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Nanobiotechnology approaches for engineering smart plant sensors

Juan Pablo Giraldo[1,2,3]*, Honghong Wu[1], Gregory Michael Newkirk[1] and Sebastian Kruss[4]

Nanobiotechnology has the potential to enable smart plant sensors that communicate with and actuate electronic devices for improving plant productivity, optimize and automate water and agrochemical allocation, and enable high-throughput plant chemical phenotyping. Reducing crop loss due to environmental and pathogen-related stresses, improving resource use efficiency and selecting optimal plant traits are major challenges in plant agriculture industries worldwide. New technologies are required to accurately monitor, in real time and with high spatial and temporal resolution, plant physiological and developmental responses to their microenvironment. Nanomaterials are allowing the translation of plant chemical signals into digital information that can be monitored by standoff electronic devices. Herein, we discuss the design and interfacing of smart nanobiotechnology-based sensors that report plant signalling molecules associated with health status to agricultural and phenotyping devices via optical, wireless or electrical signals. We describe how nanomaterial-mediated delivery of genetically encoded sensors can act as tools for research and development of smart plant sensors. We assess performance parameters of smart nanobiotechnology-based sensors in plants (for example, resolution, sensitivity, accuracy and durability) including in vivo optical nanosensors and wearable nanoelectronic sensors. To conclude, we present an integrated and prospective vision on how nanotechnology could enable smart plant sensors that communicate with and actuate electronic devices for monitoring and optimizing individual plant productivity and resource use.

To meet the demand in 2050 for food and energy from a growing human population, crop productivity is projected to require a 60–100% increase from 2005–2007 levels[1,2]. However, environmental and biological stresses impair growth and yield, leading to major crop losses worldwide[3,4]. Plant environmental stresses including drought[5], heat[6], flooding[7], salinity[4,6] and frost[6,8] are key drivers of economic losses in agriculture. Microbial pathogens and virus-related diseases also lead to devastating crop losses[9–11]. The challenge of improving agricultural productivity is magnified by the expected increase in the frequency and intensity of plant stress-related events in a changing climate[12,13]. Thus, precise management of limited resources and costly agrochemicals (for example, water, nutrients and pesticides) offers the opportunity to increase crop yields while minimizing resource losses through the use of remote sensing techniques for crop monitoring[14,15]. Improved tools for high-throughput phenotyping of desired crop traits will be needed to accelerate efforts towards developing plant stress tolerant varieties[16]. Increasing total agricultural productivity will require innovative and convergent technological approaches for managing plant stressors and resource use efficiency.

Recent advances in nanotechnology offer untapped potential to reduce the impact of major stresses on food and energy crop productivity, while optimizing the use of limited resources such as water or nutrients. In this Review, we discuss how nanobiotechnology-based sensors in plants can enable communication and actuation of electronic agricultural and phenotyping devices for optimizing crop growth and yield in response to stresses or resource deficits. We envision smart nanobiotechnology-based sensors that report plant health status through optical and wireless signals and fine-tune agricultural device responses (Fig. 1). Nanosensors are poised to enable the translation of plant chemical signals into digital information for real-time monitoring of plant health by electronic devices. These nanobiotechnology approaches for precisely detecting the onset of stress or resource deficits within individual plants will contribute to improving phenotyping technologies for plant breeding by facilitating high-throughput screening of chemical phenotypes of stress-tolerant varieties. Smart plant sensors engineered through nanobiotechnology will be able to report crop health status to existing agricultural equipment to promote a transition to automated methods for precise and efficient use of resources.

Nanotechnology, the manipulation and use of matter with dimensions smaller than 100 nm, has distinct advantages to engineer smart plant sensors. For example, engineered nanomaterials embedded in plants can be designed for monitoring signalling molecules by near-infrared (nIR) cameras in real time. Nanomaterials can also act as DNA scaffolds that overcome plant cellular barriers and deliver genetically encoded biosensors for crop research. Wearable sensors can record volatile compounds on plant surfaces and report to wireless devices. In this Review, 'sensor' is used as a general term for any probe, indicator, reporter, molecular sensor or sensing device if not termed differently in the cited reference. Optical nanosensors are nanomaterials that allow high spatial and temporal resolution for monitoring plant signalling molecules via fluorescence signals in spectral regions, such as the nIR, where living tissues are relatively transparent[17–20]. These nanosensors can be tailored to provide high stability, specificity for analytes of interest, rapid dynamics, accuracy and reproducibility in the desired analytical range for plants[21]. Recently, nanotechnology has enabled the delivery of DNA plasmids into plant cells using high aspect ratio nanomaterials as scaffolds that penetrate plant cell barriers in vivo without external mechanical or chemical aid[22,23], thus opening the door to deliver genetically encoded sensors for designing and engineering smart plant sensors. Genetically encoded sensors generally consist of a sensory module coupled to fluorescent proteins that can be detected with high spatial and temporal resolution by fluorescence imaging devices[24,25]. They are highly selective for the specific analyte and exhibit a high signal-to-noise ratio with low perturbation of biological systems in which they are integrated. Wearable sensors integrate flexible substrate materials and nanoelectronics that are advancing the fields of sensors for human health monitoring

[1]Department of Botany and Plant Sciences, University of California, Riverside, CA, USA. [2]Center for Plant Cell Biology, University of California, Riverside, CA, USA. [3]Institute of Integrative Genome Biology, University of California, Riverside, CA, USA. [4]Institute of Physical Chemistry, Georg August University Göttingen, Göttingen, Germany. *e-mail: juanpablo.giraldo@ucr.edu

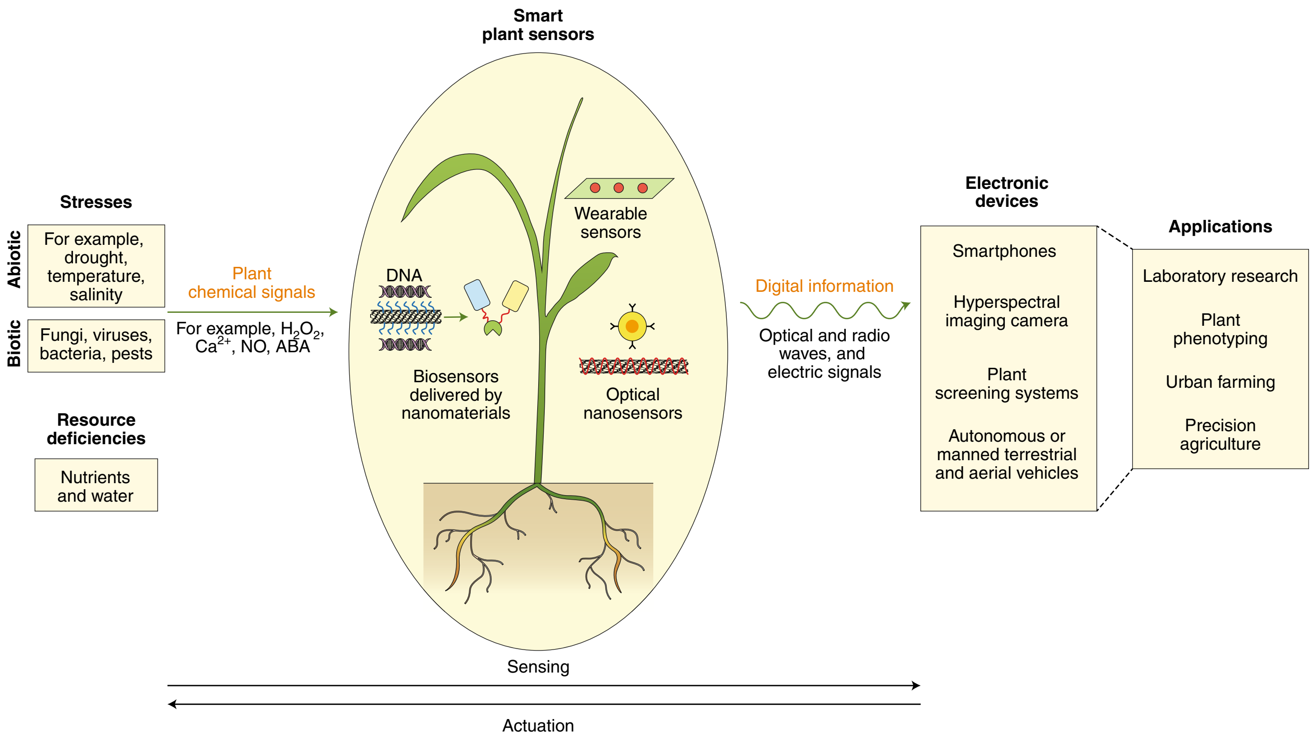

Fig. 1 | Nanobiotechnology approaches enable research and development of smart plant sensors that communicate plant chemical signals to agricultural and phenotyping equipment. Major abiotic and biotic stresses and resource deficiencies are associated with signalling molecules that communicate and regulate plant responses including ROS (for example, H_2O_2), Ca^{2+}, NO and ABA among others. Nanomaterial-mediated delivery of genetically encoded sensors enables research on signalling mechanisms of plant health that inform the design and engineering of smart plant sensors. Optical nanosensors and wearable nanotechnology-based sensors interfaced with plants allow the translation of plant chemical signals into optical and radio waves, and electric signals, which can be monitored by electronic devices. Machines with the capacity to decode spatiotemporal patterns of plant chemical signals will allow smart nanobiotechnology-based sensors to actuate agricultural devices for optimizing the plant environment. These nanobiotechnology approaches have applications ranging from research and development of technologies in the laboratory, chemical phenotyping in specialized facilities to monitoring and automation in crops for urban farming and precision agriculture.

and robotic manipulation[26] but with applications that remain relatively unexplored in plants. Engineered nanomaterials are not genome specific, making them more easily deployed across diverse plant species than species-specific molecular methods[19,20,27–30]. Nanobiotechnology approaches do not necessarily require genetically modifying whole plants to introduce new traits, which is a procedure that faces considerable governmental regulations and public debate[31,32]. Although nanomaterials are increasingly becoming subject of these regulations, studies in the past decade have reported that a wide range of nanomaterials are not inherently toxic and that detrimental effects on living organisms are dependent on specific nanomaterial composition, structure, surface chemistry and concentration[33–38]. Furthermore, we expect that exposure to nanomaterials will only be required for selected plants within an agricultural field. On these selected plants, a variety of plant structures for non-human consumption could be chosen for nanomaterial exposure. In some instances, smart nanobiotechnology-based sensor development and application would involve plant exposure to nanomaterials only in the laboratory or controlled phenotyping setups. Smart plant-sensing devices have the potential to provide unprecedented capabilities of allowing real-time monitoring of individual plant physiological status in response to stresses in safe and controlled conditions.

This Review focuses on knowledge gaps, challenges and opportunities to engineer nanobiotechnology-based sensors that report plant health status and control actuation of electronic devices for improving crop productivity and phenotyping. This field is of interest to researchers working on nanoscale science and engineering tools for agriculture, engineers aiming to address knowledge gaps in plant nanobiotechnology and biologists looking for novel tools to monitor plant biomolecules. Previous reviews discuss topics not covered in depth by this article including broad agronomic applications of nanotechnology in plants[39]; design, principles and applications of nanosensors for plant biomolecules[21] and genetically encoded biosensors in plants[24,25]. Wang et al.[39] review targeted and controlled release of agrochemicals, labelling and imaging of nanoparticles in vivo and risks associated with exposure of nanomaterials to the food chain. Kwak et al.[21] explore in more depth the mechanisms of how nanobiotechnology-based sensors for plants work and compare the pros and cons of different nanosensor technologies. Herein, we discuss nanobiotechnology-based approaches for monitoring plant signalling molecules associated with early detection of stress or resource deficiencies using nanomaterial-delivered genetic-encoded nanosensors, optical nanosensors and wearable sensors. We also provide a perspective on how smart plant sensors can actuate agricultural devices by communicating with smartphones, hyperspectral imaging cameras, wireless radio frequency devices, meteorological stations and phenotyping equipment.

Monitoring plant health in real time

Crop stress and resource deficiencies are currently monitored by assessing plant physical traits through imaging, spectroscopy and fluorescence from the visible to the infrared[16,40,41]. Although, these remote-sensing techniques provide important information about plant health status including leaf area, chlorophyll content and

Table 1 | Plant molecular targets related to health status that have been reported to be monitored by nanotechnology-based sensors

Plant signalling molecule	Concentration range (static)	Time scale	Sensor[a], indicator[b], probe[c], reporter[d] or not defined[e]	Sensitivity	Temporal resolution
H_2O_2	10–100 μM (refs. [127,128])	<1 min (ref. [129])	SWCNT[a] (refs. [20,130])	Single molecule to 100 μM (refs. [20,130])	0.5 s (ref. [20])
			HyPer2[a] (ref. [79])	5 μM (ref. [79])	2 min (ref. [79])
			roGFP-Orp1[a] (ref. [81])	0.1 μM (ref. [81])	15 s (ref. [81])
Glucose	0.1–1,000 μM (ref. [74])	10 s (ref. [63])	FLIPglu-2μΔ13[a] (refs. [63,75])	2.5–100 μM (ref. [75])	4 s (ref. [75])
			FLIPglu-600μΔ13[a] (refs. [63,75])	0.1–7.5 mM (ref. [75])	4 s (ref. [75])
			SWCNT[a] (ref. [131])	5–20 mM (ref. [131])	15 s (ref. [131])
			BA-QD[c] (ref. [29])	500–1,000 μM (ref. [29])	5 min (ref. [29])
Sucrose	1–10 mM (refs. [132,133])	20 s (ref. [75])	FLIPsuc-90μΔ1[a] (ref. [75])	0.1–5 mM (ref. [75])	4 s (ref. [75])
Ca^{2+}	200–1 × 10^7 nM (refs. [134–137])	1–10 s (refs. [59,138])	YC3.6 (ref. [77])[a] (ref. [139])[b]	10–100 nM (ref. [139])	1.5 s (ref. [77])
			GCaMP3 (ref. [72])[a] (ref. [140])[b]	200 nM (ref. [140])	2 s (ref. [72])
			R-GECO1 (ref. [80])[a] (ref. [141])[b]	<200 nM (ref. [141])	1.5 s (ref. [80])
			SWCNT[a] (ref. [88])	10 nM (ref. [88])	1 s (ref. [88])
NO	0.1–10 μM (ref. [66])	>0.1 s (ref. [142])	SWCNT[a] (ref. [20])	Single molecule to 100 μM (refs. [20,143])	0.5 s (ref. [20])
Ethylene	0.001–1 ppm per hour (refs. [144,145])	0.3 min (ref. [146])	SWCNT[a] (ref. [99])	0.5–50 ppm (ref. [99])	10 s (ref. [99])
Jasmonic acid	0.001–0.01 μM (ref. [147])	10–40 min (ref. [148])	Jas9-VENUS[a] (ref. [148])	1 μM (ref. [148])	2 min (ref. [148])
Methyl salicylate	0.01–0.1 ppm per hour (ref. [67])	Not known	Ag NPs[e] (ref. [149])	0.1–100 mM (ref. [149])	Not reported
Abscisic acid	10–100 nM (refs. [150,151])	10–15 min (refs. [57,152])	ABAleon2.1[d] (ref. [152])	100–600 nM (ref. [152])	6 s (ref. [152])
			ABACUS1[a] (ref. [153])	1 μM (ref. [153])	0.5 s (ref. [153])
pH (H^+ gradient)	pH 5.2–8.4 (ref. [154])	1 min (refs. [155,156])	GFP H148D[a] (ref. [156])	pH 4.3–7.6 (ref. [156])	1 s (ref. [156])

Reported plant signalling molecule static concentration range and timescale or expected lifetime. Sensor performance parameters such as sensitivity and temporal resolution of existing approaches, including examples of genetically encoded sensors[24], optical nanosensors and wearable nanoelectronic sensors. [a–e]The term [a]sensor, [b]indicator, [c]probe or [d]reporter was used in the cited references or this information was [e]not defined.

fluorescence[42–44], stomatal conductance[45], transpiration use efficiency[46], water potential[47] and leaf temperature[48], they are either not suitable for early detection of some types of stresses or resource deficits, lack the potential to identify specific plant stresses or are not optimal nor cost efficient for monitoring individual plants[16,44,49–51]. Chlorophyll content decline and leaf area loss are stress-induced accumulative traits that are the result of impaired plant growth. Chlorophyll fluorescence is not always suitable for early stress detection and is not a specific indicator of stress types[16]. Plant water status parameters are related to multiple stresses including drought[47], salinity[46] and pathogens[52]. Raman and infrared spectroscopy have typically low signal-to-noise ratios and require complex equipment. They provide rich chemical information about cell composition but are more difficult to interpret due to impurities and heterogeneities on or inside the plant that affect the spectral signature. However, Raman spectroscopy in leaves has recently been performed using a custom-built, portable system capable of being operated in the field[53], opening the door to applications in plant phenotyping facilities. Airborne detection tools are not optimal for monitoring individual plants[54] or crop plants grown in high-density. They are also influenced by overexposure due to sunlight or shadows, low signal-to-noise levels and interfering weather conditions[16]. Novel sensing tools that facilitate continuous monitoring of individual plants with high reliability and improved signal-to-noise ratios will complement existing remote sensing tools (Fig. 1). Electronic devices, including smartphones, hyperspectral imaging cameras and autonomous or manned vehicles, can establish direct communication with nanobiotechnology-based sensors in plants. Nanobiotechnology-based approaches provide a pathway to transduce the invisible plant stress-related chemical signals into optical, wireless or electrical signals that can be recorded by current phenotyping equipment such as greenhouse, growth room or field scanalysers.

Plant stress or resource deficit detection, based on chemical traits such as plant signalling molecules, has the potential to report the onset of plant health changes in real time and help to diagnose specific environmental or biological stressors. Key signalling molecules in plants that have been reported to be monitored by engineered nanomaterials and genetically encoded nanoscale sensors include reactive oxygen species (ROS), calcium (Ca^{2+}), glucose, sucrose, nitric oxide (NO) and plant hormones such as abscisic acid (ABA), jasmonic acid, methyl salicylate and ethylene (Table 1). Both ROS and Ca^{2+} are at the forefront of plant stress signalling[55]. Ca^{2+} is involved in most stress signalling pathways and is evolutionarily conserved among different plant species[56]. ROS have a dual role in plants, acting as a toxic molecule at high levels and playing a signalling role in a broad range of plant stress responses at low levels[57,58]. Ca^{2+} and ROS signature signals have been associated with specific plant stress responses[59,60]. Sugars such as glucose and sucrose are also important plant molecules that regulate a broad range of physiological and developmental changes[61–63]. ABA, NO, methyl salicylate and ethylene have been reported to be more specific indicators of plant stress types. ABA is an early signal of water stress[64,65]. NO, methyl salicylate and ethylene are mainly involved in plant pathogen defence response[66–68]. The plant hormone jasmonate coordinates both biotic and abiotic stress responses including salinity and freezing tolerance, drought and wounding response[69]. Other important signalling molecules that can be future targets for sensor development are salicylic acid and isoprenes. Salicylic acid is a signalling molecule involved in plant pathogen defence[70]. Plant volatile organic compounds (VOC), such as isoprenes, are associated with high light, temperature and water stress[71]. Developing and applying nanotechnology-based sensing tools for real-time monitoring of these key chemical signalling molecules in plants will improve our understanding of plant stress communication and enable plant health monitoring in the field.

Nanomaterial delivery of genetically encoded sensors

Genetically encoded sensors are capable of reporting in vivo concentrations of molecules, presence and activity of proteins, and ion dynamics. Most current biosensors enable subcellular monitoring and analysis of plant signalling molecules, which can provide critical knowledge for designing and engineering smart plant sensors. Recently, real-time imaging of calcium levels in whole plants under herbivore attack was demonstrated using genetically encoded sensors in laboratory conditions[72] (Fig. 2), providing a technology platform that could be adapted for fluorescence imaging in phenotyping facilities[41] or with modified portable field equipment[73]. Stable or transient expression of genetically encoded sensors in plants requires the generation of transgenic material in amenable plant species. The design of a genetically encoded reporter is, to a certain extent, rational and has been demonstrated in applications to plant leaves and roots for the detection of signalling molecules[63,74–79]. Genetically encoded sensing approaches in plants rely on varying mechanisms from protein–protein interactions to covalent bond modifications that influence their fluorescence intensity or wavelength in the visible range of the electromagnetic radiation spectrum (Fig. 2a,b). Currently, fluorescence resonance energy transfer (FRET)-based sensors report sugars in leaves and roots (Fig. 2c), including glucose and sucrose, with a high temporal resolution (10 s) and within physiological concentration ranges in transgenic plant model systems such as *Arabidopsis thaliana*[74–76] and rice crop plants in response to both abiotic and biotic stresses[63] (Table 1). Genetically encoded sensors have allowed imaging of calcium dynamics at very high temporal resolution (1.5 s)[72,77,78,80] and the detection of glucose at micromolar levels in roots[75]. Recently, biosensors have been reported to detect H_2O_2 in vivo with subcellular resolution in chloroplasts and nuclei[79] (Fig. 2d,e) and within physiological pH range during elicitor induced oxidative bursts with 15 s temporal resolution[81]. Overall, genetically encoded sensors offer sensitivities within the plant physiological range and temporal resolutions that allow the detection of various plant signalling molecules. Concentration changes of signalling molecules or metabolites in plant cells are dynamic but genetically encoded sensors have a temporal resolution on the order of seconds (Table 1) and could therefore report chemical signals on this time scale in real time. However, they require genetically amenable plant species to transformation methods such as gene gun particle bombardment and *Agrobacterium tumefaciens* plasmid delivery. Applications in crops have been limited by available DNA transformation methods and slow rate of optimization per species. Nanomaterial-enabled methods are being developed as an alternative approach to enable genetic material delivery into mature wild-type plants without the need to develop species-specific genetic techniques[22,23].

Delivering DNA cassettes or plasmids containing genetically encoded nanoscale sensors for expression within wild-type plant species might allow application to a broad range of crop plants that are not currently amenable to genetic modification. Single-walled carbon nanotubes (SWCNT) coated with DNA have the ability to penetrate plant lipid bilayers through passive and spontaneous translocation processes[19,82]. Thus, SWCNTs have great potential to act as a delivery chassis for exogenous DNA into plant cells. By binding the plant expression cassette to SWCNTs and application to the leaf lamina through a needleless syringe, transfection of DNA into plants is possible without *Agrobacterium* or gene gun particle bombardment[22,23]. Using a plasmid or amplified dsDNA, each containing a nuclear expression gene cassette, bound to the SWCNT, plants expressed transiently a fluorescent reporter protein without the integration of DNA into the genome[23]. How nanomaterial and plasmid properties determine transient versus stable gene expression in plants remains to be explored. These studies will inform the design of modular DNA–SWCNT transformation tools for generating fertile transgenic plants. Nuclear-based integration-dependent genetic sensors allow for new research into specific wild-type plant species. However, a uniform delivery chassis to plastids such as chloroplasts may enable a cross-species protein expression platform for mature plants due to their prokaryotic ancestry and evolutionary history in plants[83].

Nanotechnology is providing tools for engineering the relatively conserved plastid genomes of chloroplasts and mitochondria. Recently, SWCNTs delivered plasmid DNA to chloroplasts of different plant species without external biolistic aid and enabled yellow fluorescent protein (YFP) transient gene expression assessed by confocal fluorescence microscopy imaging[22]. Although, most biosensors are likely to be located in the cell cytosol, the plastid genome represents an opportunity to create translatable sensors for broader plant taxa than the nuclear genome (Fig. 2g). To date, genetically encoded sensors for chloroplasts are restrained to the few species that are able to be genetically modified[24,25]. Current chloroplast transformation techniques are limited to a small number of plant species (<12). The absence of a targeted DNA delivery mechanisms to chloroplasts, screening capabilities needed to prevent chimeric genome copies and labour-intensive calli culturing techniques hinders efforts to expand the range of transformed plant species[84]. ROS and salicylic acid are key signalling molecules produced by chloroplasts that accumulate in plants exposed to abiotic and biotic stress[85] and could act as indicators of plant health. In the future, genetically encoded nanosensors could be sent to plastids for detection of these chemical signals reporting plant health status without the need to develop species-specific methods.

Engineered nanomaterials as in vivo optical sensors

The unique optical and electronic properties of nanomaterials offer key advantages for imaging plant signalling molecules in vivo. Nanomaterials have ultra-low photobleaching, fluorescence in low- or transparent-background windows of living tissue and allow the detection of analytes with high spatiotemporal resolution down to single-molecule level and millisecond timescales[36,86]. SWCNTs have been used as building blocks for fluorescent nanosensors in mammalian systems[87] and more recently in plants[19,20,27] (Fig. 3). SWCNT fluorescence can be modulated in the nIR transparency window for plants through the interaction of the SWCNT's organic phase coating (corona) with surrounding analytes (Fig. 3a). SWCNT-based sensors are highly versatile and have been engineered for sensing various molecule classes including ROS, NO, calcium, glucose, dopamine, nitroaromatics, proteins and other small molecules[19,27,88–91]. Multiple delivery methods for these optical nanosensors can be utilized in the laboratory, phenotyping facilities or the field including needleless syringe infusion through the leaf lamina, topical delivery and vacuum infiltration. Embedding SWCNT sensors in leaves allows real-time monitoring of short-lived signalling molecules such as NO and ROS in plants[19,20]. Leaf H_2O_2 levels can be reported by SWCNT fluorescence intensity changes within selected regions of leaves (micrometres) with high temporal resolution (seconds) (Fig. 3b,c). Quantum dots (QDs) are also widely used nanoparticles for optical sensing applications. Glucose, a key target analyte for assessing plant stress and productivity, has been detected with high selectivity by QD optical probes down to the level of a few hundred micromolar[29]. QDs are fluorescent nanomaterials with bright and tunable emission range from the visible to the nIR[92]. These nanoparticles also allow facile modification of structural and surface chemical properties[92]. Functionalizing QDs with boronic acids, known to bind to sugars such as glucose, has enabled standoff

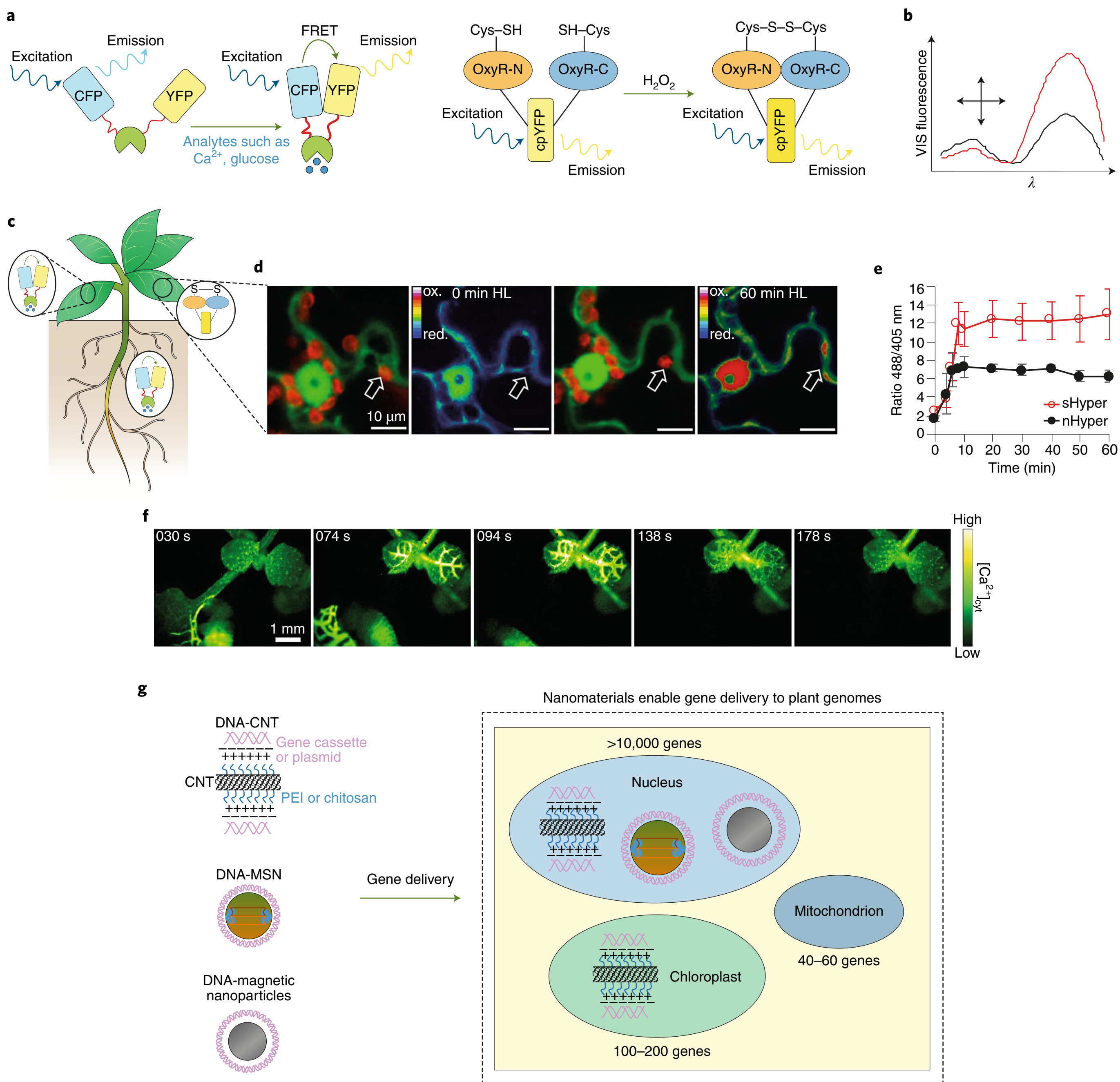

Fig. 2 | Genetically encoded nanoscale sensors for plant signalling molecules have the potential to be delivered to plant genomes by engineered nanomaterials. **a**, Schematic illustrating the mechanisms of two major types of plant genetically encoded sensors. Emission shift of a FRET sensor from donor to acceptor fluorescent protein in presence of the analyte. HyPer2 (ratiometric) sensors change their fluorescence when disulfide bonds form in response to H_2O_2. **b**, Genetically encoded sensors modulate their emission spectra in the visible range of the electromagnetic spectrum in the presence of analytes. **c**, Plants have been engineered with FRET and HyPer sensors for sensing signalling molecules such as Ca^{2+}, glucose and H_2O_2 in leaves and roots. **d**,**e**, HyPer2 sensors in leaves signal the transfer of H_2O_2 from chloroplasts to the nucleus under high light and allow monitoring of H_2O_2 temporal patterns in chloroplasts (sHyper) and nucleus (nHyper). **f**, Herbivore attack induces rapid Ca^{2+} wave across whole *Arabidopsis* plants reported by GCaMP3, a Ca^{2+} GFP sensor. **g**, Nanomaterials coated in DNA including mesoporous silica nanoparticles (DNA-MSN)[125], magnetic nanoparticles[126] and carbon nanotubes (DNA-CNT)[22,23] can be utilized as delivery systems of gene cassettes and plasmids to nuclear, chloroplast and mitochondrion genomes. Reproduced from ref. [79], SNL (**d**,**e**); ref. [72], AAAS (**f**).

optical detection of glucose and its online monitoring in plant tissue with timescales on the order of minutes[29].

Current optical standoff detection readouts from nanosensors embedded in plants suffer from low signal-to-noise ratios in comparison to optimized conditions in sophisticated microscopes and lack of targeted localization in specific plant tissues, cells or organelles. To circumvent these issues, a signal increase through mechanisms, including field enhancement by gold nanoparticles, has been proposed[93]. Plant viruses were coated with a thin gold layer to enable field enhancement without compromising the targeting capability of the virus. Although this concept has not been proven yet for standoff imaging and spectroscopy, it is a possible approach to improve

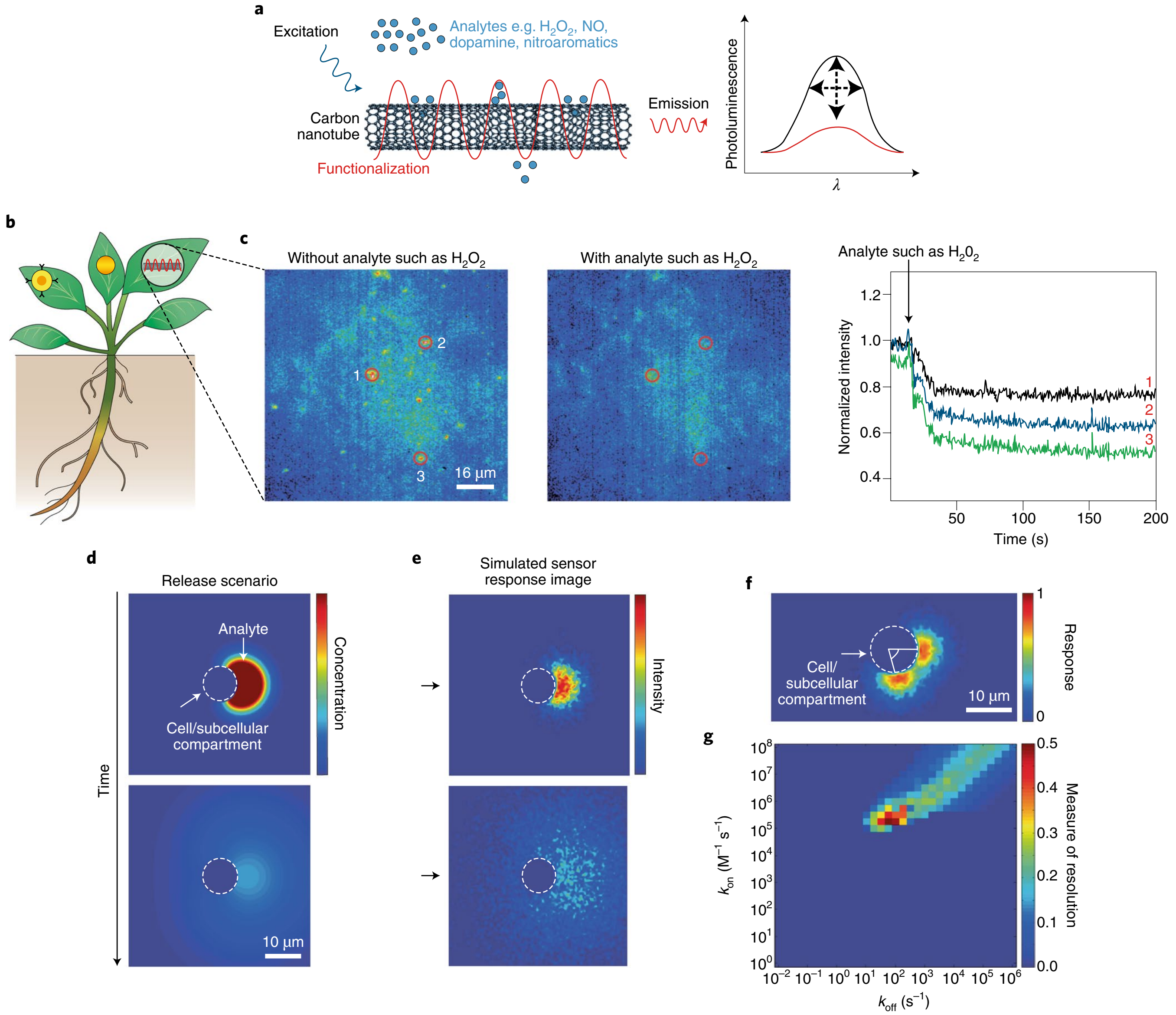

Fig. 3 | Nanomaterial-based sensors allow in vivo optical monitoring of plant signalling molecules in real time. a, Analyte interaction with SWCNT sensors with the nanomaterial carbon lattice, organic corona or both leads to changes in nIR fluorescence intensity or wavelength shifts. **b**, Plant leaves have been interfaced with optical nanosensors including SWCNT, quantum dots and nanoscale gold virus for optical sensing of plant signalling molecules. **c**, H_2O_2 monitoring by imaging SWCNT nIR fluorescence intensity changes in *Arabidopsis* leaf sections in vivo with high spatial (>0.5 µm) and temporal resolution (>0.5 s). **d**,**e**, Kinetic Monte Carlo simulations predict fluorescence responses of optical nanosensors to biological signalling events from individual cells or subcellular compartments by considering nanosensor kinetics, imaging speed and resolution limits. This allows assessment of the nanosensor's spatial and temporal sensitivity to improve sensor design. **f**, Simulated fluorescence intensity response of nanosensors for the release of an analyte from two locations on a cell or subcellular compartment. The rate constants of these nanosensors allow distinction of two release sites. **g**, Combined spatiotemporal resolution phase diagrams for optimizing biomolecule sensing in specific biological scenarios with sensors having different forward and backward rate constants. Adapted from ref. [20], Wiley (**c**); ref. [97], American Chemical Society (**d**–**g**).

the optical signal from a smart nanobiotechnology-based sensor over the inherent background in the field. In situ synthesis of artificial sensors is another intriguing concept that could circumvent the delivery of nanosensors to target tissues or subcellular compartments. Metal–organic frameworks (MOFs) are three-dimensional structures with a high surface area built from ions and organic ligands. Fluorescent MOFs generated by plants *in situ* allowed them to report acetone levels in air through fluorescence changes in the presence of these volatile compounds[94]. Alternatively, coating nanosensors with targeting moieties such as peptides[95] could allow specific localization in plant subcellular compartments.

Important plant signalling molecules, such as Ca^{2+} and ROS, are characterized by fast spatiotemporal concentration changes resulting in waves within single cells, organs or whole plants. Although optical nanosensors have been widely developed and applied in animal cells both in vivo and in vitro[96], imaging signalling molecules in neural networks of mammalian systems and in plants share similar challenges, including localized transient maxima of concentration at subcellular structures such as synapses in neurons or chloroplasts in plants[91]. A static concentration of an analyte, for example, ROS, is not likely to represent the most relevant information but the concentration changes in time and space. Consequently, sensor kinetics play an important role to determine if stress events can be resolved or not by plant signalling molecule detection. Diffusion and stochastic kinetic simulations are powerful tools to study biological scenarios and predict the fluorescence response of single or multiple sensors to spatiotemporal patterns of signalling molecules[97] (Fig. 3d,e). It has been shown that such an approach allows predicting the expected nanosensor fluorescence response (Fig. 3d,e). Based on such images, it is possible to quantify if one could resolve chemical signals, for example, to distinguish two release points of a signalling molecule from a cell or organelle (Fig. 3f). When simulations are performed for different sensor parameters such as rate constants and affinities, measures for spatial and temporal resolution can be derived (Fig. 3g). These simulations provide a rationale for the synthesis and optimization of sensors to circumvent trial-and-error approaches. Additionally, they are promising tools to correlate signalling patterns in plants with stress or resource deficiency levels. In the future, sensor responses to plant chemical signals could be tested in silico to identify patterns that are distinguishable by a given sensor. In silico approaches can pinpoint to candidate sensors and optimal properties such as selectivity, sensitivity and dynamic range that could in principle inform their design for operation under field conditions. Furthermore, simulations and modelling are crucial to translate measured plant chemical signalling patterns into the digital information that is necessary for the decisions and actions of agricultural devices.

Wearable nanotechnology-based sensors

The field of wearable sensors has been widely developed for human skin and clothing applications[26] and is creating nanotechnology-based platforms that provide minimally invasive sensors for plants[98] with costs that are significantly being reduced over time allowing increase in commercialization[26]. Sensor networks based on flexible wearable nanoelectronic circuits implanted on plant surfaces are enabling wireless communication of low concentrations of volatile molecules in real time[30]. The high sensitivity of integrated arrays of SWCNT channels and graphitic electrodes transferred to leaf surfaces of live plants can detect trace levels of airborne chemical weapons (Fig. 4). The elastic properties of wearable nanotechnology-based sensors act as a "flexible skin" that can be bent on organisms with a radius of curvature as small as ~100 µm. Wearable SWCNT–graphite sensors can be operated by radio frequency (RF) for wireless monitoring with electronic devices without power consumption in response to gas molecule concentrations down to 5 ppm (ref. [30]). Chemoresistive sensors based on SWCNTs and equipped with copper complexes work reversibly, allowing long-term monitoring of sub-ppm concentrations of ethylene, a plant hormone that acts as a key indicator of the onset of fruit ripening[99]. Carbon-nanotube-based sensing devices for plant VOCs, for example, ethylene, are now commercially available for agriculture applications but have not been interfaced directly with crops for monitoring plant signalling molecules (Fig. 4a,b). Although, highly stretchable wearable sensors based on graphene and carbon nanotubes have been reported to wirelessly monitor a wide range of gas and aqueous phase molecules, including glucose from mammalian epidermal cells[100,101], few publications to date consider their applications in plants.

Future nanotechnology-based wearable sensor approaches will require high sensitivity and increased signal-to-noise ratios under variable ambient conditions. To monitor plant VOCs associated with plant health status, the wearable sensors should be able to report ranges in very low concentrations in the ppb range (Table 1) or include concentration mechanisms to allow detection. The resistance of SWCNT–graphite electronic devices is modulated by molecule adsorption and transfer of electrons on the SWCNT surface[102]. These devices have a tunable sensitivity to gas molecules from parts per million [103] to as low as the parts per billion range[30,104,105]. Relative humidity (RH) near the plant surface results in increased sensor noise and recovery time[30]. Thus, wearable sensor performance would depend on the type of plant substrate they are interfaced with. Selectivity against analyte mixtures under variable ambient conditions, for example, humidity, temperature and wind, could be accomplished by multiplexing carbon-nanomaterial-based wearable sensors. Carbon-nanotube-based conductive inks and graphene printed on the leaf surface allow monitoring RH near the leaf epidermis and plant water status continuously through wired multimeters[28,98]. Changes in plant water transport have been reported by graphene-based wearable sensors on leaves that detect reversible changes in RH from 20–90% (ref. [98]). High-resolution patterning and transferring of tape-based graphene RH sensors on the leaf epidermis creates air gaps that allow gas exchange between the leaf and environment. The graphene sensor resistance dynamics, in response to RH, is measured and reported by a resistance, inductance and capacitance (RLC) meter[98]. Alternatively, microfluidic printed SWCNT ink on the leaf epidermis can assess plant water status and the onset of drought stress through real-time measurements of single stomatal aperture dynamics[28]. This wearable sensor is made from two printed contact pads and a stripe across a single stoma that are responsive to slight variations in stomatal opening and closing latency during drought. Other stress conditions that induce changes in stomatal conductance including flooding and salinity could be detected through wearable sensing nanotechnologies. Wired wearable sensors based on carbon nanomaterials can be designed to be operated by RF wireless signals. Thus, allowing integration of sensors for volatile compound signalling molecules in plants with potential interfering environmental signals such as RH.

Wearable sensors report basic but key parameters of crop health and have already reached a technical standard close to applications. Wearable flexible electronics based on three-dimensional sub-micrometre-thick, centimetre-scale macroporous networks[106,107] have not been interfaced with plants yet, representing an untapped sensing nanotechnology that could continuously monitor multiple plant electrochemical signals simultaneously with high temporal resolution. For example, syringe-injectable mesh nanoelectronic devices[106] could be embedded into plants using 100 µm diameter needles with minimal mechanical perturbation. Mesh nanoelectronics millisecond temporal resolution could record rapid waves of plant signalling molecules, for example, Ca^{2+} and ROS in the extracellular space, in response to stress, as it has been demonstrated for monitoring neural activity in mice brain in vivo[106].

Sensor communication and actuation with machines

Nanobiotechnology is on the verge of generating the tools for establishing real-time two-way communication channels between nanobiotechnology-based sensors merged with plants and electronic devices. SWCNT sensors embedded in leaves have converted plants into self-powered chemical detectors that report the presence of groundwater analytes via nIR optical signals[27] (Fig. 5). Plants equipped with SWCNTs functionalized with bombolitin peptides report the presence of explosive nitroaromatics, such as picric acid, to research grade cameras and even smartphones[27] (Fig. 5a). By connecting a camera to a miniaturized computer, for example, a Raspberry Pi, nanosensors embedded within plants communicate the presence of the analyte through optical signals that could trigger e-mails and text messages or communicate directly to a smartphone using Bluetooth[27]. Furthermore, monitoring of genetically encoded sensors delivered by nanomaterials to leaves with customized imaging devices for plant chlorophyll fluorescence[108] may allow reporting of stress-induced signals (for example, Ca^{2+}) in plants[72] (Fig. 2f). Fluorescence imaging of green fluorescent protein (GFP)-based Ca^{2+} sensors (GCaMP3) in whole plants was performed at 2 s temporal resolution using a 1× objective and a digital camera indicating that standoff imaging of these types of sensors would be

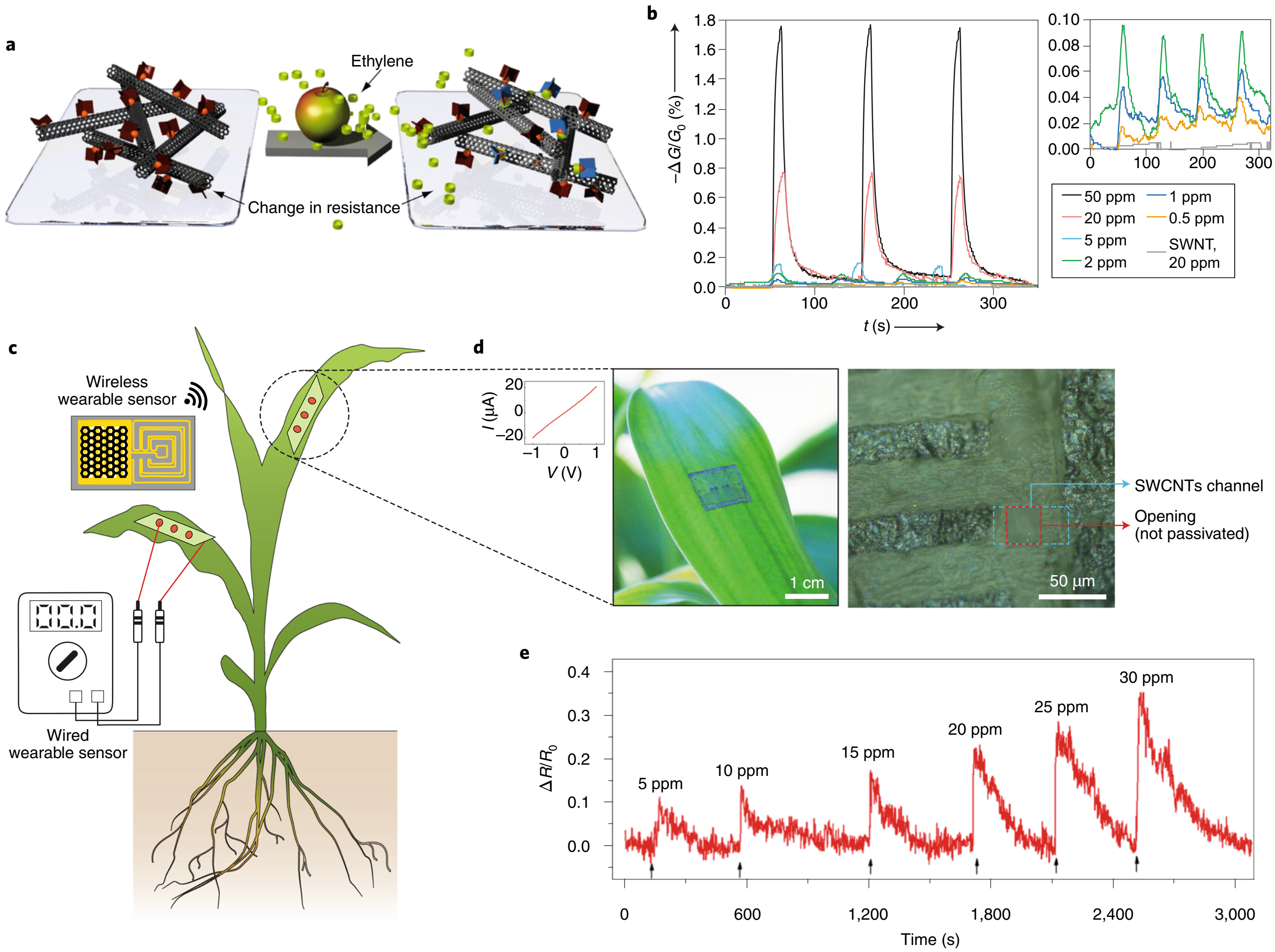

Fig. 4 | Nanotechnology-based flexible and wearable sensors for plant chemical sensing. **a**, Schematic represents SWCNT-based chemoresistive sensors for volatile molecule detection, in this example ethylene, a key plant signalling molecule. **b**, Binding of SWCNT and copper complexes to ethylene molecules emitted from plant structures, for example fruits, results in resistance changes proportional to the concentration of this volatile compound. **c**, Plants engineered with wireless and wired wearable nanotechnology-based sensors for chemical sensing have been interfaced with crop leaves. **d**,**e**, Radio frequency wireless monitoring a volatile nerve agent simulant DMMP (dimethyl methylphosphonate) with ppm concentration resolution. Reproduced from ref. [99], Wiley (**a**,**b**); ref. [30], American Chemical Society (**d**,**e**).

possible. Nanobiotechnology-based sensors are, therefore, a promising tool to create smart crops that communicate their health status to agricultural devices. However, validation of smart nanobiotechnology devices on plants has not been done outside of the laboratory.

Smart nanobiotechnology-based sensors that communicate through optical signals, wireless or wired channels have the capability to integrate with existing agricultural electronic devices, including smartphones, hyperspectral imaging cameras, high-throughput phenotyping instrumentation, radio frequency devices and meteorological stations[30,44,109–111] (Fig. 5b). Smartphones are already used to monitor crops with accurate GPS information and active communication through Bluetooth and the Internet of Things[112,113]. In combination with digital software platforms, they monitor and measure the impact of agronomic decisions on crop performance. Assisting with crop management are unmanned aerial vehicles (UAV) capable of monitoring vegetation indexes through multispectral bands from the visible to the nIR[110] and detecting water stress with a 40 cm resolution[44]. On the ground, high-throughput chemical phenotyping of plants with smart nanobiotechnology-based sensors could be possible through terrestrial platform-based multi-sensor systems with vegetation index sensors, thermal infrared radiometers, spectrometers and visible cameras[111]. Radio frequency responses of wireless sensors interfaced with plant surfaces[30] (Fig. 4c–e) are ready to be interfaced with commercially available RF identification devices. Wireless or wired sensor data can be collected and transmitted to field-deployable environmental and phenotyping stations. With internet connectivity, these stations are capable of cloud-based storage and data analysis, which are methods already facilitating data-driven decisions on farms. Remote sensing technologies with the help of computer models can complement smart nanobiotechnology-based sensors for plant stress management[114]. Large dataset technologies that transmit, process and actuate devices will synergize the integration of smart plant sensors with agricultural devices[115].

Smart nanobiotechnology-based sensors able to monitor crop health will face several challenges to ensure applicability, accuracy and durability under crop field conditions. Applications can range from urban farming and plant phenotyping facilities, to large industrial agriculture applications where they will need to report crop health status across hundreds of hectares. The required spatial resolution provided by smart plant sensors in the field, from individual

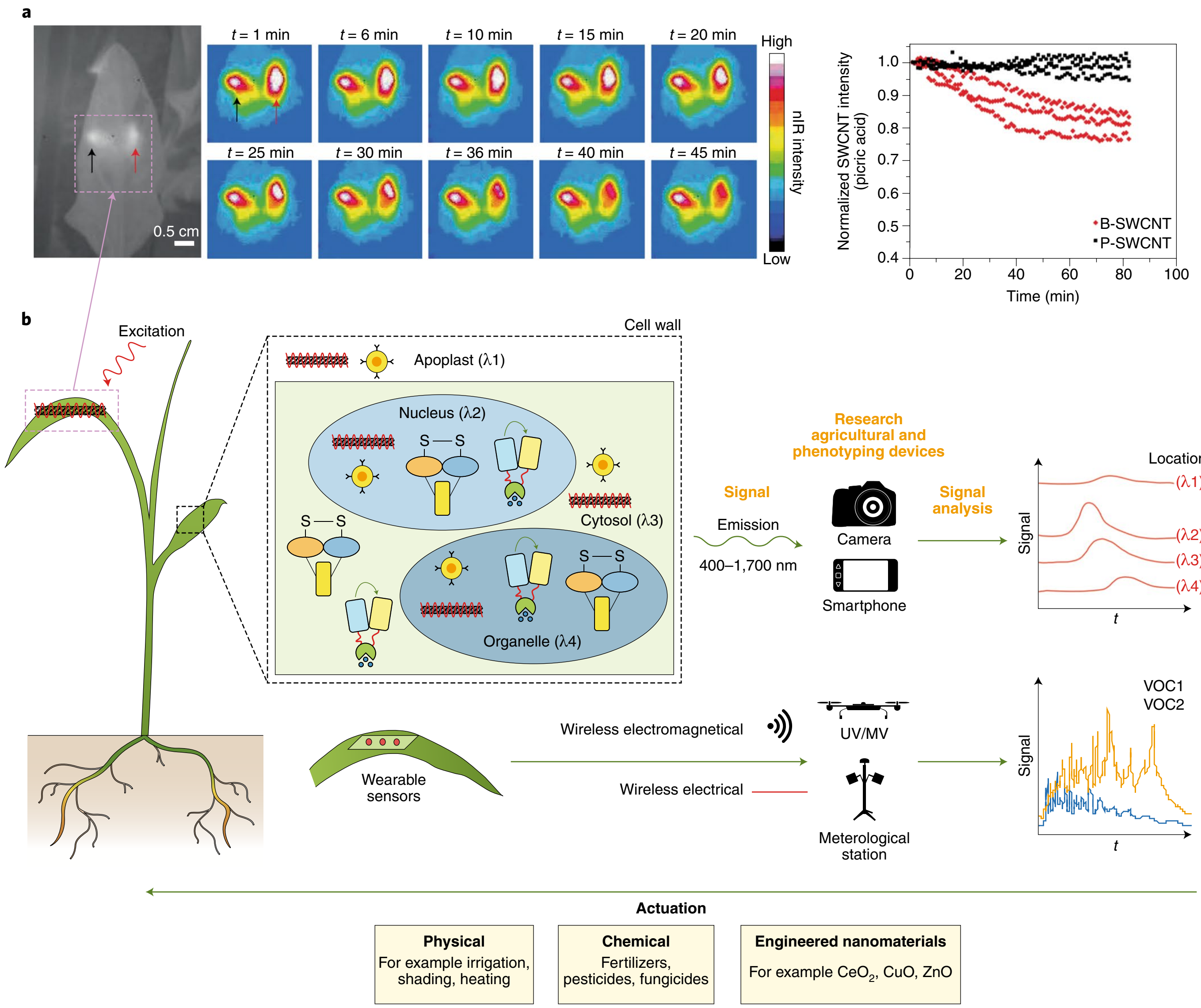

Fig. 5 | Smart plant sensor communication and actuation of electronic devices through optical and radio waves, and electrical signals. a, Plant leaves embedded with SWCNTs act as detectors of nitroaromatics, for example, picric acid. Polyvinyl-alcohol functionalized SWCNTs (black arrow) act as internal controls whereas bombolitin II peptide-coated SWCNT (red arrow) monitor picric acid in real time with a high spatiotemporal resolution by reporting changes in nIR fluorescence intensity to research-grade and smartphone-quality cameras. **b**, Smart nanobiotechnology-based sensors delivered to plant subcellular compartments have the potential to communicate health status to agricultural or phenotyping devices equipped with hyperspectral imaging cameras, smartphones, drones or meteorological stations. Machines able to decode plant subcellular spatiotemporal patterns of chemical signals could, in turn, control the actuation of agricultural devices to improve microenvironmental conditions including irrigation, shading, heating, the release of fertilizers, pesticides and fungicides, or nanoparticle-based therapeutics against stress, for example, CeO_2, CuO and ZnO. Real-time monitoring of plant chemical signalling by phenotyping systems could improve the selection of desired plant traits for high yield and tolerance to stress. Reproduced from ref. [27], SNL (**a**).

plants to groups of plants in the field, has not been determined yet and will likely depend on microenvironment variations, type of plant stress and agricultural or phenotyping sensing instruments. To accurately assess plant health status, multiple chemical signalling and environmental parameters are likely needed to be detected and analysed. Consequently, multiplexing is crucial to further improve the sensing approaches for signalling molecules described above or a combination of genetically encoded sensors, optical nanosensors and wearable sensing devices. A minimum lifetime of a growing season spanning several months is expected for smart nanobiotechnology-based sensors to adequately perform in an agricultural system. For most nanobiotechnology-based plant sensors, durability has not been tested in the laboratory or in the field. However, genetically encoded sensors integrated into the plant nuclear or plastid genome are expected to last for a plant lifetime and SWCNT sensors have been demonstrated to operate in mammalian systems for at least six months[116]. To ensure sensor accuracy in agricultural environments, plants can be embedded with both smart nanobiotechnology-based sensors for analytes of interests and non-responsive sensors to these analytes that act as internal controls. Ratiometric approaches have been applied to genetically encoded sensors and optical nanosensors for the detection of H_2O_2, calcium, glucose and other biochemicals in plants and the environment[27,29,76,77,79]. To minimize negative impacts due to downtime, the redundancy of smart plant sensors in each node will be necessary. Replaceability and reparability of smart nanobiotechnology-based sensors will require practical, efficient and scalable interfacing methods of plants with nanomaterials. However, the mechanisms of transport and delivery of nanomaterials to plants are not well understood.

Smart nanobiotechnology-based sensor output will not only allow plant health status communication with electronic devices but also actuation of agricultural equipment. Machine sensory, learning and actuation systems provide a communication platform between smart plant sensors and agricultural devices for optimizing environmental conditions through irrigation, fertilization and application of pesticides, among other crop management tools. Delivery of nanobiotechnology-based therapeutics such as ZnO (ref. [117]), CuO (ref. [118]), CeO_2 (refs. [119,120]) and nanocrystals[121] can also contribute to alleviate heat and salinity stress, pathogen infections and frost damage, respectively. Autonomous vehicles integrating sensory, decision-making and actuation of agricultural equipment have been tested in crop fields for plant invasive species detection[122]. The machine-vision systems for these agricultural vehicles allow the recording of optical signals within the emission range of the nanotechnology-based sensors described above from the visible emission, for example, genetically encoded nanosensors and QDs, to the nIR range, for example, SWCNT[123]. Optimum sensing and actuation performance of autonomous vehicles will depend on the high variability of illumination in the environment, irregular terrain conditions or plant growth status. Unlike optical sensing approaches for machine sensing, automated wireless control systems do not suffer from the interference of these environmentally related factors and have been applied to regulate water and nutrient supply in hydroponics[124]. Research on automatic actuation of agricultural equipment for fine-tuning crop health status remains relatively unexplored.

Conclusions and perspectives

Meeting the projected increase in global demand for food in this century will require interdisciplinary and convergent approaches from plant sciences and engineering to bolster sustainable agricultural production. Nanotechnology offers high spatial and temporal resolution sensors for both aqueous and volatile plant signalling molecules and delivery platforms for genetically encoded sensors to study and engineer smart plant sensors that communicate and actuate with machines. By translating chemical signals associated with stress or resource deficiencies into wireless, electrical and optical signals, smart nanobiotechnology-based sensors are poised to improve plant growth and yield while fine-tuning resource use by interacting with agricultural devices. They could facilitate faster identification of desired crop traits by allowing high-throughput screening of chemical phenotypes. Although a number of nanoscale sensors already exist for plant signalling molecules associated with abiotic and biotic stresses, few are designed for sensing plant nutrient deficiencies. Nanobiotechnology provides key advantages to solve the challenges and limitations associated with the engineering of smart plant sensors. Genetically encoded sensors are excellent tools for subcellular research, that can pinpoint to key plant signalling mechanisms of stress communication for designing smart nanobiotechnology-based sensors. These biosensors have an adequate spatiotemporal resolution for enabling real-time monitoring of plant signalling molecules but are limited to amenable species. Nanomaterials could bridge this gap by acting as genetic element delivery platforms to expand the range of species that can be transformed. Engineered nanomaterials are a pathway to create smart plant sensors without relying on genetically amenable species. Optical nanosensors can be designed for monitoring aqueous-based signalling molecules with very high spatiotemporal resolutions in the order of single molecules and milliseconds. RF nanotechnology-based wearable devices are allowing the detection of plant volatile compounds. Unlike optical-based sensing approaches, wireless wearable sensors are not subject to background interference from environmental or terrain conditions. However, their sensitivity needs to be designed to sense very low levels of volatile compounds in the order of parts per billion. To date, smart plant sensors that communicate with electronic devices have been demonstrated in the laboratory under controlled conditions. Nanoscale plant sensors have yet to be tested under real agricultural conditions in which their performance would be affected by weather, plant growth and development. If successful in field trials, nanotechnology-based sensors could provide individualized and real-time information about the onset of stresses, and plant water, nutrient, and pesticide specific needs. There is also a need for integrative studies linking smart nanobiotechnology-based sensing, plant stress, resource deficit simulations and signal analysis with actuation of agricultural devices. The ethical implications of how smart nanobiotechnology devices could transform interactions between plants and machines should also be explored. Through recently developed nanomaterial-enabled gene delivery methods in leaves in planta[22,23], genetically encoded sensors reporting plant health by inducing changes in plant pigments could be a pathway to allow farmers in undeveloped areas without access to electronics to judge plant health status by visual cues. Most of these technologies developed for smart plant sensors are not limited to agriculture and will likely be translatable to defence and environmental monitoring purposes.

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Correspondence should be addressed to J.P.G.

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