivo and in vitro measurement of plant hormones.

Methods	Types	Hormones	Measurement ranges	Limit of detection	Reproducibility (Relative standard deviations (RSDs))	Recovery	Refs.
Chromatographic analysis	Capillary Electrophoresis (In vitro)	Cytokinin, Gibberellic acid, Abscisic acid, Indole acetic acid, Naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, N-benzyladenine	40 µg/mL – 0.08 µg/mL	0.306 ng/mL	0.45–1.0%	84.6 –113.9%	[37,38]
	Gas Chromatography (In vitro)	Abcisic acid, Indole acetic acid, Jasmonic acid, Dihydro Jasmonic acid, Salicylic acid	10 ng/g – 20 ng/g	3 ng/g – 10 ng/g	1.6–11.2%	79.6 –81.7%	[39,40]
	Liquid Chromatography (High performance and Ultrapformance) (In vitro)	Gibberellic acid, Abscisic acid, Auxin, Jasmonic acid	10 <sup>-12</sup> – 10 <sup>-11</sup> g/mL	20 ng/mL – 0.030 ng/mL	0.14–7.94%	67.03 –119.83%	[41,42]
Biosensors	ABACUS, ABAlcon, SNACS (In vivo)	Abcisic acid	0.2 µM–800 µM	<400–400 nM	1.7%	87.5 –90.0%	[43 –46]
	DR5: Reporter, AuxSen (In vivo)	Auxin	3 nM–1000 nM	–	0.11%	–	[47 –49]
	BZR1-YFP (In vivo)	Brassinosteroid	100 µM	–	–	–	[50]
	ARR5, TCSn (In vivo)	Cytokinin	1 nM–1000 nM	–	45–100%	40%	[51,52]
	FP-6 × EPU, AEP, EIN3-GFP (In vivo)	Ethylene	10 µM–100 µM	34.4 µL	–	81–94%	[53,54]
	GFP-RGA, GPS1 (In vivo)	Gibberellin	0.005 µM–0.2 µM	–	0.8%	–	[55]
	Jas9-Venus, JAI3-FP (In vivo)	Jasmonic acid	50 nM–50 µM	–	–	52.4 –78.3%	[56]
	DLK2: LUC, pRATIO-SMAX1 (In vivo)	Karrikin	10 nM–1 µM	–	–	25–41%	[57]
	NPR1-FP (In vivo)	Salicylic acid	0.5 mM	–	–	56–77%	[58]
	rDAD2cpGFP, StrigoQuant, SMXL6-YFP (In vivo)	Strigolactones	10 nM–5 µM	100 fM	1–1.5%	100%	[59 –63]
Electrochemical techniques	Electrodes modifications with gold nanoparticles-based carbon nanotubes, dihexadecyl hydrogen phosphate, reduced graphene oxide (In vivo)	Indole acetic acid	1.0 × 10 <sup>-7</sup> M – 7.0 × 10 <sup>-6</sup> M	5.0 × 10 <sup>-8</sup> M	3.8%	90.0 –103.4%	[64,65]
	MeJA sensor (In vivo)	Salicylic acid	0.8 µM	0.1 µM	7–33%	71–91%	[66 –68]
	Pt electrode with Prussian blue deposition (In vivo)	Cytokinin	5 µM–75 µM	5 µM	7%	88.3 –119.1%	[69]
	Self-referencing microsensor (In vivo)	Indole acetic acid	20 µM–60 µM	0.4 µM	5.13%	99.48% –103.21%	[70]
	Nano-montmorillonite (In vivo)	Methyl jasmonate	7.0 × 10 <sup>-7</sup> mol/L – 1.0 × 10 <sup>-3</sup> mol/L	5.0 × 10 <sup>-7</sup> mol/L	>15%	96%	[1,71]
Spectroscopy	Nuclear magnetic resonance (In vivo and In vitro)	Phenylpropanoids, jasmonic acid, brassinosteroid	0.01 ng/mL – 1 ng/mL	0.005 ng/mL – 0.2 ng/mL	–	–	[72,73]
	Mass spectrometry (In vivo and In vitro)	Abcisic acid, Indole acetic acid, Jasmonic acid, Dihydro Jasmonic acid, Salicylic acid, Gibberellin	>100 kDa	0.5 fmol – 7.0 fmol	6.7–9.9%	84.6 –112.2%	[74 –77]
	Surface-enhanced Raman spectroscopy (In vivo and In vitro)	Adenosine triphosphate, indole 3-acetic acid, salicylic acid, indole 3-butyric acid	0 µM–500 µM	0.002 µM	2.4–5.3%	89.7 –105.6%	[78 –80]
Immunochemical methods	Enzyme-linked immunosorbent assays (In vitro)	Abcisic acid, Indole acetic acid	0.06 µg/mL – 1.7 µg/mL	0.06 µg/mL – 0.09 µg/mL	1.23–1.93%	90.6 –103.3%	[81]
	Liquid chromatography-Enzyme-linked immunosorbent assays (In vitro)	Abcisic acid	0.15 µg/L – 8.7 µg/L	60 ng/L	2–8%	81–90%	[82]
	Lateral flow immunoassay (In vitro)	Phytoestrogen, deoxymiroestrol	7.81 ng/mL – 1000 ng/mL	250 ng/mL	25%	84–104%	[83,84]
Wearable devices	Fiber Bragg gratings technology (In vivo)	Plant growth, temperature, and humidity factors	Temp.: –40 °C–80 °C, Humidity: 0%–100%, Light: 4500 lux–8500 lux	–	0.021%	14–17 s (recovery time)	[85,86]
	Chemo-resistive sensors (In vivo)	Ethylene	20 ppb–100 ppb	–	0.2198%	60–300 s (recovery time)	[87 –89]

the production site in plants. This is the key factor in root elongation. In addition, auxin stimulates ethylene production, cell division and differentiation [92]. Cytokinin is critically effective in the defense system during insects and pests attack and regulates the

immune system by coordinating with salicylic acid signaling. They are synthesized at plants' root tips, increasing their drought tolerance ability. They enhance cell division by concentrating on root tips, leaves, and seeds [93]. Ethylene plays various roles in plant

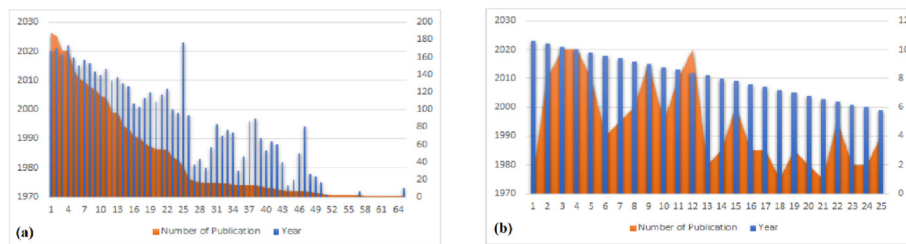


Fig. 1. The number of publications available from 1970 to 2023 on: (a). Plant hormone detection. (b). *In-Vivo* plant hormone detection. (Data source: Web of Science).

development; e.g., it reduces the stem elongation rate due to cessation. However, it increases fruit ripening, cell growth, and sex expression and regulates plant flower formation stages [94].

Gibberellic acid is usually considered a growth promoter. Its presence enhances enzyme activity, influences the nucleic acid formation and promotes growth parameters such as stem elongation, flowering, leaf expansion, and seed germination [95]. Abscisic acid helps the plant to tolerate environmental stresses and produce physiological responses. In addition, it helps to improve crop yield and quality by regulating vegetative and reproductive growth in the roots [96]. In the presence of auxin and gibberellic acid, Brassinosteroid causes cell division and elongation to help in growth. Moreover, it stimulates plants' photosynthesis, senescence, stress responses and ethylene synthesis [97].

The detection of plant hormones is necessary as they regulate plant growth and development and create the necessary measures against biotic and abiotic stress stimuli. The detection is important to understand the sites of plant hormone production, their translocation pathways, deposition mechanism, and sites. Studying the signaling pathways of hormones and their quantitative distribution in non-invasive ways helps to understand plant physio-chemical processes. The *in-vivo* sensing methods of plant hormones are getting noticed by researchers due to their non-destructive ability. Four major classifications comprising spectroscopic methods, biosensors (sensing devices with biological components), electrochemical sensors (sensing devices with electrochemical methods), and other advanced methods have been discussed for *in-vivo* detections of plant hormones.

### 3. In vivo sensing methods of plant hormones

*In vivo* bioanalytical methods are the detection techniques executed on living organisms while not taking out the samples from the body. *In vivo* and *ex vivo* detection methods are not only considered difficult to use companionily, but many sensors have yet to adopt *in vivo* detections for plant hormones. For example, biosensors and electrochemical sensors are frequently used for *in vitro* and *ex vivo* detection of plant hormones, but spectroscopy, especially SERS, and wearable devices are needed to be used *in vivo* detections. Although these techniques are not considered 'label free', their *in vivo* applications can cause toxicity; instead, biocompatible elements have proved their *in vivo* use in some fields of science, especially in the medical analysis [98]. Another method to comply with *in vivo* technique is that the *ex vivo* component was made necessary by whole-organ imaging at cellular resolution and microscopic profiling of the intact organ instead of dissecting the organ slice by slice [99]. Four major classifications comprising spectroscopic methods, biosensors, electrochemical sensors, and other advanced methods have been discussed here for *in-vivo* detections of plant hormones. The device setup, sensing mechanism, and sensing performance have been briefly explained in this review for a better understanding of the reader.

### 3.1. Spectroscopic methods

Spectroscopy is fundamentally the measurement of the spectrum generated due to the presence of atoms or molecules of the analyte in the sample [100]. Spectrum is determined by the intensity radiation pattern absorbed or emitted by the sample vs wavelength or frequency. The instrument to capture and analyze the spectrum is named as the spectrometer with the possession of a selective mechanism that splits the light beam into multiple wavelengths to only interact the specific wavelengths with the sample [101]. The most abundantly used identification techniques of spectroscopy to detect plant hormones involve nuclear magnetic resonance spectroscopy (NMR) [21], mass spectrometry (MS) [22], and Raman spectroscopy [23].

#### 3.1.1. Nuclear magnetic resonance spectroscopy (NMR)

NMR has been considered a powerful analytical technique with the ability to detect the single atom and molecule in any state of the media, either in solution or solid form [102,103]. It is used to power the most commonly available high-field microwave source today, namely the gyrotron [104]. NMR efficiency to completely analyze the molecular structure is amplified by combining infrared (IR) spectroscopy with it. Within a sample, an IR spectroscope is used to recognize the functional group, while NMR determines its type and quantifies the atoms and molecules nondestructively [105]. Though NMR's detection ability works well with many nuclei, it is very effective with the detection of carbon-hydrogen compositions. The basic modules of NMR spectroscopy are comprised of the magnet, radiofrequency oscillator, sample holder, radiofrequency receiver (detector and amplifier), and a recorder (Fig. 3) [106].

NMR is a very powerful and highly informative platform for analytical studies of metabolomics (Fig. 4 (a)) [107] and *in vivo* detections of plant hormones. In the study of the detection of chitosan for plant hormones and defense systems, NMR detected the root exudates' dynamics with the largest number of signals within the plants produced 20 days after planting (dap). Root exudates were divided into two groups and were scanned 256 times under NMR. The sensitivity was found to be different for every group, i.e., the intensity peaks were lower for phenolics groups while peaks for organic acid and amino acid were higher. The NMR signals exhibited a two-times increase in the parasitic fungus and a 1.5 times reduction in the root-knot nematodes in the root exudates with no chitosan (Fig. 4 (b)) [108].

NMR only allows the analysis and quantification of phosphorylation sites at a certain degree of incorporation [109]. Another study proposed acquiring  $^3\text{H}$  and  $^1\text{H}$  NMR spectra on a Bruker Avance-II 50 MHz spectrometer as a general procedure in the selective capture and analyses of brassinosteroid plant hormone. Tritium-labelled brassinosteroid hormone was synthesized to express in the NMR spectrum. Isotopic quantification of tritium reported with the chemical identification and comparison of  $^1\text{H}$ , standard spectrum, and  $^3\text{H}$  NMR spectrum (320 MHz, dimethyl sulfoxide- $d_6$ )  $\delta$ : 3.68 ( $1\ ^3\text{H}$ , s, 3  $\beta^3\text{H}$ ) [22]. Hydrogen-NMR spectrum was studied to

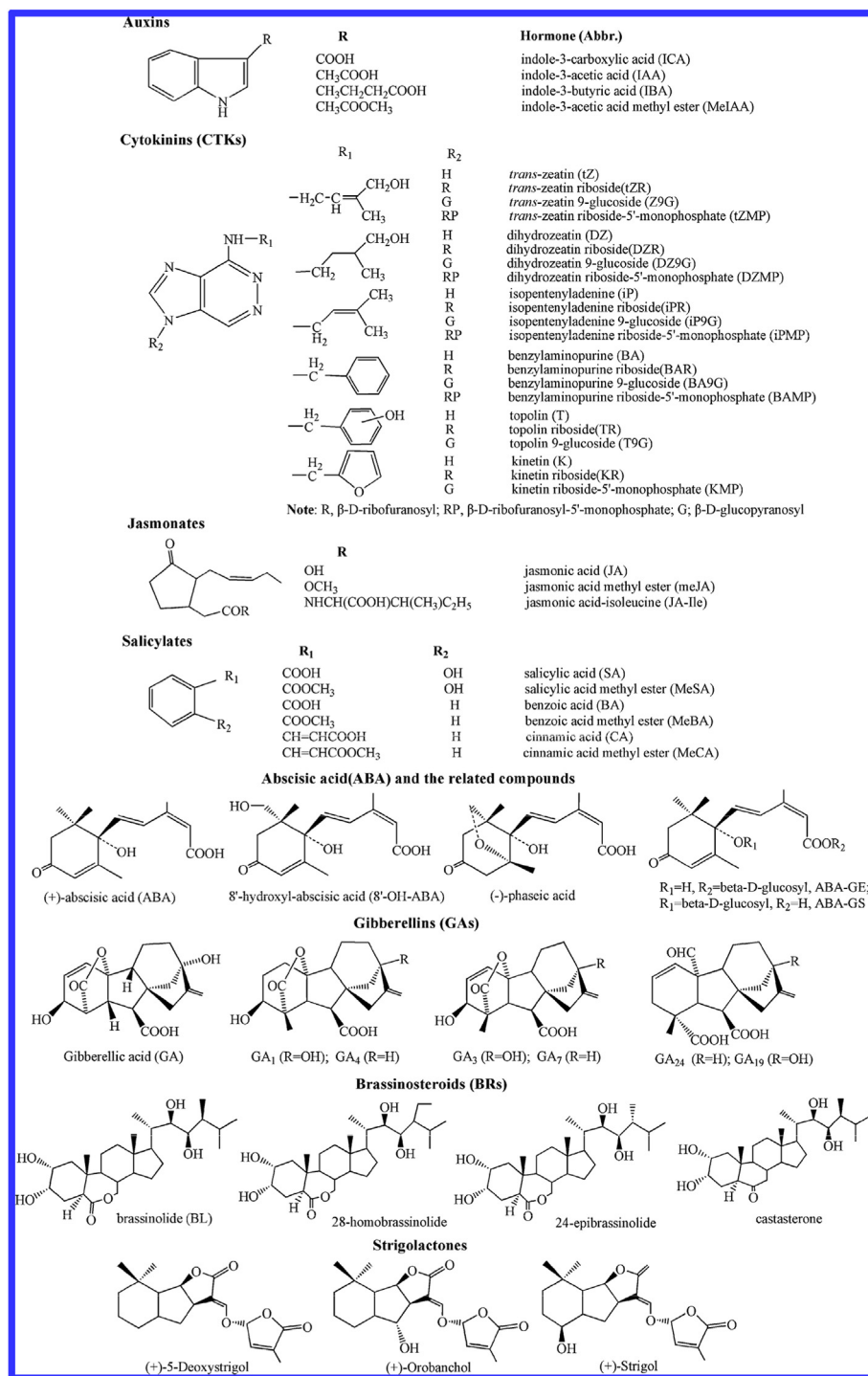


Fig. 2. Basic chemical structures of major classes of plant hormones [1,91].

determine the bioactive factors of *Abrus precatorius* and to assess the antioxidant capabilities using Bruker Biospin Avance 400 MHz NMR spectrometer. The spectrum generated several signals from the bioactive compounds and was profiled according to their chemical shift values (Fig. 5). The H NMR spectrum also elaborated and characterized many other secondary metabolites. NMR spectrum studies revealed that the *Abrus precatorius* can be used in phytopharmaceutical activities due to its antioxidant capabilities and a rich amount of major phytochemicals [110,111].

H NMR spectrum also played a significant role in the development of selective nano-sensors for plant hormone detection by providing molecular identifications. Progressively the nano-sensors were developed with the fundamental model of the corona phase molecular recognition (CoPhMoRe) concept. Unlike laborious and destructive methods, these nano-sensors were nondestructive for in vivo Auxin detections. Auxin and its derivatives were labelled with cationic polymers to check the selective response of the sensors. H NMR spectra analyzed the purity of the cationic polymers

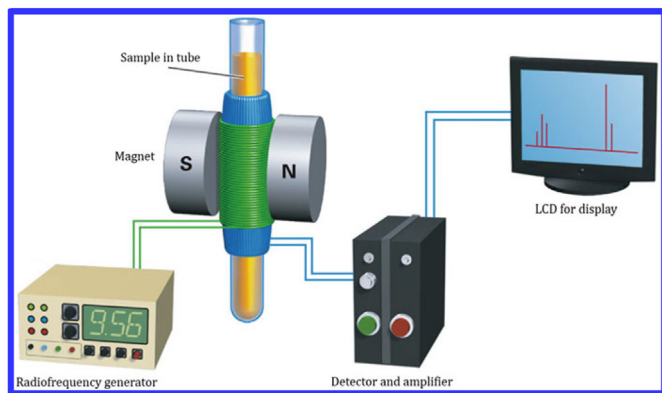


Fig. 3. Basic modules of NMR spectroscopy. Spectrum is generated between signal intensities of atoms or molecules and their frequencies [106].

with a significantly satisfactory response of 51% for Auxin and its derivatives [112].

Thus, NMR is resulted to be very effective in providing structural information, such as identifying the functional groups or sugar location in conjugate hormones. NMR is highly predictable for small molecules with the ability of analytical traceability. It is also a non-destructive method with good resolution and high flexibility. However, some limitations in NMR detections include time consumption, quite expensive materials, no ability to differentiate amongst the same compounds and, most importantly, low accuracy. NMR spectroscopy also has a low sensitivity at  $\geq 1 \mu\text{M}$  as

compared to MS, which is able to perceive the metabolites in the range of femtomolar to attomole with  $\sim 10^3$ – $10^4$  resolution [113]. This technology is still in the development phase, but many discoveries are making it great to use in detection methods. Nevertheless, the inevitable fact that limits its use is the presence of a high magnetic field which can cause interference in computer-based sensors and devices [114].

### 3.1.2. Mass spectrometry

This analytical method measures and identifies the gaseous ions in chemical and electrical fields according to the mass-to-charge ratio ( $m/z$ ) of ions in the subjected chemical substances, and the intensities plotted are named as mass spectrum [115]. In 1898 Wilhelm Wien first discovered mass spectroscopy by explaining the deflections in the charged particles due to a magnetic field. This deflection was further generated due to an electric field by J. J. Thomson in subsequent experimental years of 1907–1913 [116,117]. The principle of mass spectrometry also explained this phenomenon in which analytes from organic or inorganic compounds are ionized using electrical or magnetic methods and separated according to their  $m/z$  ratio and measures qualitatively and quantitatively [118]. The basic modules of MS are comprised of five sections, (1) high vacuum system, (2) sample handling system to incorporate the sample of the study, (3) the source of ions to produce charged particles in the form of a beam, (4) analyzer to separate beam into its components, and (5) the detector to collect the separated ion beams (Fig. 6 (a)) [119,120].

MS has comprehensive separation and high throughput analytical ability that can detect the hormone-induced signaling network with efficient separation and qualitative characteristics of

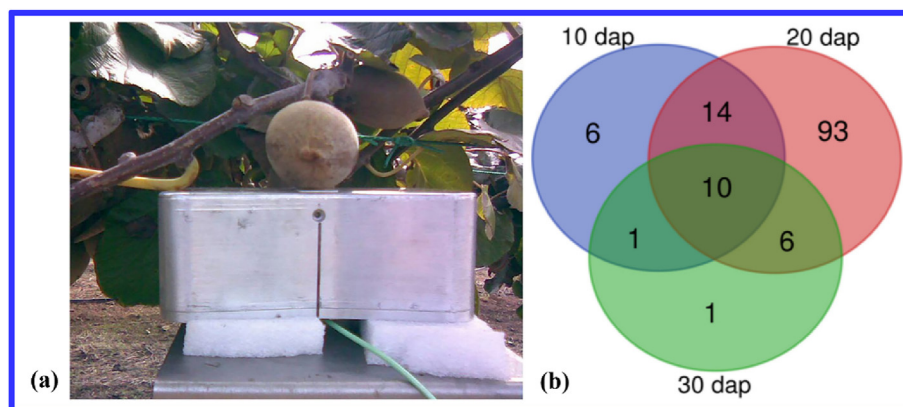


Fig. 4. NMR spectroscopy in in-vivo plant hormones detection. (a). Analytical studies of metabolomics in the intact fruit with an NMR instrument [107]. (b). In 10, 20, and 30 dap gaps, the NMR peaks for root exudates are presented with a Venn diagram. The chitosan was applied with a concentration of  $0.1 \text{ mg mL}^{-1}$ , and the release of exudates fluctuated time by time [108].

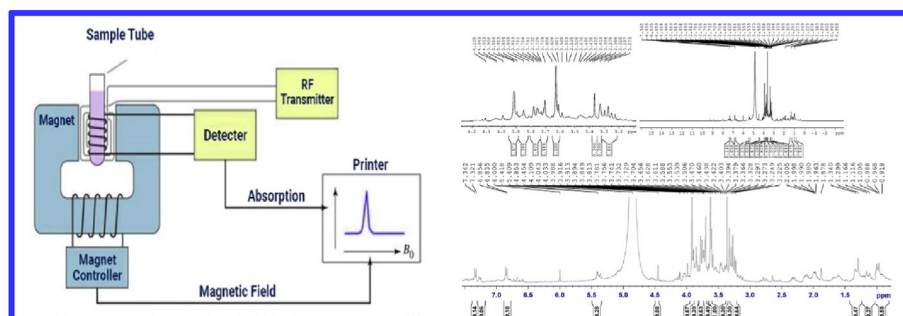


Fig. 5. H NMR spectrum generated several signals from the bioactive compounds of *Abrus precatorius* and was profiled according to their chemical shift values [110,111].

specified classes of hormones [121]. The MS has the power to eliminate the false positive signals originating from light-based assays [122]. In vivo detection study of salicylic acid phytohormone was performed under stress conditions in the seedlings of sunflower. Pt nanoflowers/ERGO microsensors analyzed and recorded salicylic acid phytohormone's high sensitivity and selectivity. The microsensor results were verified with the ultra-performance liquid chromatography-mass spectrometric results, which detected the salicylic acid in the range of 100 pM to 1  $\mu$ M with a detection limit of 48.11 pM (Fig. 6 (b)) [123].

Electrospray single quadrupole MS is recognized as a sensitive and precise quantitative method for naturally occurring cytokinin in plants, but this chromatographic bound technique makes it a destructive method. In contrast, liquid chromatography-electrospray ionization with tandem mass spectrometry approach has the ability to simultaneously determine individual or multiple classes of plant hormones [124,125]. In another precise and sensitive detection method of ion trap mass spectrometry, the four classes of phytohormones were analyzed. In a single instrument run, this nanoflow liquid chromatography-based ionization ion trap MS could detect auxins, cytokinin, abscisic acid and gibberellic acid hormones and their derivatives with 5–10 times increased sensitivity and as low as a sub-femtomole range [1,126].

The high sensitivity and selectivity of MS have simultaneously created the quantitative profiles of multiple classes of phytohormones. MS is the ideal available instrument to involve the analyte extraction as a pretreatment method without removing or damaging the enormous section of the tissues [127]. Electromembrane extraction with the combination of liquid chromatography-based mass spectrometry quantified the six acidic plant hormones and expressed the limit of detection of 0.1–10 ng mL<sup>-1</sup> [128]. Hormonal structures are the results of individual proteins and protein complexes. A technique named as immunoprecipitation was followed by tandem mass spectrometry to identify the nucleic, cytoplasmic and protein complex associated with membranes as an in vivo method [129].

The solid phase microextraction technique using the in vivo approach was used to track plant hormones and pharmaceuticals in one run to determine the stress symptoms by the hormonal response. The roots and stems of *Basella alba* L. were studied to track the jasmonic acid, salicylic acid and abscisic acid hormones with carbamazepine and ibuprofen pharmaceuticals. During the in vivo solid-phase microextraction, the analyte was analyzed using liquid chromatography based tandem mass spectrometry (Fig. 7). The evaluated analytical merits for roots and stems of *Basella alba* L. were represented as highly sensitive with  $R^2 = 0.9955$ – $0.9972$ , the limit of detection = 0.11–1.4 ng/g and the limit of quantification

0.35–4.8 ng/g [130].

The MS has versatile applications in every field of life and daily science, e.g., biotechnology uses the MS for proteins, peptides and oligonucleotides; the pharmaceutical industry, clinical and medical science, environmental science, polymer chemistry, food chemistry, and medicinal chemistry all are benefited with mass spectrometry. MS benefits them by determining the mechanisms of actions of compounds, active site identification, quantitative analysis, impurity profiling of drugs, food toxicant detections, pollutants detection in the environment, disease diagnosis, steroids, hormones, proteins, and enzymes detections and many more. The fundamental benefits of mass spectroscopy that differentiate it from other techniques are its high sensitivity, small size sample requirement, time efficiency, and the ability to differentiate the isotopes of the atom. Moreover, MS can be coupled with other techniques, i.e., chromatography techniques, to show enhanced sensitivity and acceptability. Certain drawbacks of MS efficiency include its ability to handle only pure and volatile compounds in nature [131,132].

### 3.1.3. Raman spectroscopy

This is a scattering method of spectroscopy involving the light scatters from a specific substance to provide full-fledged information on the molecular structures and chemical bonds and identifies the molecules [133]. Raman scattering basically works on the phenomenon of gain (anti-Stokes) and loss (Stokes) of energy caused by inelastic light scattering at specific wavelength and polarization angles from the substance to detect at as lowest as a single molecular level [23]. Through this phenomenal change of energy, also known as the Raman shift, the Raman spectrum is detected by Raman spectroscopy, which provides the ultimate molecular fingerprint. The structure of Raman spectroscopic instrumentation is described in Fig. 8 (a) [134].

A recently published review on the detections of plant hormones elaborated on the usage of surface-enhanced Raman spectroscopy that covered the overall efficiency of Raman spectroscopy [23]. However, Raman spectroscopy can be applied directly within the field for live analysis (Fig. 8 (b)) [135]. For effective detection of molecular properties of trace plant hormones, a pertinent substrate is needed to prepare the generation of the Raman spectra. Rapid and delicate Raman signals for flavonoid hormones were detected due to a complex substrate composed of silica nanoparticles deposited with silver nanoparticles, while ethylene-based  $\beta$ -cyclodextrin served as a ligand substrate. A hotspot on the substrate further was created for Raman shift energy transfer to detect subsistence flavonoid hormones [136].

Raman spectroscopy has also been used for the in vivo

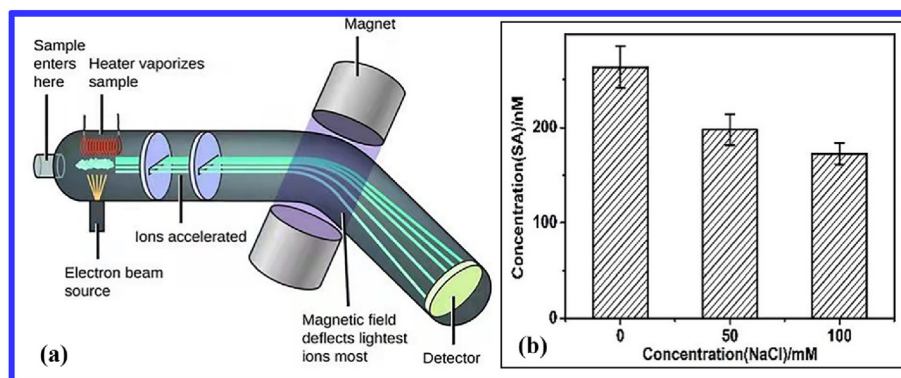
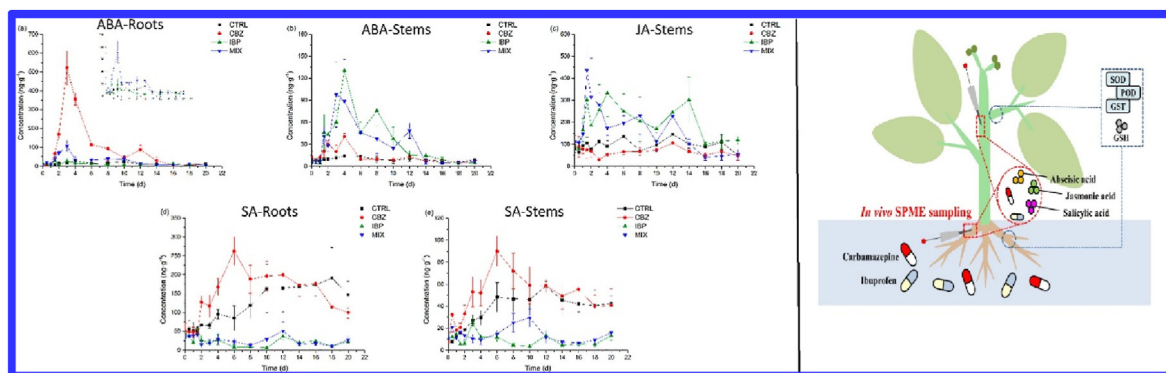
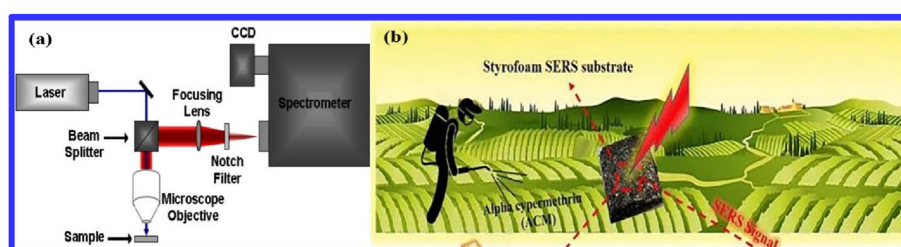


Fig. 6. Mass spectrometric in vivo hormones detection. (a). Schematic diagram of mass spectrometry [119]. (b). Ultra-performance liquid chromatography-mass spectrometric analytical graph with different levels of salicylic acid phytohormone in the seedlings of sunflowers stem [123].



**Fig. 7.** The evaluated analytical merits for roots and stems of *Basella alba* L. (a) and (b) represent the abscisic acid detection in the roots and stems, respectively. (c) depicts the jasmonic acid in stems. (d) and (e) track the salicylic acid in roots and stems, respectively [130].

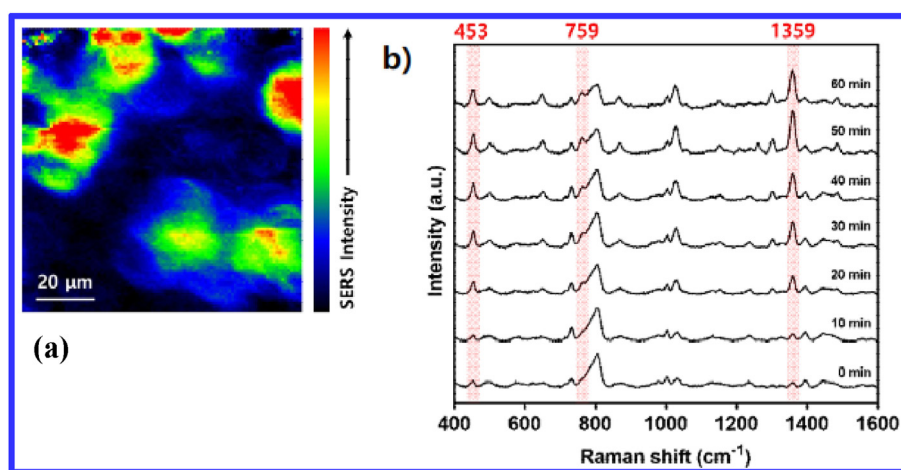


**Fig. 8.** Raman spectroscopic in vivo hormones detection. (a). Structure of Raman spectroscopic instrumentation [134]. (b). Raman spectroscopy was directly applied for real-time field analysis [135].

detections of plant hormones. The in vivo real-time determination of stress hormones against the wound stimulus was performed using silver nano-shells bound on poly (diallyl dimethyl ammonium chloride) nanoprobe in SERS. With the excitation wavelength of 785 nm, these nanoprobe resulted in enhanced SERS signals for adenosine triphosphate, indole 3-acetic acid and salicylic acid hormones. Indole 3-acetic acid, being responsible for growth and development and a major factor in the defense system of plants, resulted in three increased Raman peaks at 453, 759, and 1359  $\text{cm}^{-1}$  against the response of wound in the leaf (Fig. 9). The increase in the signals of indole 3-acetic acid over time demonstrated the efficiency of in vivo detections of plant hormones in

Raman spectroscopy [78].

Plant hormones are vital in responding to several biotic and abiotic stresses. Their chemical activities were detected and quantified by Raman spectroscopy in several experiments.  $\text{N}^6$ -Benzyladenine signifies its existence with a protective role in growth, photosynthesis, antioxidant activities and tolerance capacity of several stresses in plants [137].  $\text{N}^6$ -Benzyladenine was traced down within a complex matrix using AuNPs colloidal substrate resulting in a sensitive and rapid analysis [138]. The paper strip method immobilized with gold nanoparticles was developed using poly ( $\gamma$ -glutamic acid) for the SERS detection of brassinosteroid at very low concentrations in plants. Combining Raman



**Fig. 9.** Raman spectroscopy for in vivo detections of plant hormones. (a). Raman imaging of intensity map of nanoprobe in wounded leaf. (b). Several specific SERS signals are from different hormones and elements. Three increased Raman peaks at 453, 759, and 1359  $\text{cm}^{-1}$  represent the indole 3-acetic acid being responsible for the defense system in plants [78].



reporters with the SERS-active nanostructures when encountering the plant hormones, the changes in Raman spectra indicate trace hormones [139]. Likewise, the indole 3-butyric acid, when combined with the Ehrlich reaction, transformed into a Raman-active resonant molecule, resulting into an ultrasensitive *in vivo* determination of indole 3-butyric acid [79].

Hormones are usually structured with proteins and single-cell detections in living organisms. Detection could be performed by developing the SERS nanoprobe activated by 4-mercaptobenzonitrile. This probe provides new insights into single cells by accurately detecting the hemoglobin through *in vivo* detections [140]. Low-cost simultaneous characterization and detection of two B vitamins resulted in the sensitive identification of analyte molecules by using the SERS fingerprint method [141]. SERS detections showed enhanced values of the limit of detection in an interaction of nanomaterials and targets. Raman signals were detected at the lowest level of  $10^{-18}$  M when catechin was analyzed with high sensitivity and reproducibility [142,143]. These spectroscopic techniques have plenty of applications in all fields of science. SERS substrates development is flourishing rapidly with the wide range of periodic arrays, but the pertinent substrate with all the necessities of stable detection is still needed to develop [144]. In summary, spectroscopy is used for quantitative and qualitative analysis, enzyme assay, molecular weight determination, rate of reactions of substrates determination, dissociation constants of acids and bases and in the empirical formula of substrates determination.

### 3.2. Biosensors

The sensors were introduced to convert biological events to quantifiable signals and were named as biosensors. L. L. Clark was the biochemist who invented the first biosensor in 1950 to determine the oxygen level in the blood. However, at present, concerning detection mechanisms, three generations of biosensors are available for use. These analytical devices are structured with sensor systems containing biological and physical components, i.e., transducer and amplifier. This biological component may consist of enzymes, nucleic acid, antibodies, or hormones; thus, biosensors have vast applications in agriculture, food science and ecological services for pollution control. These biological elements communicate through the analyte being tested, and the transducer converts the biological reply in the form of the electrical signal, which is further amplified through a signal conditioning circuit known as a processor (Fig. 10 (a)) [145]. According to the usage, biosensors can be categorized into different classes, e.g., resonant mirrors, chemical canaries, biocomputers, immune, glucometers, optrodes,

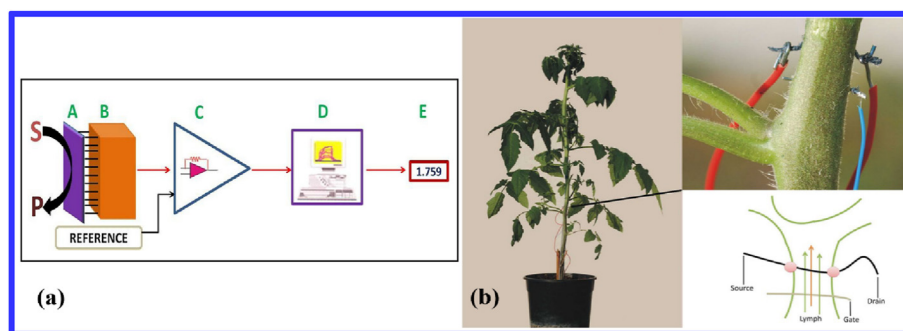
and biochips. It is relevant here to discuss the *in vivo* applications of biosensors to detect plant hormones [146]. DR5 is the first biosensor to detect plant hormones and was developed in 1997 [47].

Detection of signaling pathways of plant hormones and other molecules within an organism was brought into use through genetically encoded biosensors. Originally these biosensors were designed for animals but have now been acclimatized for plant studies [147]. These plant hormone biosensors are comprised of two components: the detector and the reporter gene. These biosensors can visualize and monitor the membrane transport processes, plant hormones, visualization of gene expression, and plant regulatory processes in real-time as *in vivo* (Fig. 10 (b)) [148,149]. Plant regulatory processes are controlled by nitrogen and phosphorus-containing molecules. These molecules are the complementary part of major biosynthetic pathways and their subcellular localization. Both organic and inorganic molecules are part of hormonal and enzymatic structures that control the plant's physicochemical processes. Genetically encoded biosensors, especially UMAMIT and FICRHR, can monitor and study the transport mechanisms of these molecules very accurately and precisely [150].

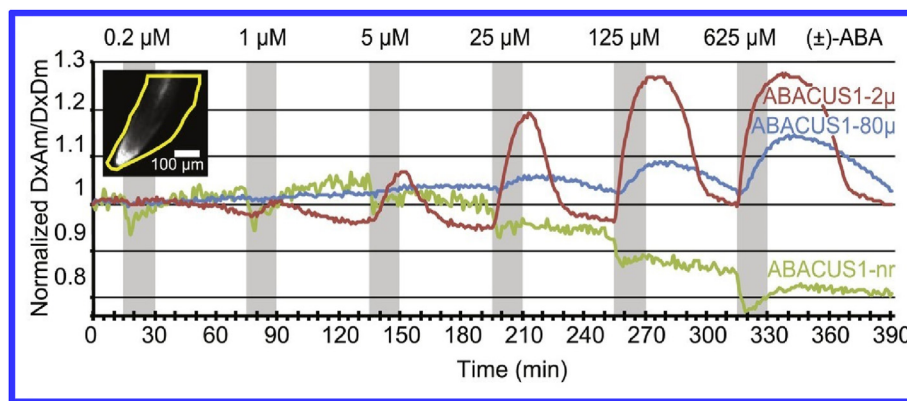
Signaling responses for different plant hormones are detected using specific promoters in genetically encoded biosensors viz., DR5rev and D2-VENUS promoter for auxin, TCSn for cytokinin, and Jas9-VENUS for jasmonate [56,151]. The working principle of all plant hormones is the same, first perceived by transmembrane or intracellular receptors, then as intermediate proteins and then to the transcription factor. Transcription factor controls the expression of plant hormone signaling pathways in the presence of reporter gene. Auxin, gibberellin, jasmonate, and strigolactones are perceived by intracellular receptors, while transmembrane receptors perceive cytokinin, ethylene and brassinosteroid. Monitoring of hormone-induced transcriptional activity is performed by using transcriptional sensors [148].

To monitor the abscisic acid, the biosensors developed were named ABACUS and ABAleon, the first-generation sensors [152]. With the same generation, another sensor to detect gibberellin was developed and named as Gibberellin Perception Sensor 1. ABACUS sensor was found to be highly ABA responsive with positive ratio changes up to ~1.3, and by exposing the roots to ABA, the sensor's brightness was enhanced twice (Fig. 11) [44].

ABA uptake was studied in the seedlings' roots using the ABAleon sensor. ABA translocation from hypocotyl to shoot and shoot to root reported a higher translocation rate of ABA in the root elongation zone rather than the maturation zone. Inside the hypocotyl, ABA hormone was found to be transported at the rate of ~16  $\mu$ m per minute against the stress of drought with the use of ABAleon sensor



**Fig. 10.** *In vivo* Detection of Plant Hormones Using Biosensors. (a). Block diagram of the biosensor. A). The substrate is converted into product, and sensing reaction is started (suitable substrate is structured for the immobilization of molecules, e.g., DNA and enzyme). B). Transducer determines and converts the sensing reaction into electric signals. C). Electric signals are amplified. D). Amplified signals are processed with computer. E). Processed signals are displayed in the form of results [145]. (b). Bioristor sensor visualizes and monitors real-time variations in the solute content of tomato plant sap *in vivo* [149].



**Fig. 11.** Expression of ABA increase in root tips enhanced from 0.2  $\mu\text{M}$  to 625  $\mu\text{M}$  proving the efficiency of the ABACUS sensor [44]. The responses of sensor represented the ABA-dependent ratio changed in the form of  $\Delta\text{DxAm}/\text{DxDm}$  with respect to time, here Am = sensor acceptor emission, Dm = sensor donor emission, and Dx = excitation of sensor donor.

[153]. An optogenetic biosensor named Gibberellin Perception Sensor 1 was developed to assess the spatiotemporal resolution and signaling of gibberellic acid in the different parts of *Arabidopsis thaliana* plants. The levels of gibberellic acid in different parts of the plants are regulated by phytochrome interacting factor during the presence or absence of light. This sensor can determine the gibberellic acid up to the nanomolar level within the molecular framework by the accumulation of gibberellic acid through the promotion of phytochrome interacting factors in the presence of light [55].

Furthermore, the affinity-based biosensor can also be applied for in vivo real-time detections of plant hormones. In this approach, microfluidics and microdialysis as a continuous sampling method integrate with assay platforms resulting in an antibody-based real-time quantitative immunosensor of practical use. These biosensors are less efficient than genetic-based ones concerning time resolution, although they are good with dynamic ranges. However, a competitive immunoassay-based biosensor with quartz crystal microbalance to determine auxin did not produce good time resolution. Likewise, for the detection of abscisic acid, an antibody-based electrochemical impedance-based electrode resulted in slow time resolution with unfavorable dependence, although the sensitivity range was 1 nM–1  $\mu\text{M}$  [154].

Different analytes variate to transduce the electrochemical, colorimetric or optical signals, affecting the evaluation of biosensors' results [155]. Biosensors have produced very stable, cost-efficient, sensitive, selective, noninvasive, rapid, quantitative, and reproducible results. The main features of biosensors involve linearity, sensitivity, selectivity, and desired response time. Furthermore, biosensors are also applicable in a wide range of daily science, e.g., healthcare, determination of metabolites, clinical treatments and disease diagnosis, industrial processing and environment monitoring, military, agriculture, pharmaceutical and veterinary applications. The sensitivity of biosensors can be enhanced by combining biotechnology and microelectronics, which can determine the vast spectrum of analytes, i.e., gases, organic compounds, bacteria, and ions [156].

### 3.3. Electrochemical sensors

Electrochemical sensors are idealized for their high functionality through excellent sensitivity, cost efficiency, and easy to operate, with the ability to be miniaturized and the potential for combining with other techniques. Compared to other techniques, electrochemical sensors are suggested as a better option for in vivo single molecule detections in plants [157]. Abundant of electrochemical methods have been developed for in-situ detection of

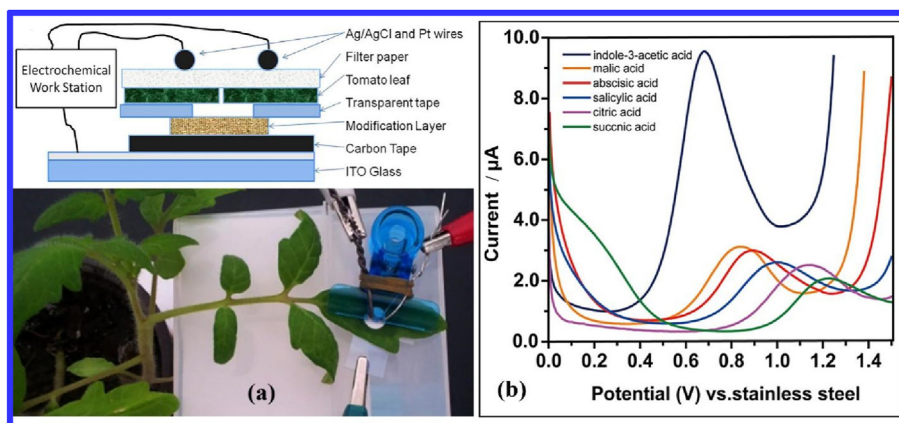
plant hormones, e.g., paper-based electrochemical sensors, electrodes modified with nano-gold/multiwalled carbon nanotubes/chitosan,  $\text{CeCl}_3/\text{dihexadecyl}$  hydrogen phosphate, and reduce graphene oxide/poly safranin T. These sensors are modified according to the phytohormone type to analyze the possible biosynthetic pathways and physiological activities of the plants.

In vivo detections were performed by developing different electrochemical sensors for various classes of plant hormones (Fig. 12 (a)). The paper-based electrochemical sensor was developed for salicylic acid detection in tomato leaves, while indole 3-acetic acid is the most widely used in vivo detected plant hormone for many of the electrochemical sensors, as reported by researchers. The electrochemical workstation was developed with carbon tape electrode, filter paper, and buffer solution. Platinum and silver chloride wires were used to tightly bind the filter paper with the leaf. With a diameter of 1.5 mm, a hole was pinched in the leaf and buffer solution through filter paper was passed to the leaves. The salicylic acid from leaves was diffused onto working carbon-taped electrodes, which were further quantified [158].

Indole 3-acetic acid was determined in soybean seedlings using the disposable stainless steel-based electrochemical microsensor. The microelectrode used was anodized stainless steel with the disposable, sensitive, and selective ability for in vivo field detections of indole 3-acetic acid. In the presence of several hormones, including abscisic acid, succinic acid, citric acid, malic acid, salicylic acid, and indole 3-acetic acid, the microelectrode showed the very low limit of detection 43 pg/mL. Moreover, the sensitivity was very high with excellent selectivity, which yielded the limit of quantity of 143 pg/mL (Fig. 12 (b)) [159].

Salicylic acid screening in infected tomato leaves was performed using carbon tape electrodes. These electrodes were modified with pencil trace by hand for in vivo differentiation of the salicylic acid amongst the normal and the infected leaves. These carbon tape-modified electrodes successfully recorded the increase of salicylic acid contents in the infected leaves against the abiotic stress. These electrodes can further be integrated with other techniques e.g., paper based analytical devices, to quantify the salicylic acid. The limit of detection for salicylic acid using carbon tape-modified electrodes was reported as  $1.0 \times 10^{-7}$  M. Although they showed similar performance to many other techniques, carbon tape-modified electrodes for salicylic acid detection are cost-efficient, possess less fabrication efforts, and, most importantly, are effective in disposable applications [160].

Glass carbon electrodes coated with multi-walled carbon nanotubes were applied for the detection of the 6-benzylaminopurine (cytokinin) plant hormone. 6-benzylaminopurine was determined using adsorptive stripping voltammetry to express voltametric



**Fig. 12.** In vivo plant hormones detection using electrochemical electrodes. (a). Electrochemical sensor developed for salicylic acid detection in tomato leaves [158]. (b). This is a differential pulse voltammogram plot to evaluate the feasibility of microelectrode for the in vivo detection of indole 3-acetic acid with the concentration of 100  $\mu\text{g}/\text{mL}$  [159].

behaviors of hormone under different controlled pH conditions as 3.0 acidic, 7.0 normal and 10.0 alkaline. Limit of detection was found 0.098  $\mu\text{M}$ , 0.165  $\mu\text{M}$ , 0.235  $\mu\text{M}$  under pH conditions of 3.0 acidic, 7.0 normal and 10.0 alkaline, respectively. While the limit of quantification for 3.0 acidic, 7.0 normal and 10.0 alkaline pH was recorded as 0.326  $\mu\text{M}$ , 0.545  $\mu\text{M}$ , and 0.776  $\mu\text{M}$ , respectively. The precision, sensitivity, and selectivity of electrodes to detect 6-benzylaminopurine decreased with the increase in pH values. This sensing performance of glass carbon electrodes coated with multi-walled carbon nanotubes for the detection of 6-benzylaminopurine plant hormone provided significant reference value for phytohormone electrochemical sensing devices under variable pH conditions [161].

Paper-based analytical devices can also do in vivo electrochemical detections of plant hormones within the lowest sample amount. Indole 3-acetic acid and salicylic acid were directly electrochemically detected in the tiny samples of plants with the low sample volume of 10  $\mu\text{L}$ . The disposable working electrodes quantified the indole 3-acetic acid and salicylic acid at the level of ng in the paper-based analytical devices [30]. Another paper-based electroanalytical device integrated with a multichannel electrochemical station was used to enhance the detection of indole 3-acetic acid and salicylic acid levels in pea seedlings under salinity stress conditions. Differential pulse voltammetry multichannel used with electrochemical graphene oxide modified carbon tape electrode depicted the reproducibility of results  $\sim 13\%$ . This technique detected the decrease of indole 3-acetic acid in the root tips of pea seedlings under salinity stress. The limit of detection for indole 3-acetic acid and salicylic acid was reported to be less than 0.1  $\mu\text{M}$  [31].

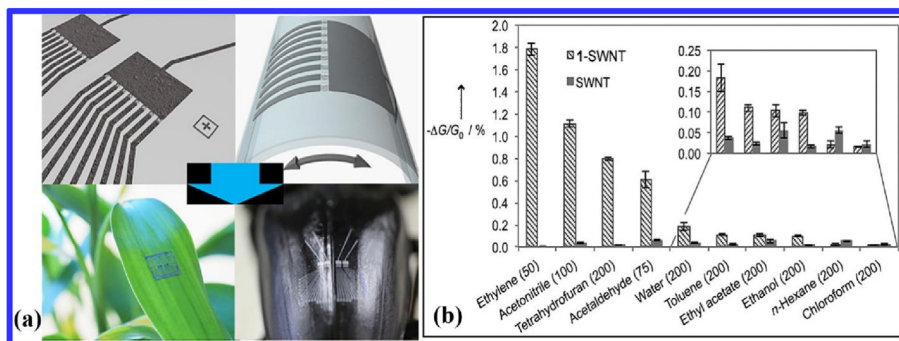
Electrochemical sensors are efficient to detect hormones in plants, but they can easily lose their activity due to complex cytoplasmic structures, and their detection life can be reduced [162]. To overcome this problem in microsensors, viz., stainless steel electrodes deposited with nanoparticles and carbon tape electrodes with carbon nanotubes have been developed. They possess the characteristics of cost efficiency, disposability, good electric conductivity, biocompatibility, and high mechanical strength, and they have been used for many biomedical tools and biosensors, thus ensuring stable performances. The commercial availability of electrochemical sensors varies according to their sensitivity and selectivity. Such as carbon-based graphite pencil electrodes have high sensitivity and selectivity, but their availability for real-time sensing is still in the process of testing. Likewise, electrochemical sensing for commercial purposes is still developing.

#### 4. Other detection methods

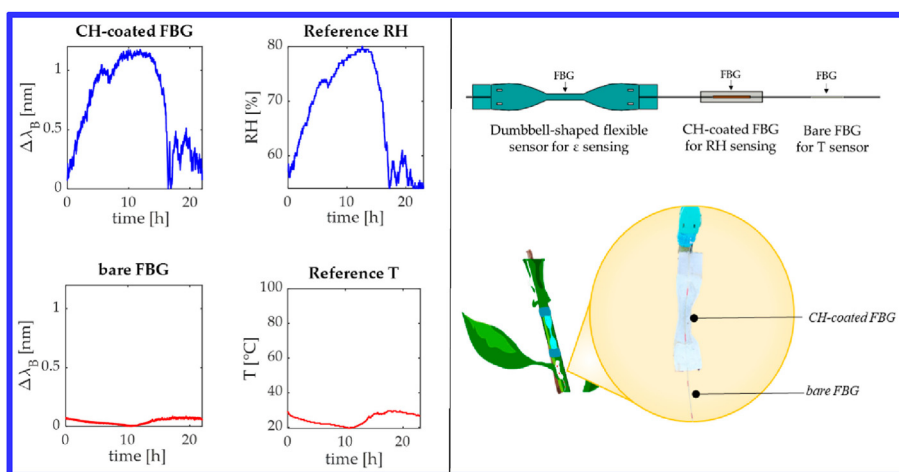
One of the most ultrasensitive advanced methods now under use is named plant wearable devices. They are the sensors for live, continuous, and noninvasive assessments by directly installing them on soft surfaces. Though most of the wearable devices are being used only for humans, some have been invented by researchers to be used in plants for health monitoring and specific needs measurements [163,164]. Wireless detection and communications of the molecules with low concentrations within the plants or the wireless real time detection of volatile molecules is performed by implanting the wearable nano-electric circuits on the surfaces of plants. These nanotechnology-based wearable devices are much elastic to be curved on the organisms up to the smallest radius of  $\sim 100 \mu\text{m}$  and play the role of flexible skin [165]. A sensor named as single-walled carbon nanotubes with graphite electrodes is a wearable device to be placed on the leaf surface to detect the gas molecules concentrations and airborne chemicals (Fig. 13 (a)). This sensor can be operated by radio frequency wireless signals to monitor the signaling molecules in live plants down to the trace level of 5 ppm [166].

Graphene and carbon nanotube-based wearable devices have been reported to monitor the wide range of volatile organic elements, plant signaling molecules, gas and aqueous phase molecules in plants [165]. Single-walled carbon nanotubes can further be modified with copper compounds to develop chemo-resistive sensors that monitor the sub-ppm concentrations of ethylene plant hormone. These sensors work reversibly, enabling them to monitor the onset of fruit ripening by detecting the ethylene concentrations for a longer duration. Single-walled carbon nanotubes with copper responded significantly higher in magnitude for the ethylene in the presence of several fruit metabolites with concentrations ranging from 75 to 200 ppm (Fig. 13 (b)). This sensory system showed efficient sensitivity responses down to 1 ppm of ethylene which is helpful to keep the ethylene level below the threshold in the storage facilities [87].

Only a few plant wearable devices are available specifically for plant hormone detection, but they rely on the ambience, stress conditions, chemicals, and the elements getting in or out of the plants. Concentrations and levels of plant hormone change with respect to abiotic stresses that plant faces [167]. To detect the organophosphorus chemicals flexible wearable device was developed using scree-printed electrodes integrated with disposable polymer gloves [168]. Another sensor was rapidly developed to determine pesticide contents on leaves and the real-time growth of



**Fig. 13.** Wearable devices for the in vivo detection of plant hormones. (a). In vivo synthesis of carbon nanotube graphite structures integrated onto live forms in nature [166]. (b). Relative responses of single-walled carbon nanotubes with copper complex 1 (1-SWNT) and Single-walled carbon nanotubes for the 50-ppm ethylene and several fruit metabolites.  $\frac{\Delta G}{G_0}$  is the change of conductivity observed by electrodes connected to a potentiostat (Concentrations are in ppm) [87].



**Fig. 14.** Fiber Bragg gratings (FBG) technology-based plant wearable sensor was experimented with to monitor the continuous and simultaneous plant growth, temperature (T), and relative humidity (RH) changes in the environment. FBG observed that RH increased from 56 to 80% and T decreased from 29 °C to 20 °C while night and vice versa in day time [85].

plants. This sensor was made with chitosan and graphite powder ink and was much flexible to be installed on the solid surface of plants [169]. Further, the integration of a sensor and the signal reading circuit was performed, and an all-in-one device was developed, which is able for quantitative nanoscale measurements in seconds [170].

These wearable devices allow detections due to concentration mechanisms and possess tunable sensitivity to gas molecules in very low concentration ranges to as low as parts per billion [165]. Furthermore, besides the hormonal and volatile organic compounds detection sensors, other wearable devices like tape-based graphene sensors and microfluidic printed single-walled carbon nanotubes have also been modified. They allow to sense relative humidity, assess plant water status, and the emergence of drought stress through real-time measurements of single stomatal aperture dynamics during the drought from the leaf epidermis [171]. Moreover, other stress conditions like flooding and salinity can be detected through wearable devices that ultimately determine the hormonal changes in the plants.

The required spatial resolution within larger areas' fields depends on microenvironmental variations, plant stresses and biosensors. The wearable nanotechnology-based sensors are vulnerable to use with a wide range of applications, from urban to industrial farming. The performance of wearable sensors is highly dependent on the variable ambient conditions. Humidity, temperature, and wind are the key factors to highly affect the wearable

devices' sensitivity, selectivity, signal-to-noise ratios, and recovery time [172]. Moreover, the wearable nanotechnology-based sensors are prone to the challenges of applicability, accuracy, and durability within the fields of larger areas. To overcome these problems, there is a need to use the more advanced wearable flexible devices to record the rapid plant signaling molecules with high temporal resolutions. Several flexible wearable sensors, i.e., centimeter-scale macro-porous networks [173], are in experimental stages, but the results showed their high temporal resolution with continuous monitoring ability of multiple plant electrochemical signals simultaneously. Likewise, a syringe-injectable mesh nano-electronic wearable device [35] that monitors mice's in vivo neural activity will be able to record the rapid waves of plant signaling molecules with the millisecond temporal resolution.

First-time use of another health monitoring wearable device has been performed in plants to measure the elongation, microclimate, and stress factors before being used for humans. This sensor is based on fiber Bragg gratings technology widely used in health care. The wearable sensor was experimented with to monitor the environment's continuous and simultaneous plant growth, temperature, and humidity factors. The plant wearable sensor was proposed for its high sensitivity, multiplexing capability, and high resistance to harsh environments (Fig. 14). Further experiments of the proposed sensor with the combination of big data and image processing will able it for in vivo detections of plant hormones directly and rapidly [85]. These wearables have large, efficient,

high-resolution display properties with flexible characteristics. Although they are deployed in the field for data collection and crop monitoring, some devices are still at the TRL stages 7 and 8, which will gradually improve their status for commercial availability.

## 5. Conclusion

This review article comprehends the *in vivo* measurements of plant hormones. Despite the presence of many *in vitro* and conventional techniques, *in vivo* methods are preferred for plant hormone detections. Spectroscopy, biosensors, electrochemical sensors, and most advanced ultrasensitive sensors, including wearable devices, have been discussed with their applications, advantages, and disadvantages in this review article. They all have a wide range of applications in detection studies, e.g., in quantitative and qualitative analysis, enzyme assays, molecular weight determination, and determining physiochemical characteristics of analytes and plant hormonal detections. Biosensors and electrochemical sensors are frequently used for *in vitro* and *ex vivo* detection of plant hormones, but spectroscopy, especially SERS, is recognized as an ultrasensitive detection tool. The electrochemical sensors can detect and quantify the trace amounts of plant hormones *in vivo*. The wearable plant device is the most sensitive and easy-to-use identification technique among them. However, there is a need for more advanced wearable, flexible devices such as centimeter scale macro-porous networks and syringe-injectable mesh nano-electronic wearable devices to be used in real agriculture conditions to rapidly detect plant signaling molecules with millisecond temporal resolution. *In vivo* plant hormone detection requires cumbersome sample preparation, but the recent development in biosensors, spectroscopies, electrochemistry, and wearable sensors have made it convenient to analyze plant hormones. Although these techniques are full of easiness yet, they still need to be improved, such as SERS needing to have stable substrates, electrochemistry needing to overcome injury remediation issues, sensors containing noise, and wearable sensors needing more spatial resolution. Although wearables are deployed in the field for data collection and crop monitoring, some devices are still at the TRL stages 7 and 8. As our understanding of the *in vivo* measurements of plant hormones, portable, wearable, and nondestructive testing technologies will improve as well, leading to higher sensitivity, faster and more reversible responses, will gain the status of commercially available and the trend of plant sensors that can make the “Internet of Plants” concept a reality.

## Contribution statements

Syed Muhammad Zaigham Abbas Naqvi: prepared and presented the work, writing- original draft. Yanyan Zhang: provided the study materials and maintained the research data. Muhammad Naveed Tahir: project administration. Zia-Ullah: reviewing and editing. Shakeel Ahmed: drafting and interpretation of the data. Junfeng Wu: project administration. Vijaya Raghavan: revising it critically for important intellectual content. Mukhtar Iderawumi Abdurraheem: management activities to annotate and reproducibility of research outputs. Jianfeng Ping: management activities to annotate and reproducibility of research outputs. Jiandong Hu and Xinran Hu: supervision and conceptualization.

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## 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.

## Data availability

No data was used for the research described in the article.

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