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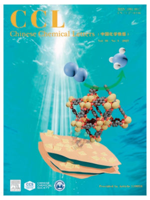

Exploring the frontiers of plant health: Harnessing NIR fluorescence and surface-enhanced Raman scattering modalities for innovative detection

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ABSTRACT

Plants play a crucial role in maintaining ecological balance and biodiversity. However, plant health is easily affected by environmental stresses. Hence, the rapid and precise monitoring of plant health is crucial for global food security and ecological balance. Currently, traditional detection strategies for monitoring plant health mainly rely on expensive equipment and complex operational procedures, which limit their widespread application. Fortunately, near-infrared (NIR) fluorescence and surface-enhanced Raman scattering (SERS) techniques have been recently highlighted in plants. NIR fluorescence imaging holds the advantages of being non-invasive, high-resolution and real-time, which is suitable for rapid screening in large-scale scenarios. While SERS enables highly sensitive and specific detection of trace chemical substances within plant tissues. Therefore, the complementarity of NIR fluorescence and SERS modalities can provide more comprehensive and accurate information for plant disease diagnosis and growth status monitoring. This article summarizes these two modalities in plant applications, and discusses the advantages of multimodal NIR fluorescence/SERS for a better understanding of a plant's response to stress, thereby improving the accuracy and sensitivity of detection.

1. Introduction

It is well-known that plants, as the cornerstone of the Earth's ecosystem, provide oxygen for human respiration through photosynthesis, establishing the foundation of the food chain, supplying vital resources essential for human survival, and thus making crop safety an urgent issue of global concern [1,2]. However, plant growth is susceptible to environmental stresses, such as salinity, extreme temperatures and drought, which may lead to adverse effects on the growth of plants, and in extreme cases, can even lead to their death. Therefore, understanding the physiological condition and health status of plants is of importance for maintaining the balance of ecosystems, enhancing the yield of crops, and ensuring food security [3-5].

Traditional strategies for monitoring plant stress including quantitative reverse transcription quantitative PCR (RT-qPCR), reveal the gene response of plants to stress by detecting the expression levels of specific genes, but they require expensive equipment and long processing duration [6]. Recently, near-infrared (NIR) fluorescence imaging and surface-enhanced Raman scattering (SERS) modalities have emerged as advanced techniques for monitoring plant stress. Moreover, those two modalities each possess unique advantages and exhibit complementary relationships [7-10]. On the one hand, NIR fluorescence imaging can non-invasively and real-time monitor the health status of plants with high resolution, which is particularly suitable for rapid screening in large-scale scenarios. On the other hand, SERS offers an effective tool for detecting metabolic products and plant hormones *in vitro* and *in vivo* with high sensitivity and specificity. NIR fluorescence technology offers macro-level physiological status monitoring, while SERS

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technology focuses on the detection of specific molecular components at a micro-level. The combination of the two modalities enables a more comprehensive and precise assessment of plant health status and plant disease diagnosis [11-15].

This paper reviews the applications of NIR fluorescence and SERS in the plant field. Initially, we explain how these two techniques assess the health status of plants through imaging and detection of plant hormones, pigments, fungi, and viruses. We next introduce the design principles of various NIR fluorescent and Raman probes. Furthermore, we summarize the latest research advances in the integration of these two modalities, aiming to gain a multidimensional understanding of the plant's response to environmental stress, thereby providing a scientific basis and technical support for the development of precision agriculture and crop improvement. Finally, we outline the future potential applications of NIR fluorescence and SERS in plants.

2. Application of NIR fluorescence imaging in plants

The application of NIR fluorescence imaging in plants demonstrates foresight and significant importance. Renowned for its provision of strong signals and high signal-to-noise ratio, this technology is particularly suitable for analyzing plant samples with high water content. Through highly specific labeling methods, NIR fluorescence imaging can accurately provide internal information about cells and biomolecules from plants with high sensitivity and resolution. This modality enables real-time detection and tracking of changes in various components within plant cells, aiding researchers in better understanding the mechanisms of plant life processes and revealing their internal complexity [16-18]. Meantime, in plant tissue cells, NIR fluorescence imaging exhibits low absorption and scattering rates, enabling deeper tissue penetration and significantly increasing imaging depth. Additionally, NIR fluorescence imaging offers multi-parameter and multi-channel imaging, allowing for the simultaneous acquisition of information on multiple parameters or biomarkers, deepening the understanding of regulatory mechanisms and interaction networks in plant bodies. Its non-invasive and non-destructive nature also effectively preserves the integrity of plant samples. Based on the multi-photon absorption effect induced by NIR light excitation, this modality possesses advantages such as deep tissue penetration, high spatial-temporal resolution and high signal-to-noise ratio, making it particularly suitable for imaging deep-seated tissues in living plants. Holding on these merits, NIR fluorescence imaging has become an indispensable tool in the detection of plant hormones, plant fungi, and other substances within organisms [19-21].

2.1. NIR fluorescence plant imaging

Compared with visible region, NIR fluorescence imaging offers higher imaging resolution, deeper penetration into biological matrices, lower optical absorption and scattering, and minimal tissue auto-fluorescence. Therefore, Qian *et al.* [22] reported an aggregation-induced emission (AIE)-active probe: APMem1 based on multiple designed strategies, for live-cell plasma membrane imaging, which achieved four-dimensional spatiotemporal imaging of plant cell plasma membranes and enabled long-term real-time monitoring of membrane morphology changes for the first time (Fig. 1A). The designed APMem-1 could rapidly penetrate through the cell wall and achieve specific staining of plant cell plasma membranes in a short time. It possessed advanced properties, including ultra-fast staining speed, wash-free characteristics, and ideal biocompatibility. Additionally, this probe exhibited excellent plasma membrane specificity and demonstrated considerable performance in terms of imaging contrast and integrity. Moreover, the frequent pH fluctuations in live cells did not significantly affect the fluorescence stability of APMem1, facilitating specific imaging of plasma membranes without interference from other factors. Validation experiments on different types of plant cells and various plants convincingly also verified the universality of APMem1. It is noteworthy that this study utilized multiple principles to design NIR emissive AIEgens. By introducing a long alkyl chain at one end and a charged group at the other end, the target probes acted as amphiphilic molecules. Moreover, the introduction of rigid groups with large steric hindrances effectively hindered the diffusion-driven penetration of molecular probes. Experiments with DMSO-treated Arabidopsis root cells before and after confirmed that APMem1 could specifically stain plasma membranes only when membrane structures were completed. Therefore, the development of this plasma membrane targeting probe with four-dimensional spatial and ultra-long-time imaging capabilities provided a valuable tool for intuitive and real-time monitoring of dynamic processes related to plasma membranes.

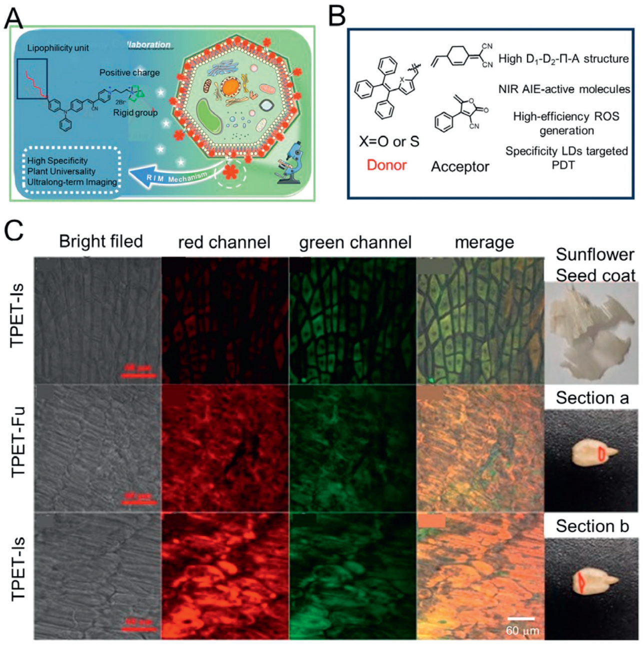

Fig. 1. (A) Schematic illustration of membrane probe design principle with ultra-high specificity and ultra-long-term imaging performance through multi-strategy collaboration, suitable for imaging plasma membranes in various plant cells and different plants. Reproduced with permission [22]. Copyright 2023, Royal Society of Chemistry. (B) The application and schematic diagram of the principle of such probes. (C) TPET-Is, TPET-Fu, TPEF-Is, and BODIPY493/503 Green co-localization images. Scale bar: 60 μm. Reproduced with permission [23]. Copyright 2022, Elsevier.

Recently, Sun *et al.* [23] synthesized three novel NIR organic compounds with AIE activity, namely TPET-Is, TPET-Fu, and TPEF-Is (Fig. 1B). These AIEgens exhibited excellent photo-stability, and strong lipophilicity, enabling high-fidelity laser-direct-structuring (LDs) imaging in plant live cells. Moreover, these AIEgens could be applied to bright imaging of LDs in oil-rich plant tissues. Therefore, this study provided a potential strategy for LDs' targeted imaging and photodynamic therapy (PDT) applications through molecular engineering with heteroaromatic bridging. It is noteworthy that the large π-conjugated tetraphenylethene (TPE) with a twisted structure and strong push-pull effect resulted in narrow molecular bandgaps, which could lead to red-shifted emission in the NIR and high reactive oxygen species (ROS) generation efficiency. These results indicated that TPET-Is, TPET-Fu and TPEF-Is with AIE activity, and NIR emission characteristics, were beneficial for biological imaging applications with low background interference.

Furthermore, the AIE-active probes were more sensitive to polarity than viscosity. Hence, these twisted intramolecular charge transfer (TICT) probes were beneficial for illuminating low-polarity subcellular LDs in plant cells, aiding in the identification of LDs in oily plants (Fig. 1C). Through staining LDs based on the principle of similar solubility, it was found that these probes could selectively target LDs due to their good lipophilicity. With their excellent lipophilicity, they could also be employed for high-resolution imaging of LDs in oil-rich plants (such as sunflower seeds). Although AIE probes have promising advantages, they also face challenges: they tend to aggregate in aqueous environments, potentially causing background fluorescence interference; the plant cell wall may hinder their penetration, affecting the depth of imaging; and changes in the intracellular environment can also affect their luminescent properties [24]. Despite facing challenges, we believe that this technology has the potential to better revolutionize agricultural practices, and we are committed to in-depth research and continuous innovation to fully leverage its potential.

2.2. Detection of plant hormones

Plant hormones are the key signaling molecules that regulate plant growth and physiological processes of plants [25]. NIR fluorescence offers advantages such as rapidity, accuracy, and non-destructiveness. By detecting specific fluorescence signals of hormone molecules in plant tissues, rapid determination of plant hormone levels can be achieved without the need for cumbersome pretreatment steps. This efficient detection method provides a powerful tool for physiological research on plants, enabling a deeper understanding of the mechanisms regulating plant growth and development, as well as facilitating real-time monitoring of plant responses to environmental stressors. In turn, this provides a scientific basis for agricultural production, promoting improvements in crop quality and yield [26,27].

To understand the dynamic growth of plants and their adaptive responses to external conditions, Strano *et al.* [28] fabricated amphiphilic polymers encapsulating single-walled carbon nanotubes (SWNTs) to form corona phase nanosensors for detecting plant growth hormones based on corona phase molecular recognition strategy (Fig. 2A). Corona phase molecular recognition (CoPhMoRe) technology utilizes specially designed cationic polymers to wrap SWNTs, forming a corona phase with unique molecular recognition sites. After screening, it has been found to detect specific small molecules with high selectivity. These recognition sites can specifically bind to target molecules through electrostatic interactions and other intermolecular forces, triggering characteristic changes in the near-infrared fluorescence of SWNTs. Meanwhile, SWNTs displayed high photo-stability and promising fluorescence in the NIR range, away from the auto-fluorescence range of chlorophyll. Moreover, SWNTs maintained good stability even after penetration for 7 days, making them as ideal probes for *in vivo* sensing in plant tissues. Notably, molecular recognition properties of these nanosensors were realized by the molecular probe adsorption (MPA) methodology to measure the surface area of adsorbed analytes. Based on this nanosensor, the accumulation of 2,4-dichlorophenoxyacetic acid (2,4-D) in sensitive dicotyledonous cabbage leaves could be observed after addition of 2,4-D to hydroponic culture media for 5 h, while no absorption was seen in resistant monocotyledonous rice leaves (Fig. 2B). Meanwhile, this nanosensor indicated that 1-naphthalene acetic acid (NAA) was absorbed more rapidly, suggesting that the levels of NAA and 2,4-D in plant cells depend on the diffusion rates and differential activity or expression of the influx and efflux carrier proteins at the cellular and tissue levels. Therefore, these nanosensors were capable of monitoring the concentration of endogenous growth hormones synthesized within live plants, providing a new platform for real-time acquisition of hormone levels in other plants, and aiding our understanding of the dynamic growth of plants and their adaptive responses to external conditions.

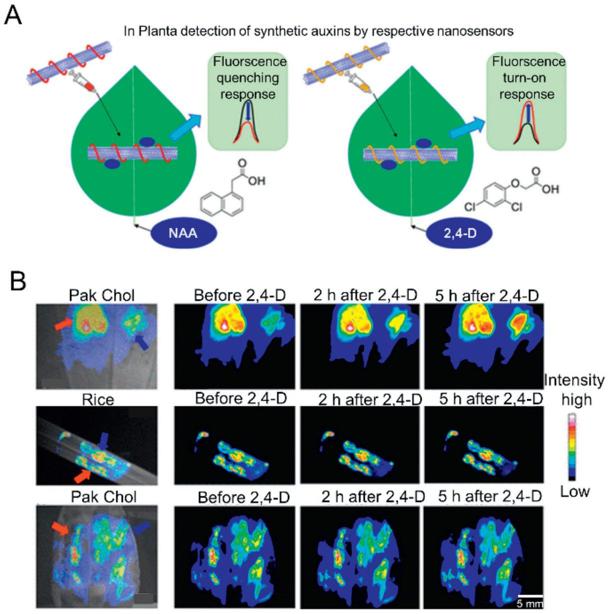

Fig. 2. (A) Schematic diagram illustrating the detection of synthetic auxins in plants using corresponding nanosensors. (B) Under 785 nm laser excitation, pak choi was infiltrated by a reference sensor, 2,4-D sensor and NAA sensor, and rice leaf was infiltrated by a reference sensor and 2,4-D sensor. Scale bar: 5 mm. Reproduced with permission [28]. Copyright 2023, American Chemical Society.

Although NIR fluorescence has demonstrated advantages in the detection of plant hormones, some challenges such as the concentration range of hormones and the influence of internal plant structures remained. Additionally, current NIR fluorescence fails the simultaneous detection of multiple plant hormones or high-sensitivity detection of trace hormones. To address these issues, future research can focus on several aspects: Firstly, by integrating diversified sensing technologies such as SERS modality and electrochemical sensors, simultaneous monitoring of multiple plant hormones can be realized, thereby comprehensively understanding the dynamic changes of hormones during plant growth and development. Furthermore, nanomaterials and molecular imaging technologies can be utilized to improve detection sensitivity and spatial resolution, enabling precise monitoring of trace hormones and providing a more comprehensive and reliable solution for plant hormone detection. Holding onto these efforts, there is no doubt that NIR fluorescence will promote more excellent projects on plant hormone detection in the future.

2.3. Detection of plant fungi

In China, fungal diseases account for 70% to 80% of all plant diseases and are widely distributed across various regions. Plants often exhibit symptoms such as rotting, necrosis, wilting, and deformities after infection. However, by the time these symptoms appear, the plants are already adversely affected [29,30]. Therefore, Cai *et al.* [31] reported the first example of an NIR-Ib fluorescence probe not only for investigating plant transpiration but also for identifying fungal pathogens. Unlike traditional NIR-Ia fluorescence imaging (700–900 nm), this new fluorescence modality (NIR-Ib) relied on dyes emitting between 900 nm and 1000 nm region. Hence, compared to NIR-Ia fluorescence imaging, NIR-Ib fluorescence imaging exhibited significantly improved spatial resolution, signal-to-background ratio (SBR), and penetration depth.

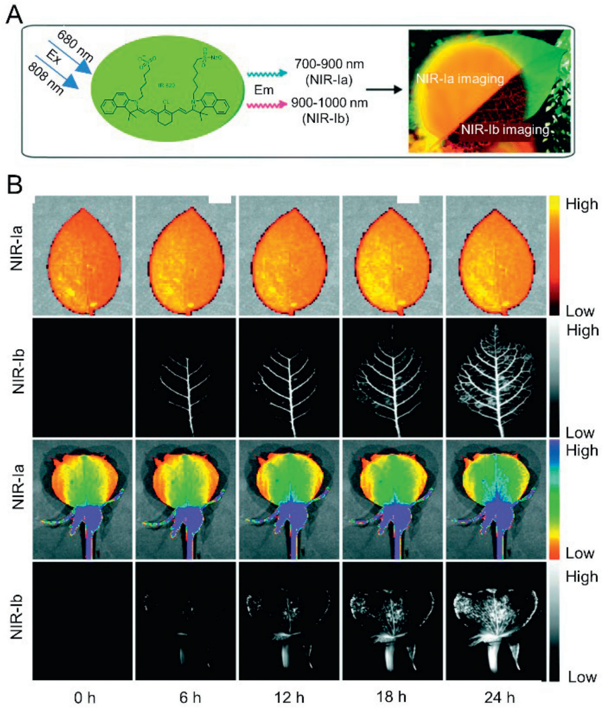

Fig. 3. (A) Chemical structure of IR-820 dye and schematic diagram illustrating NIR-Ia and NIR-Ib fluorescence imaging. (B) NIR fluorescence images (NIR-Ia and NIR-Ib) showing the leaf and flower of *R. rugosa*. Reproduced with permission [31]. Copyright 2018, The Royal Society of Chemistry.

With a heptamethine cyanine dye (IR-820) (Fig. 3A), they demonstrated the clear advantages of NIR-Ib fluorescence imaging of petals, leaves, and stems of various plants with high-quality figures (Fig. 3B). By imaging of stems infected with fungi, it found that fungal pathogens in NIR-Ia images were not prominent, but appeared as clusters of bright spots in NIR-Ib images, with clear characteristics in terms of shape, size, and morphological features, indicating that NIR-Ib fluorescence imaging could be used not only for detecting fungal pathogens but also for species differentiation of fungal pathogens. The emergence of NIR-Ib fluorescence technology has brought new possibilities and potential applications for plant fungal detection [32,33].

2.4. Detection of other substances in plants

Dynamic monitoring and high-resolution characteristics of NIR fluorescence imaging have led scientists to attempt its application in detecting various substances in plants. For example, nitric oxide (NO) plays multiple physiological roles in plants, involving root growth, flowering, responses to oxidative stress, pathogen infections, and salinity tolerance [34-39]. Therefore, real-time fluorescence monitoring NO in plants not only provides deeper understanding of the physiological status and stress response mechanisms of plants, but also offers important references for plant growth management and disease control [40]. Recently, Wu *et al.* [41] designed a NO-activatable probe with a benzothiadiazole-core fluorophore (BNDA), which exhibited strong NIR-II fluorescence emission and photoacoustic signals. To confer good water dispersibility and biocompatibility to BNDA, a host-guest supramolecular complex BNDA-HbCD was prepared through host-guest complexation using 2-hydroxypropyl-β-cyclodextrin (HbCD) (Fig. 4A). Moreover, the designed BNDA-HbCD displayed good selectivity and anti-interference performance in detecting NO against various potential interfering substances. Furthermore, NIR-II fluorescence imaging and photoacoustic imaging were performed on different groups of bean sprouts and it found that the different levels of NO in the bean sprouts corresponded to different fluorescence intensities (Fig. 4B).

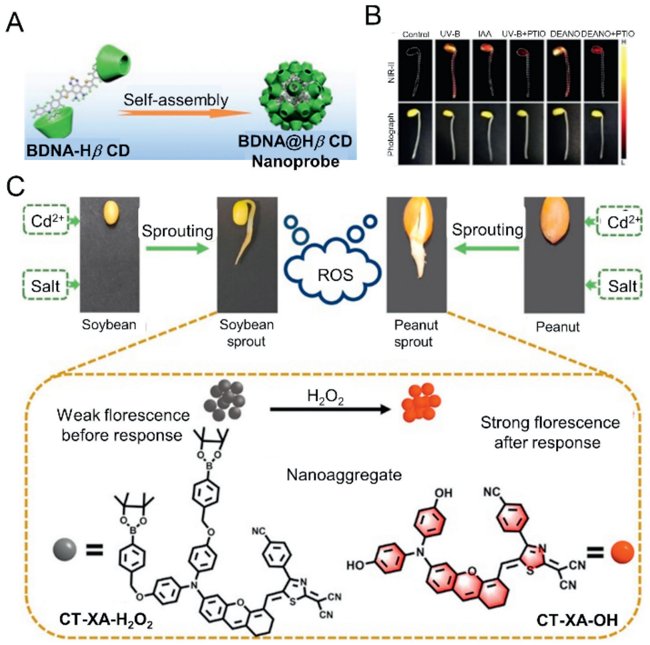

Fig. 4. (A) Illustration depicting the synthesis process of the nanoprobe BNDA@HbCD. (B) NIR fluorescence images (NIR-II) and white-light photographs of soybean sprouts from various experimental groups. Reproduced with permission [41]. Copyright 2022, Elsevier. (C) Illustration depicting the fluorescent imaging process of the probe CT-XA-H_2O_2 for detecting oxidative stress induced by Cd^{2+} ions or high-level salt (NaCl) in soybean sprouts and peanut sprouts, through the response to the *in vivo* biomarker H_2O_2. Reproduced with permission [44]. Copyright 2022, Wiley-VCH.

Environmental pollution, such as heavy metal ions and high salt stress, threats to the growth of plants. Hydrogen peroxide (H_2O_2) serves as another endogenous biomarker within plant bodies, utilized for tracking oxidative stress induced by heavy metal ion contamination (Cd^{2+}) or high salinity (NaCl) [42,43]. Wu *et al.* [44] reported an activatable NIR-II fluorescent probe, named CT-XA-H_2O_2, utilized for monitoring oxidative stress in plant sprouts induced by Cd^{2+} contamination or high NaCl salinity through detecting endogenous biomarker H_2O_2. In the presence of H_2O_2, the boronate moiety within the probe CT-XA- providing warning and monitoring for oxidative stress induced by capability for imaging and detecting *in situ* biomarkers within plant sprouts, providing warning and monitoring for oxidative stress induced by Cd^{2+} or NaCl (Fig. 4C). The authors further demonstrated that the response of the CT-XA-H_2O_2 probe to H_2O_2 is unaffected by other common ions in soil and certain reactive oxygen species. Based on the imaging results of soybean and peanut sprouts, the higher levels of Cd^{2+} and NaCl resulted in increased fluorescence intensity, corresponding to elevated stress levels in plants. These results highlighted the ability of CT-XA-H_2O_2 to track non-biological stress inducing by NaCl in soybean and peanut sprouts through *in vivo* NIR-II fluorescence imaging.

Recently, Giraldo *et al.* [45] reported a NIR nanosensor: HeAptDNA-SWNT (Fig. 5A). Through non-covalent functionalization, SWNTs were encapsulated in a layer of DNA aptamer sequences and hemoglobin, which endowed its high sensitivity and specificity in detecting plant H_2O_2. Due to the presence of hemin, HeAptDNA-SWNT could effectively catalyze a Fenton-like reaction, generating hydroxyl radicals from H_2O_2 and quenching its fluorescence emission in a concentration-dependent manner similar to

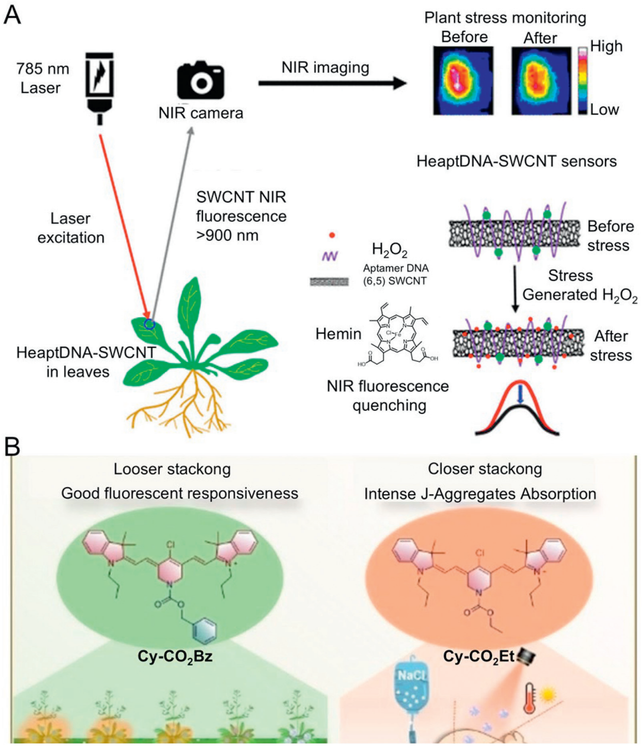

Fig. 5. (A) Monitoring plant health in real-time using SWCNT sensors for detecting H_2O_2. SWCNTs, functionalized with a DNA aptamer specific to hemin (HeAptDNA-SWCNT), exhibit a decrease in NIR fluorescence when they interact with H_2O_2 produced during plant stress. The spatial and temporal variations in NIR fluorescence intensity in leaves containing HeAptDNA-SWCNT sensors are remotely captured using an NIR camera to evaluate the plant's health condition. Reproduced with permission [45]. Copyright 2020, American Chemical Society. (B) Cy-CO_2Bz and Cy-CO_2Et compounds are utilized for tracing salt stress in plants and for enhancing photothermal therapy of tumors. Reproduced with permission [47]. Copyright 2022, Wiley-VCH.

protonation reactions, facilitating quantitative analysis. Moreover, the nanosensor demonstrated specific recognition of H_2O_2, as its NIR fluorescence signal was unaffected by substances such as Ca^{2+}, sucrose, glucose, methyl salicylate, abscisic acid, and jasmonic acid. It is noteworthy that HeAptDNA-SWNT is a reversible sensor capable of real-time reporting of physiological levels of H_2O_2 in plant tissues. More importantly, the NIR emission intensity of HeAptDNA-SWNT significantly decreased (~9.4%) at an H_2O_2 concentration of 10 µmol/L, and with increasing H_2O_2 concentration, the NIR emission intensity further decreased to ~12.9%, achieving highly sensitive responses within the physiological range of plant H_2O_2concentrations (10–100 µmol/L). In brief, such nanosensor was capable of real-time detection and quantitative analysis of biological processes in plants, providing a new tool for early prevention of plant diseases.

Salt stress refers to the condition where the salt content in the soil exceeds the upper limit of tolerance for crops, thereby affecting normal plant growth. Therefore, to better construct a plant salt stress monitoring system [46], Yang *et al.* [47] reported two types of aza-IR780 heptamethine cyanine dyes containing *N*-amidomethyl groups, which could form *J*-aggregates under NaCl induction. Among them, Cy-CO_2Bz exhibited a good fluorescent response to NaCl, serving as a sensitive indicator for tracking active root tips and the overall degree of salt stress, while effectively avoiding background signals produced by chlorophyll. *N*-Ethyl oxycarbonyl substituted Cy-CO_2Et formed tightly packed *J*-aggregates under NaCl conditions, significantly increasing the molar absorptivity (Fig. 5B). Compared with the monomers of anthocyanins, both the absorption and emission bands of *J*-aggregates underwent a red shift, resulting in promising *in vitro* and *vivo* fluorescence performance. Sodium salts containing only halide and nitrite ions promoted the formation of broad absorption bands, while sodium salts containing only halide ions caused Cy-CO_2Et to form *J*-aggregates. Therefore, NaCl exerted strong regulatory effects on the formation of these two anthocyanin aggregates. With the increase of NaCl concentration in water, the intensity of the absorption band (770 nm) of Cy-CO_2Bz monomer gradually decreased, while the absorption intensity of *J*-aggregates increased. Cy-CO_2Bz could effectively penetrate into Arabidopsis tissues, and with the increase of probe concentration, the fluorescence image produced by the leaves gradually decreased, consistent with the observation of aggregation-induced quenching *in vitro* studies. By observing confocal laser scanning microscopy images of Arabidopsis root tips and *in vivo* images, it found that after further treatment with NaCl stress, significant fluorescence changes could be observed in Arabidopsis.

NIR fluorescence imaging enables non-invasive observation of gene protein expression and various cellular activities in plant organisms. Compared with magnetic resonance and radioisotope imaging, NIR fluorescence imaging has low photon detection thresholds, resulting in higher sensitivity, making it capable of detecting minute components in plant tissues. Each probe is designed specifically for a particular molecular target, resulting in strong specificity and the ability to avoid interference from other components in plant cell matrices. Additionally, NIR fluorescence imaging has low scattering rates, allowing for strong tissue penetration, providing scientists with deeper understanding and cognition of substances in plants that are either undiscovered or difficult to detect. Unlike bioluminescence imaging, NIR fluorescence imaging does not require genetic modification, making the operation relatively simple. Currently, NIR fluorescence imaging is still in the early stage of plant science, which presents significant challenges [48].

3. The application of SERS in the field of plants

Nowadays, SERS and SRS technologies are playing an increasingly role in the field of plant health monitoring. Herein, we primarily focused on SERS technology [49,50]. SERS technology combines the high sensitivity of Raman scattering with surface enhancement effects, enabling high sensitivity and high-resolution imaging of molecules in plants at the microscale. Based on SERS technology, non-destructive and real-time analysis of chemical components on the surface and inside plants can be achieved. Due to its highly sensitive nature, SERS can accurately detect trace active substances in plant samples. These active substances include but are not limited to secondary metabolites inside plants, hormones, pigments, and fungi. Based on SERS, researchers can observe the spatial distribution and concentration changes of these active substances in plant tissues. Moreover, SERS also act as powerful tool for studying plant physiological metabolism, resistance mechanisms, medicinal value, and more, contributing to a deeper understanding of internal chemical information in plants and advancing progress and applications in the field of plant science.

3.1. SERS plant imaging

The SERS imaging of cells in plant tissues is of paramount importance for gaining insights into plant-microbe interactions, studying plant health, disease resistance mechanisms, and microbial ecology in agriculture and ecosystems [51]. Firstly, SERS imaging enables researchers to monitor and evaluate microbial communities within plant tissues in real time, both on the surface

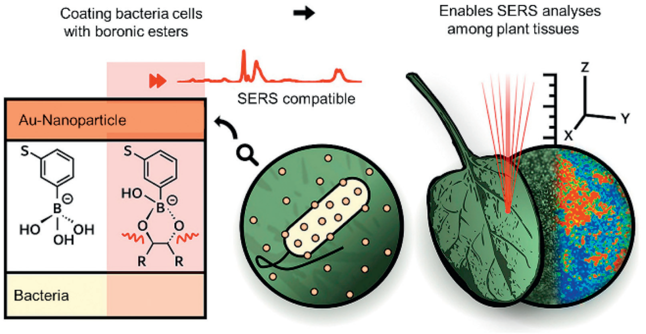

Fig. 6. Schematic diagram of SERS imaging analysis of bacterial cells between plant tissues. Reproduced with permission [54]. Copyright 2020, Elsevier.

and internally. This is crucial for the early detection of plant pathogen infection and for evaluating plant health conditions. Secondly, it provides insight into the location of microbes within plants. Through SERS imaging, the precise positioning of bacterial cells within plant tissues could be identified with high resolution, aiding in understanding microbial distribution and colonization patterns within plants. This is significant for research on plant-microbe interactions, ecology, and pest control. Thirdly, SERS not only offers morphological information about bacterial cells, but also allows for monitoring of microbial metabolic activities. Analysis of SERS signals provided information about the physiological status and metabolic products of microbes within plant tissues, in which is helpful to understand microbial functions and activities. Lastly, it has implications for agriculture and environmental monitoring. For example, SERS imaging enables analysis of microbial communities in soil to improve soil health and ecosystem management. Additionally, SERS imaging can be utilized for monitoring environmental microbes, assessing environmental pollution, and evaluating ecosystem health [52,53].

However, effective imaging and *in situ* monitoring strategies for bacteria within plant tissues without damaging the plant tissue are still limited. Faced with this issue, He *et al.* [54] pre-labeled bacteria with 3-mercaptopropionic acid to allow for complexation with silver nanoparticles, forming panoramic chemical images of bacterial populations through SERS (Fig. 6), which realized *in situ* monitoring of bacterial cells in plant tissues on a large scale. The results indicated that spinach leaves exhibited the strongest SERS signal depth, possibly due to the high optical transparency of the tissue, allowing better laser penetration. Despite the rough and random surface of jackfruit, SERS microscopy could still image bacterial distribution on its surface. Additionally, it also found that peanut shells adsorbed the bacterial inoculum, resulting in noticeable discoloration upon contact with the shell. Therefore, SERS could be used for analyzing bacterial communities in plant tissues and researchers could scan large areas of bacterial colonies in natural substrates. Thus, SERS was expected to stimulate new research in studying bacterial distribution, internalization, and attachment in plant structures and bacterial-plant interactions. However, this method still has limitations when dealing with thicker leaves and is insensitive to certain restricted structures, necessitating further improvement.

3.2. Plant hormone detection

Gibberellins, auxins, cytokinins, abscisic acid and ethylene are among the key hormones that aid plants in coping with environmental stresses such as drought, salinity, and pest infestations, thereby enhancing their resilience [55]. Hence, understanding and mastering the mechanisms of plant hormones is paramount for plants including facilitating agricultural production, increasing crop yields, and effectively ensuring sustained plant health and growth [56]. For example, when the levels of abscisic acid (ABA) deviate from normal, it can significantly impact wheat yield [57]. To address this issue, Hu *et al.* [58] designed a SERS aptamer sensor 1, incorporating 4-mercaptobenzoic acid (4-MBA) between gold cores and silver shells to stabilize SERS signals and prevent external matrix interference (Fig. 7A). Meanwhile, they used biotinylated complementary DNA (cDNA) strands coupling with Fe_3O_4 magnetic nanoparticles (MNPs) as capture probes and ABA aptamer terminally modified with Ag-S covalently bonded thiol groups linked to Au@Ag nanoparticles (NPs) as signal probes. In the presence of ABA, the aptamer preferentially bonded to ABA molecules, thus releasing the signal probe by the capture probe, resulting in the decreased SERS intensity of 4-MBA upon magnetic separation. This allows for the quantitative measurement of ABA levels in samples based on the inverse relationship between 4-MBA's SERS intensity and ABA concentration, achieving high sensitivity and selectivity in ABA detection. Notably, this new ABA detection strategy offered a wider linear range, lower detection limits, and significantly higher sensitivity, even outperforming chromatography by five orders of magnitude and requiring only a few seconds for detection. Selectivity tests demonstrated the sensor's excellent specificity towards ABA even in the presence of other endogenous hormones like gibberellin A3 (GA3), indole-3-acetic acid (IAA), and cytokinin (CTA). This high-performance SERS aptamer sensor not only enabled sensitive detection of ABA in plants but also held promise for analyzing other endogenous hormones in plants.

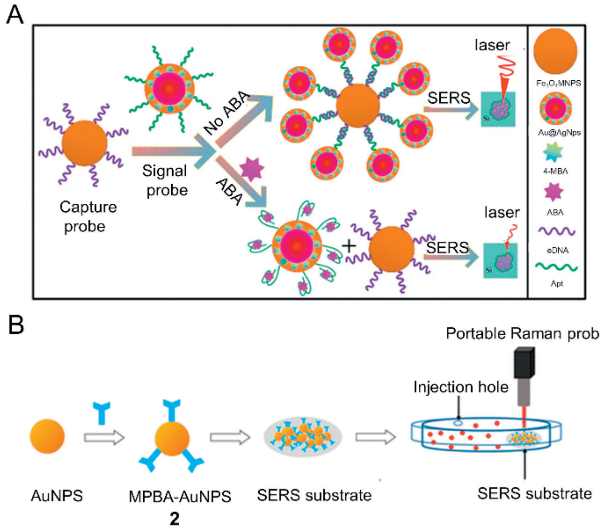

Fig. 7. (A) Schematic diagram of the detection principle of abscisic acid using SERS aptamer sensor. Reproduced with permission [58]. Copyright 2022, Springer. (B) Detection scheme for the plant volatile biomarker methyl salicylate using SERS. Reproduced with permission [61]. Copyright 2022, American Chemical Society.

Furthermore, methyl salicylate (MeSA) is a volatile organic compound (VOC) released in significant quantities by plants during pathogen infection [59]. The quantitative detection of MeSA enables the rapid diagnosis of plant diseases. Hence, the development of a rapid, sensitive, and reusable MeSA detection method could well meet the demands of plant disease monitoring [60]. Zhao *et al.* [61] fabricated a SERS sensor 2 utilizing 4-mercaptophenylboronic acid (MPBA)-functionalized AuNPs for MeSA detection (Fig. 7B). Utilization of MPBA-functionalized AuNPs as SERS probes, the SERS signal of MPBA decreased in the presence of MeSA vapor, which was attributed to the weak interactions between MPBA and MeSA. By measuring changes in the SERS signal, MeSA vapor can be detected with high sensitivity. More-

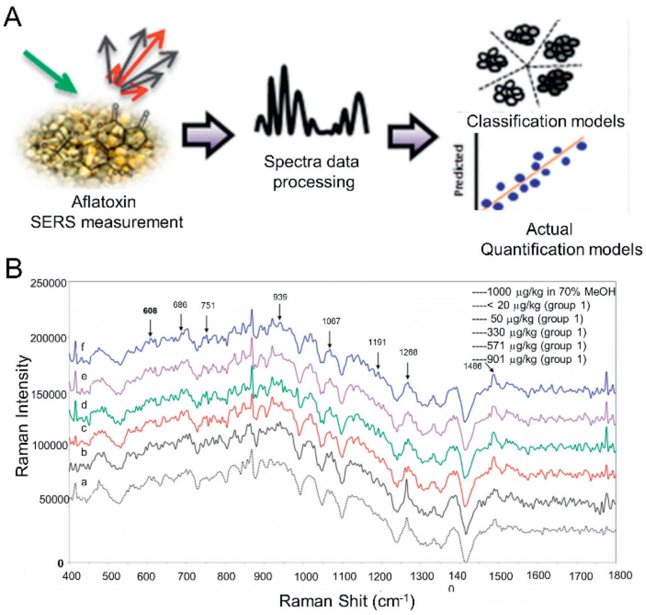

Fig. 8. (A) Schematic diagram illustrating the detection of plant aflatoxin using the Raman spectroscopy technique. (B) SERS spectra of 1000 μg/kg pure aflatoxin extraction solvent (70% MeOH) and aflatoxin-contaminated samples at different concentrations below. Reproduced with permission [64]. Copyright 2014, American Chemical Society.

over, this sensor displayed good reproducibility, reusability, and specificity towards other common leaf volatile organic compounds. Thus, this research not only provided a novel tool for the rapid and sensitive detection of plant volatile compounds but also supported studies on signal transduction between plants and plant disease monitoring.

3.3. *Detection of plant fungi*

Early detection and accurate identification of fungi in plants are crucial for safeguarding crop health and the introduction of SERS technology offers new potentials for fungal detection. For example, researchers have successfully utilized SERS to detect various fungi in plants, including powdery mildew pathogens and aflatoxin [62]. For instance, aflatoxin is a common fungal toxin in corn that poses health risks to humans and animals. However, the employ of current SERS in the aflatoxin detection still faces some technical challenges including the preparation and stability of metal substrates [63]. Therefore, Murray *et al.* [64] proposed a simple and rapid SERS method for on-site detection of aflatoxin in corn (Fig. 8A). Initially, the authors prepared corn powder samples containing different concentrations of aflatoxin which were then extracted using a methanol/water extraction solvent. Subsequently, Ag NPs were employed to prepare SERS substrates, followed by mixing the SERS substrates with the sample extract solution for SERS measurement. The results indicated that SERS possessed high sensitivity, specificity, and rapidity, serving as a high-throughput analytical tool for the rapid detection and analysis of corn samples (Fig. 8B).

Despite this successful example, much effort should be paid to promote this field [65]. Firstly, there is a need to encourage more research institutions to engage in relevant studies. Secondly, interdisciplinary cooperation can be strengthened, integrating expertise from biology, spectroscopy and nanotechnology to collectively tackle the challenges. Additionally, efforts should be made to encourage and support the conduct of relevant research projects, allocating more funds for in-depth research on optimization of SERS technology. Through these efforts, it can expect more breakthroughs in SERS technology research in the field of plant fungi detection, further expanding its application in agricultural production and food safety [66-68].

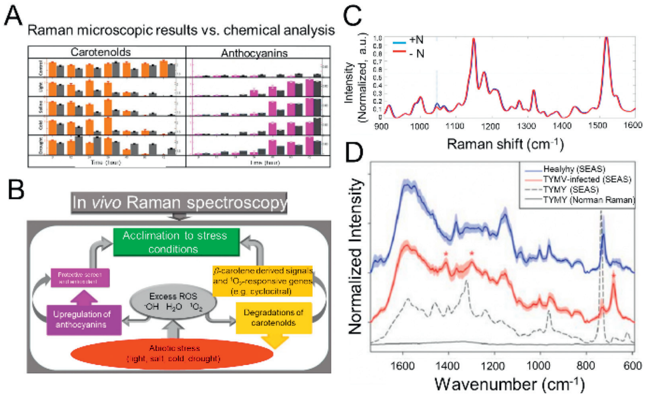

Fig. 9. (A) Bar chart showing fitting coefficients for carotenoids (in brown) and chemical extraction values of carotenoids (in grey) as a function of non-biological stress duration. (B) Diagram demonstrating concurrent detection of anthocyanins and carotenoids in plants *via* Raman spectroscopy technology. Reproduced with permission [71]. Copyright 2017, Proceedings of the National Academy of Sciences of the United States of America. (C) Transfer 3-week-old wild-type seedlings into +N and -N hydroponic culture media respectively, and measure Raman spectra after 3 days of cultivation. Reproduced with permission [74]. Copyright 2020, Frontiers Media S.A. (D) Normal Raman spectrum (black) and SERS spectrum (dashed) of pure turnip yellow mosaic virus (TYMV). SERS spectra of 1 μL extracts from healthy leaves (blue) and TYMV-infected leaves (red). Reproduced with permission [75]. Copyright 2013, Wiley-VCH.

3.4. *Detection of other substances in plants*

Plant pigments only impart various colors to plants but also play key roles in photosynthesis and plant growth and development [69]. Through SERS technology, plant pigments can be detected and characterized with high sensitivity, which facilitates the detection of pigments at the level of individual plant cells. Therefore, the importance of SERS modality in studying plant pigments is evident, as it reveals molecular-level information about plant pigments, providing crucial support and insights for further exploration of plant life processes [70].

Plant growth is easily influenced by changes in the natural environment, such as drought and cold, which lead to alterations in the physiological, biochemical, and morphological characteristics of plants. Currently, many methods for assessing plant biochemical properties rely on destructive chemical analyses, which are time-consuming, and may involve toxic chemicals. To overcome these challenges, Scully *et al.* [71] proposed a high-throughput stress phenotype analysis method based on Raman spectroscopy technology. From the SERS detection results, they observed the changes in the concentrations of carotenoids and anthocyanins in plants under four common abiotic stress conditions: high soil salinity, drought, low temperature, and light saturation (Fig. 9A). Thus, SERS enabled high-throughput stress phenotype and early stress detection in plants (within 48 h), with higher sensitivity and the ability to simultaneously detect carotenoids and anthocyanins (Fig. 9B). Based on these results, anthocyanins and carotenoid change all four abiotic stress factors and they have been also found to act as signaling molecules for plant stress. Finally, the further chemical analyses also validated the accuracy of SERS detection, which confirmed the feasibility of SERS for these pigments.

Nitrogen is one of the crucial nutrients for plant growth and development and it primarily exists in the soil in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), which plants absorb through their roots. Enabling them to withstand such as drought and high temperatures. Therefore, accurate monitoring and management of plant nitrogen status are crucial for achieving sustainable and efficient agriculture [72,73]. Park *et al.* [74] recently utilized Raman technology to achieve rapid diagnosis of nitrogen status by detecting nitrate levels in plant leaves. Through studying Arabidopsis thaliana and two leafy vegetable species, it found that Raman spectroscopy accurately detected changes in nitrate levels caused by nitrogen deficiency (Fig. 9C). Arabidopsis thaliana and two leafy vegetables were selected as models and were cultivated on nitrogen-containing and nitrogen-free media, observing leaf yellowing due to nitrogen deficiency. Then, the total chlorophyll and nitrate contents in the leaves were measured using Raman spectroscopy and it discovered a strong Raman peak in nitrate molecules. Therefore, it proposed using the nitrate Raman peak as an indicator of plant nitrogen status. Finally, by comparing wild-type Arabidopsis thaliana with mutants, it found a significant decrease in the intensity of the nitrate Raman peak in mutants, consistent with the decrease in their nitrate content.

Plant viruses disrupt normal physiological processes that cause serious harm to plant growth and development. For example, turnip yellow mosaic virus (TYMV) affects cruciferous vegetables such as turnips, radishes, and cabbage. Traditional polymerase chain reaction require lengthy analysis time and complex sample processing steps. Raman spectroscopy technology, with its rapid, non-destructive nature and ability to provide rich structural and chemical information, is considered a potential method for plant virus detection. Chung *et al.* [75] utilized Raman spectroscopy technology and SERS technology to measure and compare cabbage plants infected with TYMV and healthy plants. The results showed that Raman could differentiate between infected and non-infected plants, providing a potential alternative method for screening plant virus infections (Fig. 9D). As seen in the SERS spectrum, the characteristic peaks of TYMV were significant and intense, demonstrating the high sensitivity of SERS technology for TYMV detection. Furthermore, by comparing the differences in metabolite compositions of healthy and infected leaves SERS not only effectively identified infections, but also visualized the discriminative ability between healthy and infected samples in a two-dimensional space. This study provided a reference for further studying the metabolic pathways of plant virus infections and plant physiology.

4. The combining modality of fluorescence and SERS in the field of plants

The combination of Fluorescence imaging and SERS modalities can provide detailed and precise information on various aspects of plant physiology, growth monitoring, disease detection, and screening of active compounds [76]. For example, fluorescence imaging can be used to monitor the photosynthetic efficiency of plants, while SERS technology can be used to detect specific chemical substances on the surface or within plants, such as pesticide residues or heavy metals. In the given example, Kwak's research team [77] simply integrated these two techniques to monitor the health status of plants. They synthesized an intracellular SERS sensor, termed sensor 3, by first reducing silver nitrate onto thiolated silica NPs using hexadecylamine to synthesize silver nanoshells (AgNS), enhancing NIR Raman scattering intensity (Fig. 10A). Subsequently, they modified the surface of AgNS with a water-soluble cationic polymer, poly(diallyldimethylammonium chloride) (PDDA), to improve water compatibility. Once loosely coiled PDDA polymer chains attracted relevant signal molecules from plants, these molecules were localized near the surface of AgNS, resulting in highly enhanced Raman signals. Through electrostatic interactions, sensor 3-PDDA attracted endogenous ATP (eATP) from within the plant, then captured eATP near the surface of AgNS by forming multiple hydrogen bonds, achieving the goal of monitoring eATP within the plant. It is noteworthy that sensor 3 could enter plants through stomata and localize within intercellular spaces, enabling faster detection of low concentrations of relevant signal molecules. Due to their different SERS absorption bands, ATP, salicylic acid (SA), IAA, and folic acid (FA) were simultaneously detected with increasing concentration, even in the presence of other molecules, demonstrating the feasibility of sensor 3 for simultaneous detection of multiple chemical analytes. Experimental evidence showed that due to the strong interaction between the thiol group of glutathione and the AgNS surface of the sensor, sensor 3 easily detected the transport of endogenous glutathione on the cell wall, manifesting as a SERS signal at 643 cm^{-1} (Fig. 10B).

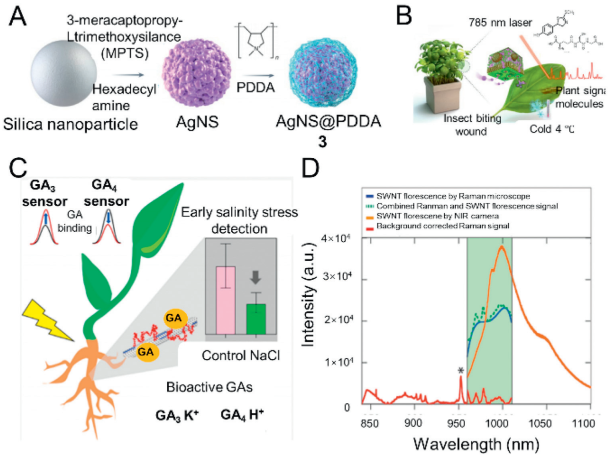

Fig. 10. (A) Schematic diagram of sensor design utilizing **3**. (B) Diagram depicting SERS detection by the sensor under non-biological stress. Reproduced with permission [77]. Copyright 2023, Springer Nature. (C) Detection of early salinity stress by GA family. (D) Representative spectra of GA3-SWNT sensor obtained using NIR camera (orange) and coupled Raman/NIR fluorescence spectrometer (green dots). Reproduced with permission [80]. Copyright 2023, Springer American Chemical Society.

Subsequently, scientists further ingeniously combined NIR fluorescence imaging with SERS imaging for monitoring the gibberellins (GAs) within plant organisms. GAs are a crucial class of hormones for plant growth and sensitive signaling molecules in living plants. When plants face environmental stresses (*e.g.*, salinity and drought), the GA levels in plant tissues fluctuate, particularly in the roots. GAs containcarboxyl groups, and most plant hormones containing carboxyl groups primarily exist in deionized forms in plants [78,79]. Based on this feature, Strano *et al.* [80] synthesized a library of SWCN sensors encapsulated by different amphiphilic polymers using the corona phase molecular recognition platform. The designed carbon nanotubes emitted NIR fluorescence and embedded with charged styrene derivative monomers, enabling specific recognition of GAs through electrostatic interactions and hydrogen bonding. In plant or cell environments, two screened specific sensors were separated in roots of basil plants containing GA3 and GA4, respectively, and both of them exhibited fluorescence enhancement after a certain period (Fig. 10C). By contrast, the reference sensors for the GA family showed no change, confirming the specificity of detection. Meanwhile, the Raman G-band in the sensors could not only be used to label biological cells but also serve as an internal reference for fluorescence emission normalization (Fig. 10D). The intensity of the Raman of G-band of carbon nanotubes was linearly related to concentration at low concentrations, allowing for fluorescence standardization of carbon nanotubes using the Raman G-band in plant samples, thereby eliminating fluorescence variations caused by uneven concentrations. This innovation successfully addressed the challenge of requiring active and reference sensors to be placed simultaneously on the same tissue in the past, significantly simplifying instrument requirements and enabling GA measurement without the need for a reference.

In conclusion, the combined use of fluorescence imaging and SERS technology provides a powerful tool for plant science research and agriculture, offering detailed information on plant physiology and chemical states. This contributes to improving crop yield and quality, as well as ensuring food safety. With advancements in technology and cost reduction, the combined application of these two technologies is expected to be more widely adopted in the future.

5. Summary and perspective

As our understanding of plant biology deepens, insights into the molecular dynamics and biological mechanisms of living plants have become increasingly crucial. This not only helps to reveal the mysteries of plant growth and development but also aids in understanding how they regulate metabolism and respond to environmental stress. This paper reviews the design principles, synthetic strategies, and applications in plant research of various types of fluorescent probes, and discussed the design principles and potential applications in the plant field of various types of Raman sensors. This review particularly emphasized the important role of fluorescence detection and SERS modalities in the imaging and detection of plant chemicals, hormones, pigments, fungi, viruses, *etc.* [81,82].

Both of fluorescence and SERS detection technology have unique advantages and limitations. They can complement each other in certain aspects, jointly promoting the progress of plant scientific research and agricultural production. For example, NIR fluorescence technology comprehensively assesses the physiological state of plants through macroscopic monitoring methods, while SERS technology uses its microscopic detection capabilities to accurately identify specific molecules within the plant body. The combination of these two technologies provides strong support for a comprehensive assessment of plant health status, significantly improving the accuracy of plant disease diagnosis and the precision of growth monitoring, bringing a new and efficient tool for plant health management [83].

The combined application of NIR fluorescence and SERS technologies in the field of plant detection has indeed demonstrated significant potential, but it also faces a series of challenges. Firstly, the auto-fluorescence of plants can interfere with the detection of SERS signals or NIR markers, affecting the clarity of imaging and posing obstacles to precise analysis [84]. Future research directions should consider integrating with NIR-II region fluorescence technology to circumvent these interferences [85-88]. Moreover, although SERS technology is renowned for its high specificity and sensitivity, the issue of signal reproducibility remains to be addressed, limiting its application in quantitative analysis [89]. The emergence of digital colloidal enhanced Raman spectroscopy (dCERS) technology, which employs single-molecule counting, provides a new solution for achieving high sensitivity and accurate quantification of ultra-low concentration molecules [90]. In the field of plant health detection, we propose the development of intelligent monitoring devices for plants, inspired by the concept of wearable devices such as smartwatches for humans. These devices would be capable of real-time monitoring of plant biological indicators, providing immediate and accurate data support for plant health management, thus leading to revolutionary progress in the field [91]. This innovative monitoring method could not only enhance the efficiency and accuracy of plant detection but also is expected to have a profound impact on agricultural technology.

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.

CRediT authorship contribution statement

Shu Tian: Writing – original draft, Methodology, Data curation. **Wenxin Huang:** Writing – review & editing, Formal analysis, Data curation. **Junrui Hu:** Writing – review & editing. **Huiling Wang:** Visualization, Validation, Formal analysis. **Zhipeng Zhang:** Writing – review & editing, Resources, Formal analysis. **Liying Xu:** Writing – review & editing, Data curation. **Junrong Li:** Writing – review & editing, Visualization, Supervision, Methodology. **Yao Sun:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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