nature reviews chemistry

https://doi.org/10.1038/s41570-022-00443-0

Review article

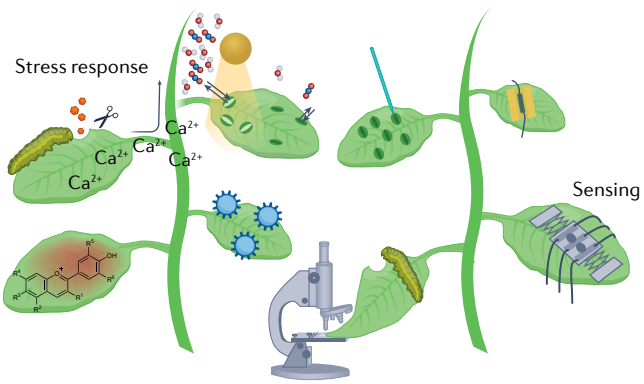
Check for updates

Continuous monitoring of chemical signals in plants under stress

Philip Coatsworth [1] ✉, Laura Gonzalez-Macia [1], Alexander Silva Pinto Collins[1], Tolga Bozkurt [2] & Firat Güder [1] ✉

Abstract

Time is an often-neglected variable in biological research. Plants respond to biotic and abiotic stressors with a range of chemical signals, but as plants are non-equilibrium systems, single-point measurements often cannot provide sufficient temporal resolution to capture these time-dependent signals. In this article, we critically review the advances in continuous monitoring of chemical signals in living plants under stress. We discuss methods for sustained measurement of the most important chemical species, including ions, organic molecules, inorganic molecules and radicals. We examine analytical and modelling approaches currently used to identify and predict stress in plants. We also explore how the methods discussed can be used for applications beyond a research laboratory, in agricultural settings. Finally, we present the current challenges and future perspectives for the continuous monitoring of chemical signals in plants.



Sections

[1]Department of Bioengineering, Royal School of Mines, Imperial College London, London, UK. [2]Department of Life Sciences, Royal School of Mines, Imperial College London, London, UK. ✉e-mail: p.coatsworth18@imperial.ac.uk; guder@ic.ac.uk

Introduction

Time is a factor that is often overlooked in applied and fundamental plant science but is a key component of molecular processes and chemical signalling in plants, especially in the context of stress[1] (Fig. 1). Biotic stresses, such as attack from pathogens and herbivores, lead to well-defined immune responses, with bursts and fluctuations in levels of pH, Ca^{2+} and reactive oxygen species (ROS) varying over time[2–5]. Abiotic stresses, such as drought and salinity, also produce time-dependent chemical changes, including reduced transpiration, differing CO_2 concentrations at stomatal pores and oxidative stress throughout the plant[6,7]. As some stress markers take time to accumulate, real-time continuous measurements would give more information on not only the physiological state of a plant but also how it reached that state. Some chemical signals with short lifetimes, such as the superoxide radicals, may be missed in measurements taken at set intervals but would be observed in continuously recorded high-frequency measurements[8]. Plant signalling is generally not studied in a continuous, time-resolved fashion and most of the information that time-dependent chemical signals carry with specific time signatures is, therefore, lost. To make up for this, many mechanistic studies and diagnostic tests require measurement of a large set of chemical species captured at a single time point to provide a more complete picture about a stressor or the underlying molecular processes countering the induced stress. Measuring many chemicals at the same time is, however, not a trivial task and often costly. Many conventional assays used in the quantification of chemical signals in plant research are also destructive, likely impacting the results[9]. By measuring a small number of chemicals continuously in a non-destructive manner, with spatiotemporal fidelity, we may acquire the same level of (if not more) information about a stressor than making many single-shot measurements. This in turn may enable early identification of stresses with low-cost and simpler tools, alongside development of new insights into the molecular mechanisms — all without disturbing the whole plant by destructively extracting samples, such as leaf discs.

Continuous-time measurement techniques commonly used in plant monitoring can be categorized into two types: electrochemical and optical. Electrochemical sensors use electrically conductive, modified electrodes to detect specific chemical species and are placed in, on or near the plant. Optical methods can be used without plant modification — by simply measuring the optical properties of the surface of the plant, or following genetic or chemical manipulations, for example, by adding nanomaterials or introducing genetic transformations to produce fluorescence under certain chemical conditions[10]. Both measurement types have been used in vivo for the detection of a range of chemical species, including ions, radicals, organic and inorganic molecules in living plants under stress. Most of the examples in the literature for continuous-time monitoring of chemical species are proofs of concept from academic laboratory-based research. There is, however, still a lot of progress to be made before these sensor designs could be produced commercially and used in real-world scenarios. Time-resolved, continuous measurement of chemical species in whole plants will also aid the prediction and identification of the specific stresses in real time, with the potential to intervene and introduce measures to control stress in a closed-loop fashion. There are forecasting tools already available to farmers today that predict the likelihood of disease due to local weather conditions and nearby presence of diseases. Monitoring of chemical signals within plants to identify abiotic and biotic stresses in real time, however, would allow management and monitoring of stresses at a fundamentally new level[11]. Mathematical tools and machine learning models would also allow detailed analysis of data concerning the chemical signals within the plants, which can be combined with environmental parameters (such as weather) to provide more robust prediction and understanding of stress at the molecular level[12–14].

In this Review, we describe how ions, molecules and radicals are produced and how their concentrations change in response to the presence of various biotic and abiotic stressors. We then discuss existing and emerging electrochemical and optical continuous, time-resolved sensing techniques used for the detection of these stress-related chemical species in whole live plants. The modelling and analysis of the plant physiological data are put into context for use in agricultural applications and, finally, the challenges that must be overcome to move this technology into the commercial world are presented.

Chemical stress response signals

Stress in plants can be broadly separated into biotic stress, which is stress caused by living organisms such as pathogens or herbivores, and abiotic stress, which is stress caused by environmental factors. Both types of stress can lead to reduced growth, damage and death of plants, impacting yields. Both stressors may produce visual symptoms that are similar in appearance, but the chemical signals involved often differ in composition, pathway and intensity over time[15]. A summary

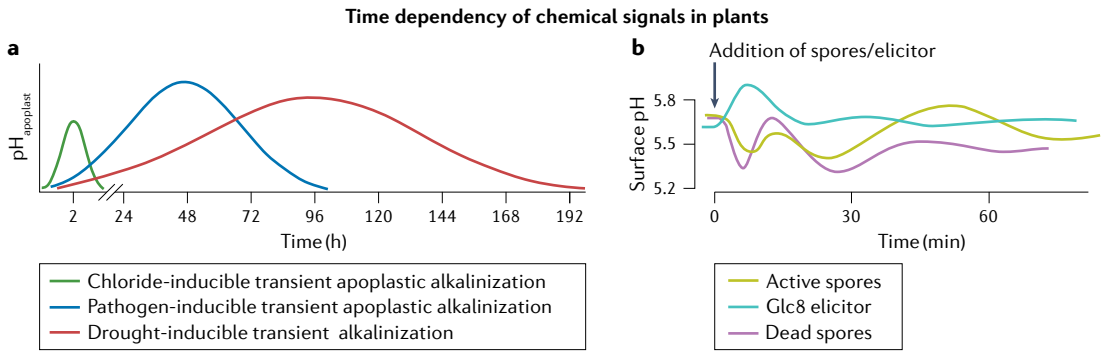


Fig. 1 | Duration, magnitude and complexity of chemical signals in plants are often highly dependent on the stressor and can be captured through continuous, time-resolved measurements. a, Changes in apoplastic pH of plant cells with different stressors. **b**, Surface pH of barley roots in response to *N*-acetylchito-octaose (Glc8) elicitor or dead or active spores of *Piriformospora indica*. Part **a** adapted with permission from ref.[1], Elsevier. Part **b** adapted with permission from ref.[5], American Phytopathological Society.

Table 1 | Key chemical markers in plant stress response

Class	Chemical marker	Typically relevant concentration range and effect on plant	Refs.
Ionic	Na^+	>200 mM: salt stress, leading to oxidative damage, reduced growth, reduced yields	23,24
	pH	Increase in pH upon pathogen detection	21,22
	Nitrogen source (NO_3^-, NH_4^+)	<2.50% dry weight: deficiency; >6.00% dry weight: excessive or toxic	20
	Ca^{2+}	Increase in Ca^{2+} signalling and cytosolic Ca^{2+} influx upon plant immune responses, herbivore feeding, physical damage, cold shock	22,27,29,30
Inorganic	H_2O	Drought stress leads to stomatal closure, oxidative damage	24,33
	CO_2	>800 ppm external CO_2 leads to stomatal closure	31
Inorganic/radical	ROS	Burst of ROS upon pathogen detection; ROS build-up from abiotic stress (heat stress, drought, excess light, UV light, salt stress, extreme cold)	24,34–38,112
Organic	Phytohormones	Found in fmol to pmol g^{-1} fresh weight; functions: antiherbivore, signalling, regulation of ageing processes	42,45–47
	VOCs	Increased release of VOCs upon pathogen or herbivore attack, physical wounding	48–51
	Pigments	Defence against abiotic stresses (UV light, oxidative damage); anthocyanins accumulate upon environmental stresses (pH, nutrient deficiency, extreme cold, water stress, UVB radiation)	52–54
	RNA molecules	Plant-to-parasite RNA transfer to alter gene expression systems	55–57

ROS, reactive oxygen species; UV, ultraviolet; VOC, volatile organic compound.

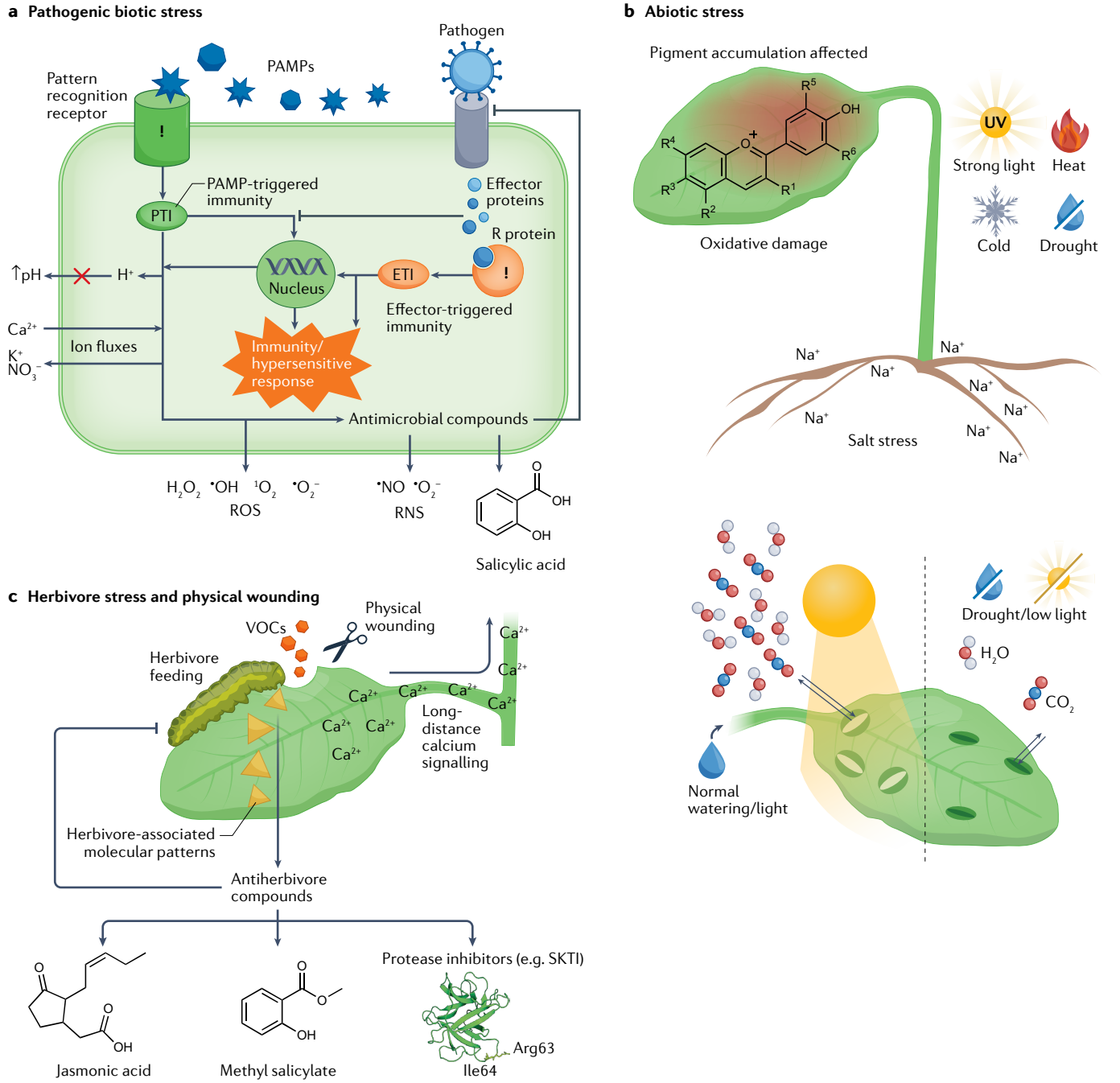
of the most important chemical markers in plant stress response is provided in Table 1.

Pathogens, including bacteria, fungi and oomycetes, structurally contain pathogen-associated molecular patterns (PAMPs) that are detected by specific pattern recognition receptors located on the surfaces of plant cells. Feeding by herbivores, including insects such as caterpillars and aphids, is detected in a similar way to pathogens by recognition of elicitors or herbivore-associated molecular patterns found in the saliva of the herbivore and leads to the release of specific antiherbivore compounds and an increase in chemical signalling[16]. Detection of a PAMP initiates a signalling cascade that leads to activation of the plant's primary immune response, PAMP-triggered immunity (PTI), resulting in an increased concentration of ROS, calcium influx and production of antimicrobial compounds (discussed below). Pathogens that are specifically adapted to the host plant often deliver effector proteins that either operate on the cell surface or are translocated inside the host cells to inhibit and counter PTI responses. Plants have developed highly specialized resistance proteins to detect these effector proteins and initiate a stronger immune response, called effector-triggered immunity (ETI). ETI typically has more severe immune outcomes compared with PTI and is often viewed as the final line of defence. ETI is often accompanied by hypersensitive response, leading to cell death, associated with prevention of spread of the pathogen[2,17] (Fig. 2a). Recent breakthroughs have shown that PTI and ETI are tightly linked, where, in some cases (such as antibacterial immunity), PTI potentiates ETI, amplifying the strength of the overall immune response[18]. The extent to which PTI boosts ETI is not fully understood, but time-resolved monitoring of biochemical changes in infected cells with non-disruptive methods should provide new perspectives to study PTI–ETI crosstalk by enabling precise measurements of ion fluxes in a spatiotemporal manner.

Ions in stress signalling

Ions serve multiple purposes in plants, where they provide nutrition and act as chemical signals, but they can also cause damage to a plant's health. The xylem transports water and water-soluble ions (such as nutrients) and compounds up from the roots to the rest of the plant, where water eventually leaves the plant through the stomata, driving water uptake through transpiration. Typical nutrient ions include K^+, Mg^{2+}, nitrate (NO_3^-) and phosphate (PO_4^{3-}), where the latter two act as sources of nitrogen and phosphorus, respectively. If the soil concentrations of these nutrients are too high or too low, plants may suffer from nutrient stress or nutrient deficiency, respectively, leading to reduced growth and yields[19,20]. Alongside internal nitrate concentration being an indication of uptake, nitrate exposure also induces gene expression: within minutes of exposure to very low nitrate concentrations (down to 10 μM), responding genes encode nitrate transporters and enzymes, including nitrate reductase, nitrite reductase, glutamine synthase and ferredoxin-dependent glutamate synthase[21]. Biotic stress can also affect plant ion concentrations and K^+ and NO_3^- cellular efflux has been observed after exposure to the PAMP Pep-13, from the oomycete *Phytophthora*. Additionally, extracellular pH has an impact on cellular transport of organic molecules and ions, including nutrients such as nitrates. pH is controlled by H^+-ATPase proton pumps across the plant plasma membrane and the energy that comes from the proton gradient across the membrane drives the uptake of nutrients[21]. Extracellular pH is also affected by biotic stress, where pathogen detection causes the proton pumps to stop, leading to extracellular alkalinization and cellular H^+ influx[22].

High levels of ions can be detrimental to plants. This is especially true of Na^+, one of the most damaging ions to crops, where high Na^+ uptake and transport around the plant is associated with salt stress, resulting in reduced growth and yields (Fig. 2b, top). NaCl concentrations over 200 mM in the growth medium are unsuitable for the survival of most plants and increasing salinity is linked to, and can be aggravated by, drought[23]. Salt stress can cause a build-up of ROS, leading to oxidative damage to the plant[24]. Chloride toxicity is less understood, where Cl^- acts as a micronutrient at low levels (0.2 to 0.4 mg g^{-1} dry weight) but can be toxic at higher levels (4 to 35 mg g^{-1} dry weight)[25,26]. Despite this, average Cl^- levels in plants are often much higher than the required micronutrient level, and even crossing the recorded toxic range, with no observed effect on growth.



Another key signalling ion in disease and stress response is Ca^{2+}, with cellular levels of Ca^{2+} being affected by numerous biotic and abiotic stressors. Plant immune responses (both PTI and ETI), physical damage and cold shock lead to cytosolic Ca^{2+} influx or increased Ca^{2+} signalling[22,27]. Ca^{2+} receptors around the plant respond to these increased Ca^{2+} levels, activating protein kinases (enzymes that add phosphates to proteins) that regulate many stress response gene functions[28]. Herbivore feeding results in a distinctly long-distance, time-dependent, 'nervous-system-like' signalling response[29]: glutamate increases at the site of feeding or physical wounding and travels along the phloem of the plant. This activates glutamate-like receptors in cells that line the vasculature, triggering the influx of Ca^{2+} into these cells and the synthesis and accumulation of jasmonates, a class of phytohormone with antiherbivore effects[30].

Inorganic molecules and radicals

Water and CO_2 are key to basic plant physiological processes, including photosynthesis and respiration. They could therefore be considered key stress signalling molecules, as their levels in the plant and in the surrounding environment are directly related to basic physiological processes, including the behaviour of the stomata (Fig. 2b, bottom). For example, photosynthesis is affected by both available light levels

Fig. 2 | Overview of chemical signals in response to stress in plants. a, Pathogen-associated molecular patterns (PAMPs) detected by pattern recognition receptors initiate PAMP-triggered immunity (PTI), a response that effector proteins from the pathogen suppress. Resistance proteins (R proteins) detect these effector proteins and initiate effector-triggered immunity (ETI), leading to a range of responses, including alkalinization, Ca^{2+} influx, ion efflux, burst of reactive oxygen species (ROS) and production of antimicrobial compounds, such as reactive nitrogen species (RNS) and salicylic acid. **b**, Abiotic stress, such as strong ultraviolet (UV) light, drought, cold, heat and salt stress, leads to a change in pigment accumulation and ROS build-up, resulting in oxidative damage (top). Drought and low-light conditions result in stomatal pore closure, leading to reduced flux of water vapour and CO_2 to the surrounding air (bottom). **c**, Wounding from cutting or herbivore feeding leads to release in volatile organic compounds (VOCs) and increase in long-distance Ca^{2+} signalling throughout the plant. Additionally, herbivore feeding leads to release of herbivore-associated molecular patterns, resulting in release of antiherbivore compounds, such as jasmonic acid, methyl salicylate and protease inhibitors (such as soybean Kunitz trypsin inhibitor (SKTI)). Part **c** is adapted from ref.[168], CC-BY 4.0.

and environmental CO_2 levels, which in turn changes internal stomatal CO_2, leaf H_2O levels and stomatal aperture[31]. Heat stress also affects photosynthetic processes, including CO_2 assimilation and electron transfer[32]. Drought stress leads to stomatal closure to preserve water, which results in a lower relative humidity around the leaves[33].

Plant stress responses often lead to sudden increases, or 'bursts', of reactive molecules and free radicals, including ROS and reactive nitrogen species (RNS). ROS, including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$), are involved in messaging, stress response and maintaining plant health when produced in lower concentrations or on a short timescale. Upon recognition of avirulent pathogens, an initial small, transient burst of ROS occurs within minutes, followed by a larger and longer-lasting burst, correlating with disease resistance. Virulent pathogens lead to the same initial muted response, but the latter, larger burst is suppressed due to the introduction of effectors that interfere with PTI[34–36]. Abiotic stresses also influence ROS levels in plants, where drought, excess visible and ultraviolet (UV) light, heat stress and Na^+ salt stress have all been shown to lead to a build-up of ROS, causing oxidative damage to the plant[24,37]. Temperatures below freezing can lead to ice formation in plant cells and reduced production of ROS-scavenging enzymes, leading to a harmful increase in ROS concentration[38]. RNS, including the nitric oxide free radical ($NO^{\bullet}$), act as stress mediators and antimicrobial agents. $NO^{\bullet}$ levels have been observed to increase upon pathogen exposure and heat stress. Excess RNS can also have a damaging effect on a plant's health, akin to excess ROS[39–41].

Organic molecules

Phytohormones are chemical messengers occurring in low concentrations (fmol to pmol g^{-1} plant fresh weight) within plants that control and coordinate many processes, including growth, stress response, messaging and reproductive development[42]. Examples of phytohormones involved in stress response include indole-3-acetic acid/auxins, abscisic acid, salicylic acid, jasmonic acid/jasmonates, methyl jasmonate, methyl salicylate and ethylene. For long-distance travel, phytohormones are often moved via the xylem or phloem through transporters. Some phytohormones, such as indole-3-acetic acid, can transfer from cell to cell via transporters[43]. During ETI, the infected organ activates salicylic-acid-based signalling throughout the whole plant, inducing system-acquired response and thus giving the plant enhanced resistance to future pathogenic infections[44]. Insect feeding leads to increased production of jasmonic acid and methyl salicylate, both of which have antiherbivore effects[45,46] (Fig. 2c). Indole-3-acetic acid, abscisic acid and salicylic acid have been shown to regulate stress response for abiotic stresses, including low humidity, high salinity and mechanical cutting. Ethylene is unique among the phytohormones; being gaseous and the smallest and simplest in structure, it primarily regulates many age-related processes, such as fruit ripening, flowering, seed germination and senescence of flowers and leaves. Also involved in stress feedback loops, ethylene production is affected by biotic and abiotic stresses, including pathogens, salt stress, metals and air pollutants[47]. Ethylene can diffuse freely through lipid membranes and it is detected by cells far from its location of production[43].

Volatile organic compounds (VOCs) are released by plants through the leaves, flowers, roots and fruits. First identified around 1700, common VOCs released by plants include terpenoids, benzenoids, C6-aldehydes, alcohols and derivatives of fatty acids and amino acids. VOCs are released in low concentrations, with only some aromatic compounds strong enough for the human olfactory system to detect, and the composition and intensity of VOCs can be indicative of plant stress. VOCs can act directly to repel or intoxicate pathogens or herbivores, attract predators of attacking herbivores and warn neighbouring plants by inducing defence responses[48] (Fig. 2c). For example, increased levels of C6-aldehydes, alcohols, terpenes and terpineol have been detected from injured or infected tomato leaves or stems, with infection from *P. infestans* resulting in an extreme rise in (*E*)-2-hexenal, suggesting that measuring VOC profiles could predict specific infections[49]. VOCs can also act as airborne signalling molecules. For example, production of protease inhibitors can be triggered by airborne methyl jasmonate (a derivative of the jasmonate class of phytohormones) released from nearby plants[50,51].

Pigments have many roles in plant stress defence and response (Fig. 2b, top). For example, carotenoids are considered a first line of defence against ROS due to their ability to quench singlet oxygen and anthocyanins can block dangerous UV light and act as an osmotic regulator[52]. Anthocyanins are also induced or accumulate at different levels in different areas of the plant, depending on environmental stresses such as pH, nutrient deficiency, cold, water stress and visible or UVB radiation[53,54].

There are also reports of bidirectional cross-kingdom transfer of various RNA molecules (including small RNAs, messenger RNAs and long non-coding RNAs) between plants and fungi, plants and parasitic plants, and plants and insects[55–57]. The emerging paradigm is that both host plants and their parasites use various forms of mobile RNA molecules to alter the gene expression systems of their opponents for their own benefit, whereas adapted parasites have counter mechanisms to eliminate RNA-related defences[58–60].

Measuring chemical markers of stress

Most procedures reported in the literature to monitor chemical signals in plants use cell suspensions, which enable the study of stress responses at a cellular level with a wide range of available techniques[61,62]. Sensors for use with live whole plants are less common, however. Ions and most phytohormones are generally aqueous and found internally in plant organs, where implanting a standard electrode may induce a wounding response. VOCs, CO_2, ethylene, H_2O and other

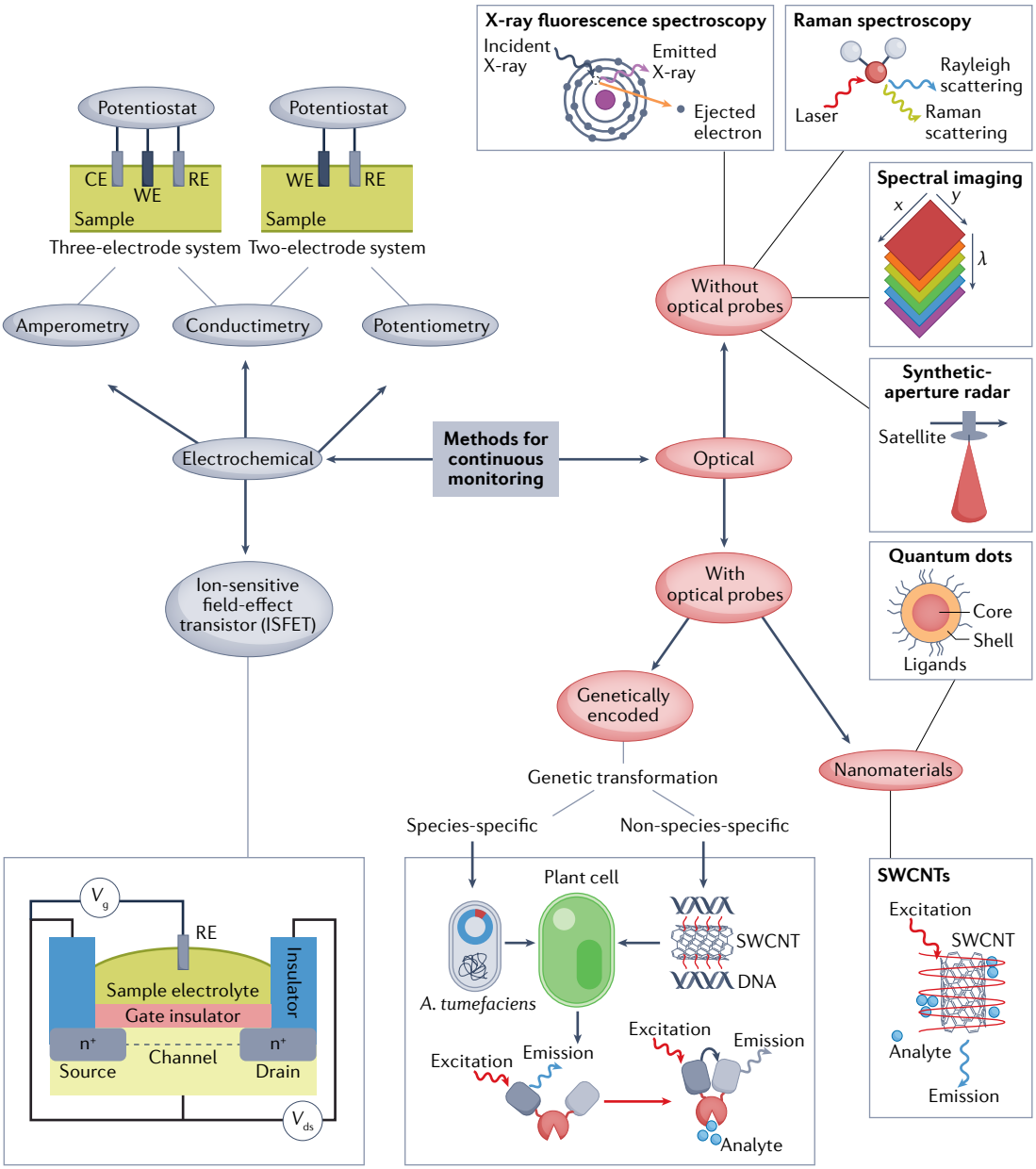


Fig. 3 | Methods for continuous monitoring of chemical signals in plants. Continuous monitoring can be broadly categorized into electrochemical and optical methods. Electrochemical methods vary widely in their complexity, from simple, low-cost, two-electrode or three-electrode systems to microfabricated field-effect-transistor-based sensors[76,78]. Optical methods include those without addition of optical probes, such as spectroscopic or imaging techniques, and those that involve addition of nanomaterials or genetically encoded sensors[10,52,63,67,68]. *A. tumefaciens*, *Agrobacterium tumefaciens*; CE, counter electrode; RE, reference electrode; SWCNT, single-walled carbon nanotube; WE, working electrode.

gaseous signals must be measured externally, where the lack of electrolyte or the need for custom sensor geometries are problems to be considered. Sensors suitable for use in whole plants, however, enable in vivo and continuous monitoring of chemical signals in response to stress; we focus on these types of sensors in this Review (Fig. 3). A summary of the main techniques used for plant chemical sensing is provided in Table 2.

Optical sensors

Optical sensors can avoid many of the problems typically associated with whole-plant sensors due to the non-contact nature of measuring light. Optical sensors for continuous monitoring typically involve the introduction of optical probes, such as engineered nanomaterials or genetically encoded molecular sensors[10,63]. These optical probes typically produce fluorescence, the intensity of which changes depending

on the surrounding chemical environment and can be detected by fluorescence imaging devices, such as with fluorescence microscopy. Engineered nanomaterials, including single-walled carbon nanotubes (SWCNTs) and quantum dots, can provide remarkably high spatiotemporal resolution for measuring chemical species, sometimes down to individual molecules. They have been added to plants to respond to chemical changes inside the plant, where their fluorescence occurs with low to no background absorption from living tissues. Nanomaterials are commonly delivered by needleless syringe, vacuum infiltration or topical application.

Genetically encoded molecular sensors typically consist of a sensory module coupled to a fluorescent protein (for example, green fluorescent protein (GFP))[10]. Genetically encoded sensors can be introduced through genetic transformation. Transformation typically requires species-specific techniques, such as transfection using *Agrobacterium tumefaciens* or insertion using gene gun particle bombardment[64,65]. Recent efforts with nanomaterials have led to deliverable genetically encoded molecular sensors without the need for species-specific techniques: after needleless syringe application, SWCNTs coated with DNA can passively and spontaneously penetrate plant lipid bilayers[10,66].

Optical sensing can also be performed without the addition of optical probes. Plant surfaces can be measured directly by methods such as Raman spectroscopy, X-ray fluorescence spectroscopy or spectral imaging, where changes in the optical properties of the plants that can be observed from the outside may relate to certain internal chemical changes[52,67,68]. Where images of whole plants or organs (such as leaves) are captured, computer vision (including object recognition) can be used to categorize and predict stress[12–14].

Optical sensors have both advantages and limitations: a lack of heavy physical components means that the plant's growth is generally unaffected (although smaller second-order and third-order molecular interactions would be largely unknown for the methods that require optical probes) and stress due to wounding is avoided. However, interference with cellular functions can occur, including gene silencing by strong promoters[69]. Going beyond the laboratory, there is also a trade-off between coverage and precision, where optical sensor usage ranges from satellite imaging of whole fields to microscopy of individual cells[70]. Many of the optical techniques discussed require laboratory-based equipment (for example, microscopes, positron emission tomography (PET) scanner), limiting their practicality for on-site or field-based sensing[30,71]. Furthermore, optical sensors are susceptible to light scattering by plant tissue and interference from external light sources[69]. Additional limitations of optical sensing include the need for a source of light (and, sometimes, a specific wavelength) and the use of expensive and complex readers to transduce the response from the analyte into a measurable signal[29].

Electrochemical sensors

Electrochemical sensors convert changes in the concentration of chemicals into measurable electrical signals and form the second major class of sensors used for the continuous measurement of chemical signals in plants. Electrochemical sensors typically consist of two or three conductive electrodes in contact with the plant under study. There are three common properties evaluated in electrochemical solution-based sensing in plants (that is, internal measurements in stems, leaves or roots). First, conductivity and impedance, where two-electrode (counter electrode (CE), working electrode (WE)) or three-electrode (CE, WE, reference electrode (RE)) setups are used with techniques such as conductimetry (where the electrical conductivity of a solution is measured) or impedance spectroscopy (where the change in electrical impedance is measured at the WE or in the material between two interdigitated electrodes). Second, potential via potentiometry. Potentiometry is a static electrodic technique and involves measuring the potential between a WE and a RE, commonly in an open-circuit configuration. The potential difference between the RE and the WE changes proportionally to the concentration of the target chemical species in the sample. For example, potentiometric sensors can use ion-selective

Table 2 | Summary of the main techniques used for plant chemical sensing

Class	Techniques	Analytes	Proximity	Plant organs	Sampling frequency	Refs.
Electrochemical	Potentiometric, amperometric (two and three electrodes, field-effect transistors)	pH, ions, CO_2, reactive oxygen species	Insertable (organs, cells or stomata)	Leaves, stems, roots	<second	5,31,37,85,86, 104,112
		Ethylene	In same container	Leaves	<second	47
	Conductimetric (resistive, bioimpedance spectroscopy)	Relative humidity, ethylene	Wearable, direct application, in same container	Leaves	<second	33,80,105,106
Optical	Microscopy with insertion of optical probes (nanomaterials, genetic transformation)	pH, ions, reactive oxygen species, phytohormones	Microscopy: µm–cm Invasive insertion of material	Leaves, roots	<second	30,89,118–120, 126–128
	X-ray fluorescence spectroscopy	Plant composition (ions, nutrients)	cm–m	Leaves, stems	Minutes to hours (on demand)	68
	Raman spectroscopy	Plant composition (pigments)	mm	Leaves	Minutes to hours (on demand)	67
	Positron emission tomography	Na^+	cm–m	Whole plant	Minutes to hours (on demand)	71
	Spectral imaging	H_2O, chlorophyll	cm–m	Leaves, stems	Minutes to hours (on demand)	137
	Satellite imaging	H_2O	Remote (resolution cm–km)	Leaves	Hours to days (set intervals)	110

electrodes, where the WE is modified with an ion-selective membrane for sensing certain ionic species. The RE remains at a constant potential, normally in contact with a saturated aqueous solution, with the glass-based saturated calomel and silver/silver chloride electrodes being common REs for traditional electrochemical sensing[72]. For plant-based sensing, pseudo-REs are often used, as they are not glass-based and do not contain harmful chemicals, unlike the mercury found in the traditional calomel electrode. Common materials for pseudo-REs include wires made of platinum, silver or Ag/AgCl (ref.[73]). They have also been produced using conductive inks, commonly Ag/AgCl, printed or deposited onto a flat substrate, such as a ceramic. Known as 'pseudo'-REs due to their limited chemical stability, Ag/AgCl pseudo-REs are often coated with a membrane containing chloride ions to improve their stability for continuous monitoring[72,74]. Third, current via amperometry. Amperometry is a dynamic electrodic technique, where the current is measured between two polarized electrodes (a WE and a CE) at a set voltage. The polarization potential is typically controlled by the use of a RE. In traditional amperometric sensors, the WE is a highly conductive and inert material, such as gold or carbon. A sensor can be made analyte-specific by coating the WE with molecular recognition elements that enable or catalyse redox reactions of certain species. WEs can also be functionalized by immobilizing nucleic-acid-based or antibody-based biomolecules on the surface of the electrodes, although not all recognition elements are suitable for continuous measurement[75]. CEs are commonly made of an inert, highly conductive material, such as platinum, ideally with a larger surface area than the WE, ensuring that all processes occurring at the CE do not limit the kinetics of the electrochemical processes under study on the WE[76]. Amperometric measurements can be performed using only two electrodes at low currents, where the CE and the RE are one electrode, although signal drift can occur due to changing reference potential[77].

In addition to the traditional two-electrode and three-electrode sensing approaches outlined above, continuous ion sensing can also be achieved using ion-sensitive field-effect transistors (ISFETs)[78]. The current flowing through a field-effect transistor (FET) is controlled by an electric field, where an ISFET replaces the metal gate with an ion-sensitive membrane (ISM). Compared with a classical setup of a two-electrode potentiometric ion sensor that produces a voltage as the analytical signal, ISFETs produce a current proportional to the amount of charge on the surface of the ISFET. ISFETs can be highly sensitive but are more complex and expensive to produce, as they require microfabrication in a silicon foundry.

Chemical signals in plants also involve volatile molecules, which can be monitored continuously using impedance-based measurement techniques. A conductive material, such as graphene or SWCNTs, is placed between two electrodes of a higher conductivity[79–81]. The impedance of the sensor depends on interactions between the sensing material and gaseous molecules in the environment. The selectivity of the sensor is normally tailored by the addition of metal complexes or nanoparticles with ligands into the sensing material, which interact with the analyte. Externally placed sensors have additional challenges because the collection of gaseous analyte molecules is difficult in an open environment, hence some setups use an enclosed chamber to increase the target gas concentration and accelerate its detection.

Other sensor types

While electrochemical and optical sensors form the two main classes of sensors used for the real-time detection of chemical signals in plants, there are other sensors used for gaseous analytes that do not fall into those two classes. Gravimetric sensors, incorporated into electronic nose (e-nose) systems for the detection of VOCs, use piezoelectric sensors, where the change in mass absorbed on the sensor due to gas absorption results in a change in the resonant frequency[82]. Microcantilevers, one form of microelectromechanical systems also found in e-nose systems, typically consist of a cantilever layered with an analyte-sensitive layer. The layer will shrink or swell upon analyte absorption, bending the cantilever, the deformation of which can be measured to determine the analyte concentration[83,84].

Ions

H^+ is a crucial chemical species for signalling in plants and, hence, continuous sensing of pH is important for understanding the chemical basis of stress signalling. Using pH-sensitive microelectrodes formed of glass capillaries, the pH of root hairs and root cortex of various plants was measured continuously under different chemical and pathogenic conditions by Felle et al.[5,85,86] (Fig. 4a). The tip (about 5 µm in diameter) of a glass capillary was filled with a pH-sensitive gel (consisting of PVC and a pH-sensitive cocktail) and backfilled with a pH-sensitive cocktail in KCl (0.5 M). A second, internal capillary filled with KCl (0.5 M) provided a reference voltage. The whole sensing electrode was then inserted into cells with a pressure controller to measure the internal pH. However, these glass microelectrodes are too fragile for general use. Because of this, recently, a more robust ISFET-based device has been proposed as an insertable sensor for measuring internal pH in the stems of plants, although both the glass capillaries and the ISFET device have the potential to cause injury-related stress[87] (Fig. 4b). Genetically encoded fluorescent sensors are a popular optical measurement method for pH and have been used to measure a diverse range of plant processes, including exocytosis (discharge of vesicle materials into extracellular fluid), ion uptake in roots, cell growth, homeostasis, response to stimuli and protein trafficking[88]. Changes in light emission originating from within the plant may, however, result from both changes in pH and the concentration of the genetically encoded molecular sensor. This issue is addressed with ratiometric molecular sensing, which typically uses two proteins: for example, pHusion (cytosol located) and apo-pHusion (targeted to the apoplast) use enhanced green fluorescent protein (EGFP) and monomeric red fluorescent protein (mRFP1) to measure rapid homeostasis upon external changes in pH, demonstrating the buffering capacity of plant cells[89] (Fig. 4c). Single optical sensing proteins are also being explored, such as pHRed, a system that exploits the dual excitation of a mutant of red fluorescent protein mKeima to show cell acidification upon decreasing external glucose concentration[90]. The variety of pH-sensitive fluorescent protein sensors being developed opens up the future of continuous optical monitoring of chemical stress signals, homeostasis and physiology of plants.

Genetically encoded fluorescent protein sensors have also been used to continuously measure cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$). GcaMP3, a fluorescent-protein-based $[Ca^{2+}]_{cyt}$ sensing molecule, was used by Toyota et al. to study stress signalling[30]. Their approach revealed a hormone-like role for glutamate in long-distance, time-dependent calcium signalling. For example, mechanical damage by scissors and caterpillar were both shown to produce an immediate local increase in $[Ca^{2+}]_{cyt}$, followed by a response at distal leaves over a minute later (Fig. 4d). Their work with apoplastic fluorescent glutamate ($[Glu]_{apo}$) sensing protein also revealed that mechanical damage increased glutamate concentration at the site of wounding. This initial local increase was shortly followed by the glutamate signal extending through the

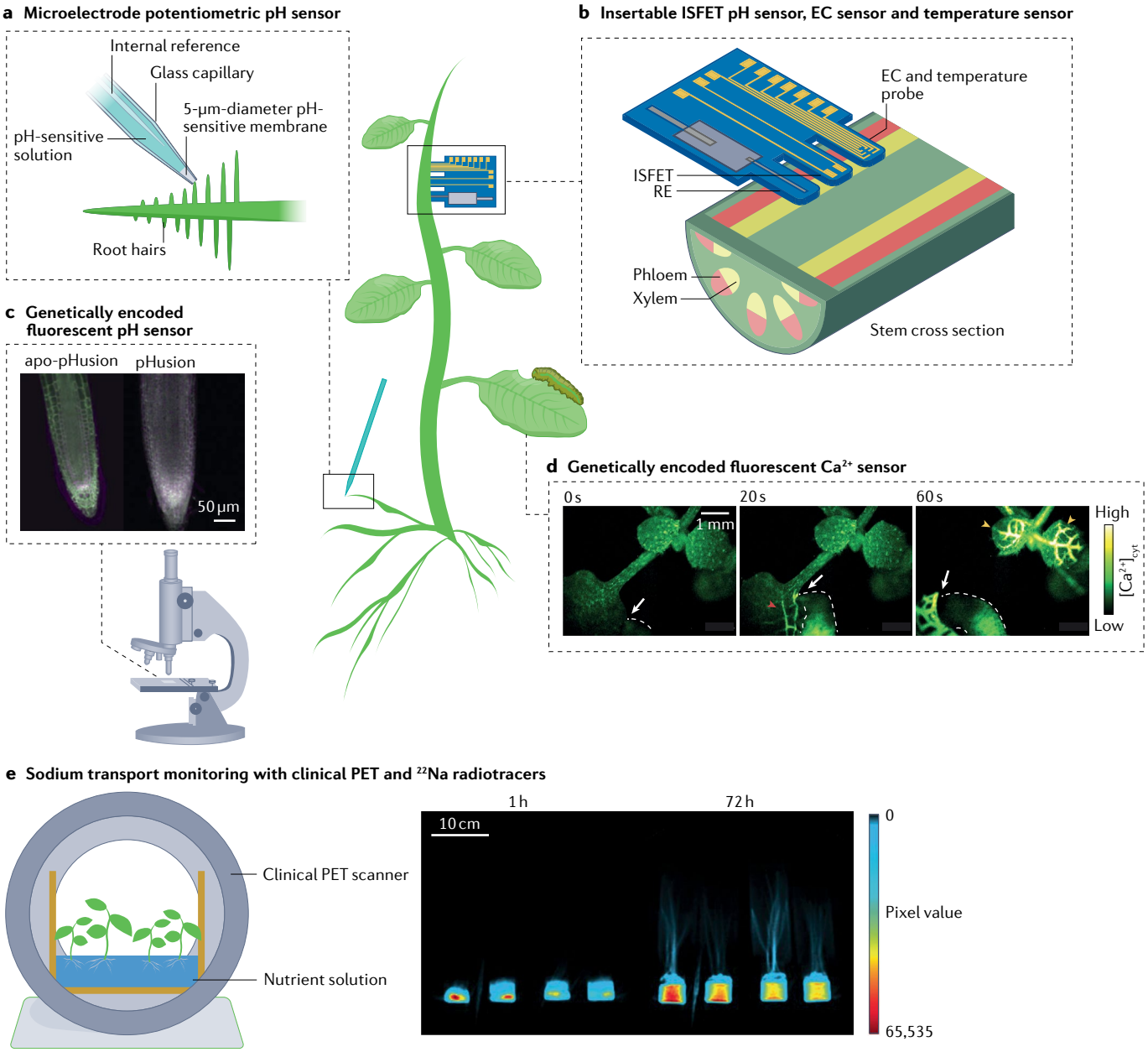


Fig. 4 | Sensors for monitoring ions as stress signals in plants. **a**, A microelectrode-based pH sensor for the measurement of root hair pH (not to scale)[5]. **b**, An insertable ion-sensitive field-effect transistor (ISFET)-based pH sensor combined with electrical conductivity (EC) and temperature sensors to measure xylem properties[87]. **c**, A ratiometric, fluorescent, genetically encoded pH sensor to measure intracellular and extracellular pH changes: confocal overlay images of enhanced green fluorescent protein (EGFP; green) and monomeric red fluorescent protein (mRFP1; magenta) showing sensor signal of apo-pHusion (left) and pHusion (right). **d**, A fluorescent, genetically encoded Ca^{2+} sensor to measure local (red arrowhead) and long-distance (yellow arrowheads) Ca^{2+} signals upon herbivore (dashed outlines) feeding (white arrows). **e**, A clinical positron emission tomography (PET) scanner used to continuously monitor sodium transport dynamics. RE, reference electrode. Part **c** is adapted from ref.[89], CC-BY 4.0. Part **d** is reprinted with permission from ref.[30], AAAS. Part **e** is adapted from ref.[71], CC-BY 4.0.

vascular system and activating glutamate-like receptor channels, causing an influx of Ca^{2+}. This remarkable research enabled by time-resolved continuous monitoring of chemical signals suggests that glutamate acts much like a hormone associated with stress signalling in plants, unlike its mammalian role as a neurotransmitter[29,30].

For the optical measurement of the concentration of Na^+ in plants, a common ionic source of abiotic stress, the use of radioisotopes and PET are more popular than fluorescence-based sensing (Fig. 4e). Measurements using PET have the advantage of continuously detecting the complete flow of Na^+ into, around and out of the plant in 3D, rather than

simply the internal concentrations that genetically encoded fluorescent sensors are able to detect. PET scanning has been used with ^{22}Na tracers to show how Na^+ accumulates in the shoots of salt-sensitive rice but does not transport beyond the roots in salt-resistant reeds[91]. PET has also been used to demonstrate in barley how nutrient deficiency drives an increase in Na^+ uptake, how $BaCl_2$ inhibits Na^+ uptake and the presence of a diurnal effect on Na^+ uptake that had not been previously reported[71]. Although PET scanners are necessarily large and expensive, they have greatly improved the 3D understanding of time-dependent Na^+ transport in plants compared with traditional techniques, which are often invasive or destructive and only capable of measuring Na^+ in certain organs without temporal resolution. For example, insect stylectomy can be used for the chemical analysis of pure phloem sap but can only be used on the shoots of plants[91].

In comparison with optical sensors, continuous electrochemical sensing in whole living plants has not yet been explored fully for Ca^{2+} and Na^+, presumably due to complexities of developing a robust insertable sensor. This is unlike in healthcare and fitness, where wearable sensors have been used to measure these ions in human perspiration[92–102]. In vitro experiments with plant materials have been performed and, though in the early stages of development, they show potential for future application in insertable sensors. For example, the concentration of Ca^{2+} in the extracted sap from sesame leaves was found to be different under normal or nutrient-deficient conditions using a reduced graphene oxide (rGO)-aerogel-based electrochemical sensor, although insertion into leaves in whole plants was not investigated[103]. Printed carbon electrodes were coated with rGO aerogel followed by an ISM and measured potentiometrically against a printed Ag/AgCl RE. Due to the simple fabrication method, this design could be adapted to produce insertable-style sensors to measure internal ion concentrations, although damage to the ISM from mechanical damage is a potential concern.

Insertable ISFET-based nitrate sensors have been developed for use in planta, measuring changes in nitrate levels in the xylem dependent on light, irrigation and fertilization of soil. Despite still potentially causing physical stress upon insertion, these ISFET-based sensors are more durable in nature, and with the possibility of interchanging the ISM, these devices open the doorway for future continuous nutrient and ion insertable sensors[104].

Inorganic molecules and radicals

H_2O and CO_2, two inorganic molecules that are key to many basic plant functions, have been measured using a range of electrochemical sensing methods, monitoring both their direct levels and their effect on plant functions. The glass pH microelectrode by Felle et al. previously discussed was modified with an outer section containing a carbonic anhydrase solution and, while still being too fragile for most uses, displayed some remarkable results for continuously tracking CO_2 concentration in stomatal pores (Fig. 5a). CO_2 dissolves in the microelectrode solution and decreases the solution pH, where the carbonic anhydrase speeds up the pH-changing reaction. Placing the electrode tip into stomatal pores of *Vicia faba* (chosen for its large stomatal aperture), the CO_2 concentration in substomatal cavities was shown to change dramatically with changing environmental light and CO_2 concentration[31].

Sensors for H_2O vapour are simpler and more streamlined in comparison with CO_2 sensors: relative humidity sensors typically consist of a conductive material, such as graphene or gold, deposited onto a flexible substrate, such as polyimide or poly(ethylene terephthalate)[33,105]. The electrical properties of the sensors change in a concentration-dependent fashion to relative humidity. The electrical properties of the relative-humidity-sensing material may also change upon exposure to other chemical species, alongside mechanical deformations under normal use in the field (such as twisting, bending, stretching). The flexible relative humidity sensors are fixed onto the leaf of the plant, commonly the abaxial side (due to the increased density of stomata). These sensors have been used to measure the relative changes in the levels of water vapour released from the plant, which is impacted under conditions such as drought[33,106] (Fig. 5b).

A more recent example of measuring the effects of water loss, albeit with measurements taken 'on demand' rather than continuously, involved vapour-coating *p*-doped poly(3,4-propylenedioxythiophene) (PProDOT-Cl) electrodes directly onto the leaf surface of a wide range of plants. These electrodes were long-lasting and reliably measured deep-tissue damage via bioimpedance spectroscopy caused by drought and UV damage[107]. The idea of placing a sensor directly on the subject has been explored further: a non-specific gas sensor consisting of a graphite-SWCNT FET was placed directly onto the leaves of 'lucky bamboo' (*Dracaena sanderiana* cv. *Virens*). Using the graphite-SWCNT FET, despite high levels of noise being present in the plant measurements, concentrations of dimethyl methylphosphonate (DMMP) as low as 5 ppm were detected[108].

The water levels within plants were indirectly continuously measured using an electromechanical sensor printed directly onto the guard cells: the resistance across contacts corresponded to stomatal aperture, where stomatal closure and opening were induced with watering and drought[109] (Fig. 5c). Although unlikely to be used widely due to the precision required for placing the electrodes, this research truly shows the innovations being made in the field of plant stress measurement.

Continuous optical monitoring of H_2O levels within plants can be achieved at a lower temporal frequency with satellite imagery. Synthetic-aperture radar can provide information on crop moisture content, soil moisture and the effects of water or drought stress on plants. Geostationary satellites provide imagery of a specific area with a temporal frequency of minutes or hours, whereas polar orbiting satellites provide information on a location with a frequency of hours or days. As synthetic-aperture radar has sensitivity towards other agricultural factors, such as crop biomass, crop height and plant density, retrieving specific parameter information can be challenging: for example, for the isolation of soil moisture information, other parameters such as crop cover, surface roughness and soil texture will provide noise[110]. Satellite-based measurements techniques, however, are highly scalable, allowing the monitoring of large plots of land at a low cost in exchange for lower spatial resolution.

H_2O_2, found in aqueous form in plants, is commonly measured both electrochemically and optically. Platinum, a typical electrode material used to monitor the oxidation of H_2O_2 electrochemically, is suitable for biological systems due to its biocompatibility and inertness[111]. To increase selectivity to H_2O_2, platinum electrodes have been modified by the addition of Pt microparticles and poly-*o*-phenylenediamine film. These modified electrodes have been used as insertable sensors placed in leaf tissue of oilseed rape to amperometrically continuously measure bursts of H_2O_2 resulting from biotic (*Sclerotinia sclerotiorum* infection) and abiotic (UV light exposure) stress[37,112] (Fig. 5d). Measuring internally in leaf tissue material produced a large amount of noise in measurements, likely due to the amount of oxidizable components that are present in plants. Despite the noise, multiple oxidative bursts were detected, up to 25 h after stress was induced. Pt is expensive, limiting its feasibility in disposable electrochemical

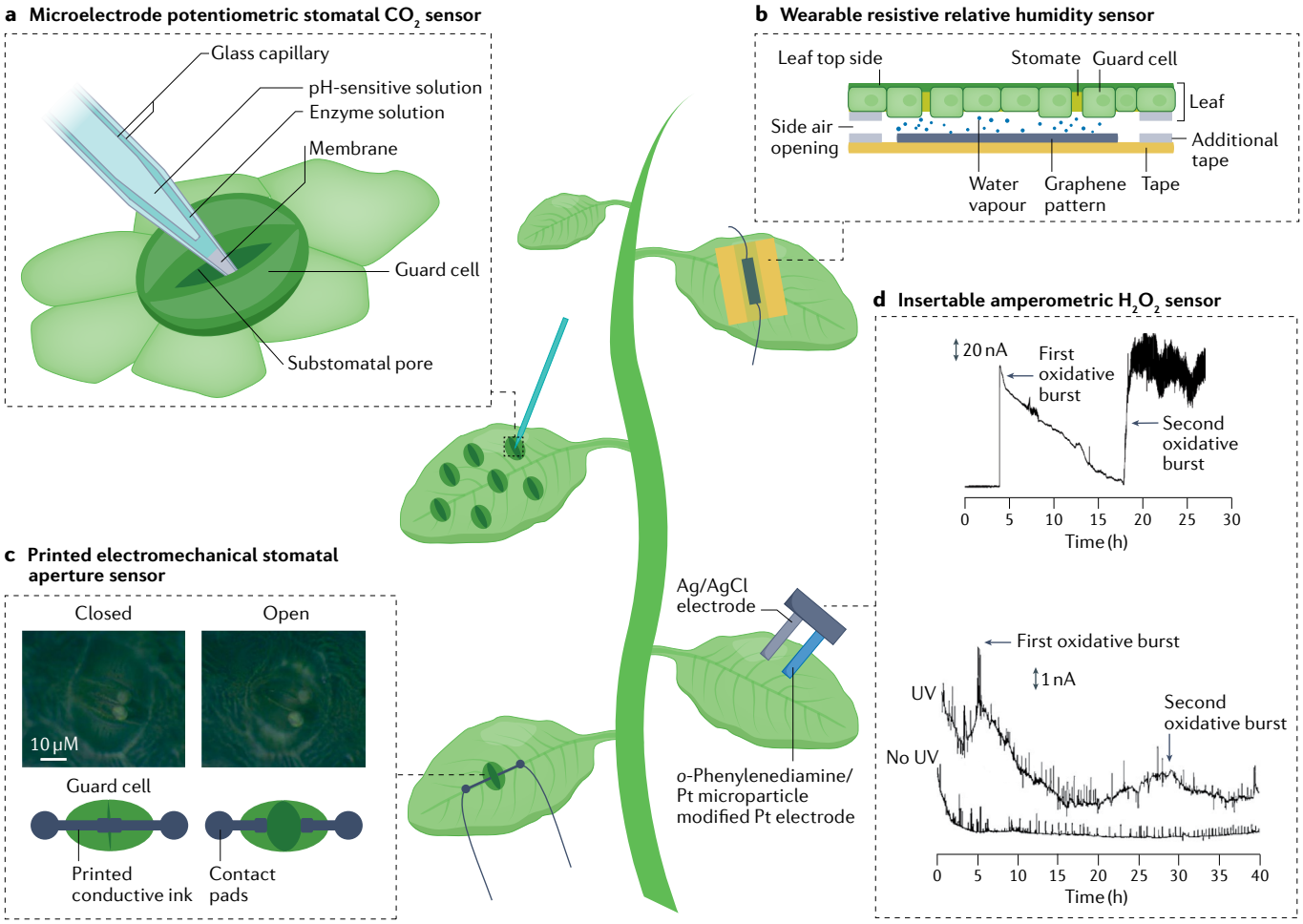


Fig. 5 | Sensors for monitoring radicals and inorganic molecules in plants. a, A miniaturized CO_2 sensor for measurements within the stomatal pore[31]. **b**, A graphene-on-tape relative humidity sensor for plant leaves. **c**, An electromechanical sensor directly printed onto guard cells to measure stomatal aperture under drought conditions. **d**, An insertable *o*-phenylenediamine/Pt microparticle modified Pt sensor for measurement of reactive oxygen species, where the graph shows the sensor's detection of oxidative bursts as a result of fungal infection (top) and ultraviolet (UV) radiation (bottom). Part **b** reprinted with permission from ref.[106], Wiley. Part **c** adapted with permission from ref.[109], RSC. Part **d** adapted with permission from ref.[37] and reprinted from ref.[112], Elsevier.

H_2O_2 sensors. Because of this, enzymes such as horseradish peroxidase can be immobilized on carbon electrodes for electrochemical sensing of H_2O_2 (ref.[113]). Relatively poor stability of enzymes, however, limits the use of enzyme-based electrochemical approaches for continuously measuring the levels of H_2O_2 internally in plants. Prussian blue ($Fe_4[Fe(CN)_6]_3$) is another common material layered on electrodes to sense H_2O_2 and allows electrochemical oxidation of H_2O_2 to occur at 0 V versus Ag/AgCl, reducing noise from interfering chemical species, although it is yet to be used for continuous stress sensing in plants[114].

Optical genetically encoded fluorescent sensing materials have only been used to measure H_2O_2 continuously in individual cells in vitro, small tissue samples or *Arabidopsis* seedlings[115–117]. Currently, the best methods for continuous H_2O_2 sensing in whole, mature plants use engineered materials: 2′,7′-dichlorodihydrofluorescein (H_2DCFDA) is a probe that is oxidized by several radicals and ROS (H_2O_2, $O_2^{\bullet-}$, $HO^{\bullet}$, peroxynitrite ($ONOO^-$) and NO) to the fluorescent species 2′,7′-dichlorofluorescein (DCF), enabling continuous monitoring of multiple oxidative bursts from a variety of stimuli (such as light stress, physical injury, pathogen infection)[8,118]. By introducing H_2DCFDA to *Arabidopsis* through fumigation, mechanical wounding was shown to induce an oxidative burst in both the local leaf and systemically, similar to the glutamate Ca^{2+} pathway reported by Toyota et al.[8,30]. In this case, however, an oxidative burst was not induced systemically after the local burst, but instead leaves younger than the local leaf often displayed an oxidative burst earlier. Another popular method for continuous optical sensing of ROS uses SWCNTs due to their fluorescence in the near-infrared region, away from the chlorophyll autofluorescence region[118–120]. SWCNTs can be infiltrated into leaves and, through functionalization, made specific to an analyte of choice. Ratiometric SWCNT-based sensors have been developed by separating single-chirality SWCNTs sensitive to specific analytes (H_2O_2 or NO) and pairing with an invariant SWCNT emitter acting as a reference, thereby negating problems arising from noise and uneven geometry of leaf surfaces, whilst allowing absolute analyte calibration[120].

Organic molecules

Traditionally, most organic molecules are measured using standard chemical detection techniques, such as liquid chromatography (LC) or gas chromatography–mass spectrometry (GC–MS). These techniques, however, do not allow high-frequency continuous monitoring for chemical species in plants and often involve expensive and specialized instruments. For continuous monitoring, optical and electrochemical techniques have recently been reported for the measurement of phytohormones. Indole-3-acetic acid, abscisic acid and salicylic acid, phytohormones that regulate stress response, have been measured using techniques including differential pulse voltammetry and electrochemical impedance spectroscopy for plant samples[121–125]. Real-time measurements of phytohormones in live plants using these electrochemical techniques have not yet been reported, however, as the sensors are either not sensitive enough or require the analyte to be destroyed or immobilized. As phytohormones are produced by plants in low concentrations (fmol to pmol g^{-1} plant fresh weight), it is often more favourable to induce a chemical response, allow the concentration to build up over time and take a single sample with maximum analyte. Continuous measurement of the phytohormones indole-3-acetic acid, abscisic acid and jasmonic acid have been performed with a variety of genetically encoded optical molecular sensors under abiotic stresses (salt stress, osmotic stress, physical cutting) and environmental changes (drop in humidity)[126–128] (Fig. 6a).

Ethylene, a gaseous and the smallest phytohormone, has been measured continuously with plants using SWCNT-based electrochemical sensors, although not for measuring chemical signals associated with stress: for example, SWCNTs mixed with a copper(I) complex have been used as a chemiresistive sensor for measuring ethylene as a signal for the regulation of ripening of fruits[80]. Ethylene production in flower blooming and senescence was also measured continuously using a SWCNT-based sensor decorated with Pd catalysts via Wacker oxidation[47]. The presence and concentration of ethylene was reversibly detected by the modulation of the concentration of charge carriers in the nanotubes by the catalytic aerobic oxidation reactions. Ethylene was able to be detected as low as 0.5 ppm, low enough to measure the release of ethylene from fruits and flowers in containers. Ethylene concentrations required for fruit ripening can lie from 0.1 to 1 ppm, however, suggesting that increased ethylene sensitivity may be required in some cases[47,80]. Gas sensors such as these have the added advantage of using materials that may not be biocompatible (such as SWCNTs), as they are not in direct contact with the plant. There are inherent limitations from the sensors being placed further from the plant, where typically the sample will be placed in a container to collect any gaseous analyte.

A range of VOCs in low concentrations are released by plants. E-noses have been developed over the past two decades as a more accessible form of VOC analysis to traditional GC–MS — the sensitivity and specificity of expensive GC–MS is traded off for lower cost, lower analytical performance and continuous sensing of e-nose-type sensors[82]. Rather than identifying the individual compounds, e-noses form a VOC profile from a source through interactions between the VOCs and an array of gas sensors, commonly conductivity, optical or gravimetric sensors. Attempting to mimic the olfactory system of animals to detect odours, each sensor has a specific sensitivity and, when combined and calibrated, they can be used to discriminate different compounds. Each compound produces a distinct sensor 'fingerprint'[82]. E-nose systems have been used to detect the VOC fingerprints of insect-damaged and age-damaged wheat, postharvest fungal-infected blueberries and basal stem rot disease in oil palm in field experiments[129,130]. Although usually handheld and portable, e-nose systems often require a sealed and controlled environment. Temperature, humidity and other gases present, which can cross-react with the sensors, can make it difficult to detect low concentrations of target VOCs[131]. Developing the e-nose system further, a wearable chemiresistive VOC sensor array consisting of functionalized rGO has recently been reported by Li et al.[81] (Fig. 6b). Ligand-modified gold nanoparticles embedded into the rGO interacted with VOCs through hydrogen and halogen bonding, changing the overall resistance of the rGO. By using eight uniquely modified sensing units with different ligands, 13 individual common plant VOCs (including late blight markers, green leaf volatiles, phytohormones and aromatics) were fingerprinted and used to detect infection from *P. infestans* ('tomato blight'). As perturbation from sources including wind and wildlife are common for crops in field scenarios, a stretchable kirigami-inspired support was used to reduce the effects of disturbance.

Microcantilevers have been used to continuously detect hexanol, a VOC released by crops with green leaves upon biotic stress (attack from pests and disease)[84]. A microfabricated Au cantilever was layered with poly(methyl methacrylate), a polymer that swells upon absorption of a target analyte (hexanol or ethanol), pushing the cantilever towards a Pt contact. The device acted as a switch and, therefore, only activated once a threshold analyte concentration was reached. This setup enabled the sensor to function with no required power until the point of detection (an important consideration for use in remote locations), after which the circuit was completed and a reporting device (such as a radio transmitter) could activate. The current hexenol threshold (around 1,237 ppm) far exceeded actual VOC concentrations localized to damaged leaves (around 10 ppm), limiting the use of the device in in vivo applications, although the threshold can be reduced to around 60 ppm by using a folded-beam design.

The detection of other plant molecules such as pigments (for example, anthocyanins and carotenoids) has been performed optically using Raman spectroscopy due to its non-destructive nature and ability to investigate multiple chemical signals simultaneously[52]. Raman spectroscopy is used to gather chemical and structural information of samples through the scattering of light by chemical bonds[132]. Traditional Raman techniques have been used to observe changes in carotenoid and anthocyanin concentrations due to salt stress, light stress, drought and cold temperature, although the equipment required is not suitable for fieldwork[52]. A smaller, portable Raman sensor was designed to clip onto the leaf of the plant and measure plant metabolites, such as carotenoids, anthocyanins and nitrates (Fig. 6c). The device was used for rapid and real-time monitoring of nutrient deficiency in a range of leafy vegetables, demonstrating the importance of devices designed for non-specific crops[67]. SWCNTs have been used to measure the increase in the level of polyphenols, compounds consisting of multiple phenol units and, commonly, flavonoids and tannins that are released for chemical defence against pathogens and herbivores[133].

Analysis and modelling of plant stress

Stresses lead to responses on a molecular level, but the changes elicited in plants can manifest over a large range of spatiotemporal scales: from the cellular level to whole plants and fields, over periods ranging from seconds to days. In agriculture, continuous measurements with high temporal frequency (that is, measurements separated by minutes) are rare and most analyses rely on temporally sparse datasets with hours,

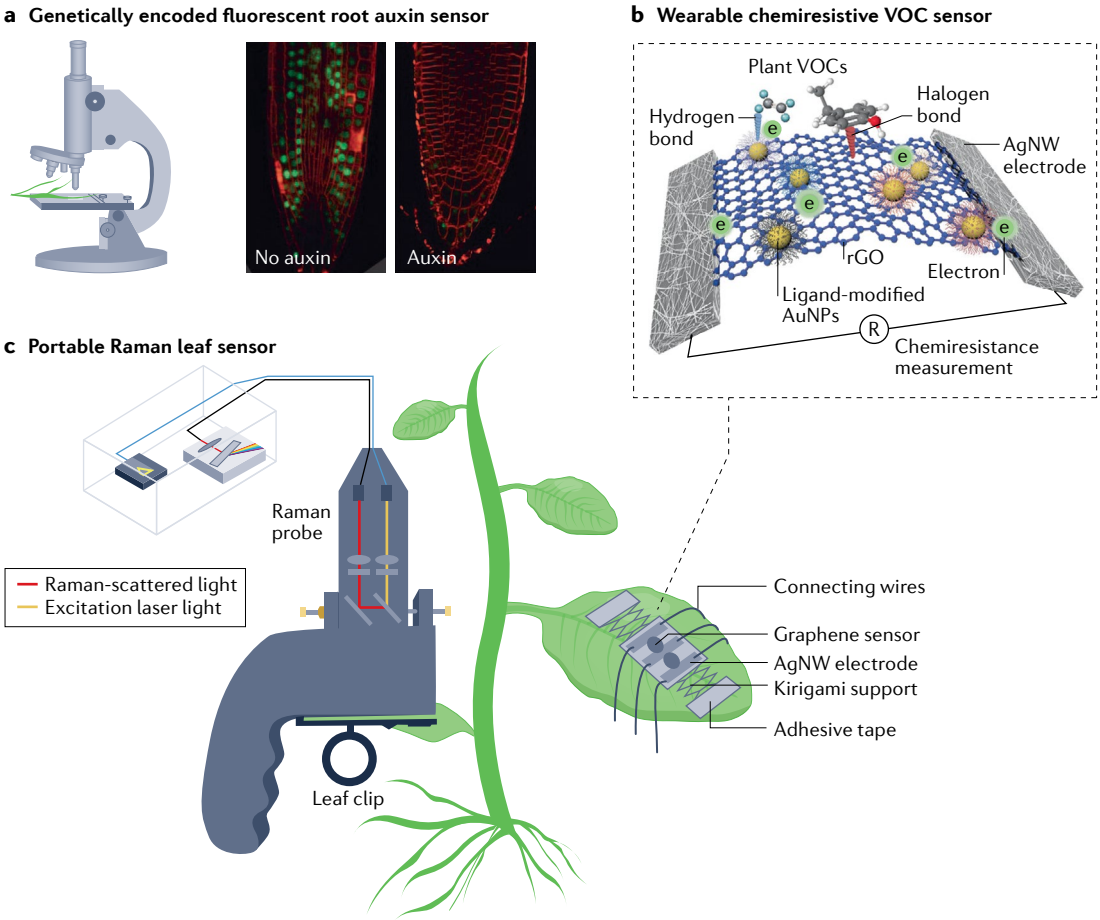


Fig. 6 | Sensors for monitoring organic molecules in plants. a, A genetic fluorescent sensor for the measurement of auxin in plants. **b**, A kirigami-supported, reduced graphene oxide (rGO)-based wearable volatile organic compound (VOC) sensor. **c**, A handheld Raman sensor for the monitoring of pigments in leaves. AgNW, silver nanowire; AuNP, gold nanoparticle. Part **a** reprinted from ref.[126], Springer Nature Ltd. Part **b** reprinted with permission from ref.[81], Elsevier. Part **c** adapted from ref.[67], Springer Nature Limited.

days or weeks between each measurement. Spectroscopic optical remote sensing from satellites, unmanned aerial vehicles, agricultural vehicles and handheld optical spectrometers are the primary methods that can provide information on the chemical and physical status of plants on the kilometre scale[134,135]. Spectral information can be used to assess plant health through different vegetation indices (formulae that relate measurements at two or more wavebands to biophysical characteristics)[136]. For example, the popular normalized difference vegetation index is calculated from visible and near-infrared bands and can be used in the estimation of parameters such as biomass, chlorophyll concentration and stress[137]. Additionally, vegetation indices can be useful at smaller scales; they have been shown to be viable for monitoring water stress in greenhouse-grown tomato plants and even for early detection of salt stress (within 15 min of exposure) in *A. thaliana* seedlings before human visual detection[138]. Imaging has also been combined with machine-learning approaches to analyse stress; image and spectral data can be fed into algorithms that classify plants into, for example, 'diseased' or 'healthy' through pattern recognition[139,140]. Deep learning, a subset of machine learning, has been used to classify stresses from large image datasets in several studies, such as for the identification of different diseases in cucumber plants and estimation of stress severity in arabica coffee leaves[139,141,142]. Accuracy for predicting disease was generally between 93% and 96%, with prediction of disease severity being lower at around 86%, although these experiments did not predict real-time disease onset. Current challenges in stress detection include small learning dataset size of individual diseases and species, performance under non-standard lighting and, most importantly for real-time detection, high computational complexity, leading to slow detection speed[12].

On the individual plant to cellular scale, analysis of metabolites such as phytohormones, phenolics (a class of resins) and ROS can give insight into physiological processes — known as metabolomics[143,144]. As metabolomics requires a relatively complete chemical profile of the internal chemistry of the plant, traditional methods with low temporal frequency are typically used for analysis, including nuclear magnetic resonance spectroscopy, mass spectrometry, liquid chromatography and gas chromatography[134,145]. Metabolic fingerprinting involves the use of statistical techniques to find patterns or 'fingerprints' in the chemical composition of a (plant) sample[145,146]. Fingerprinting has been used to investigate abiotic stress in many crop plants and combined with

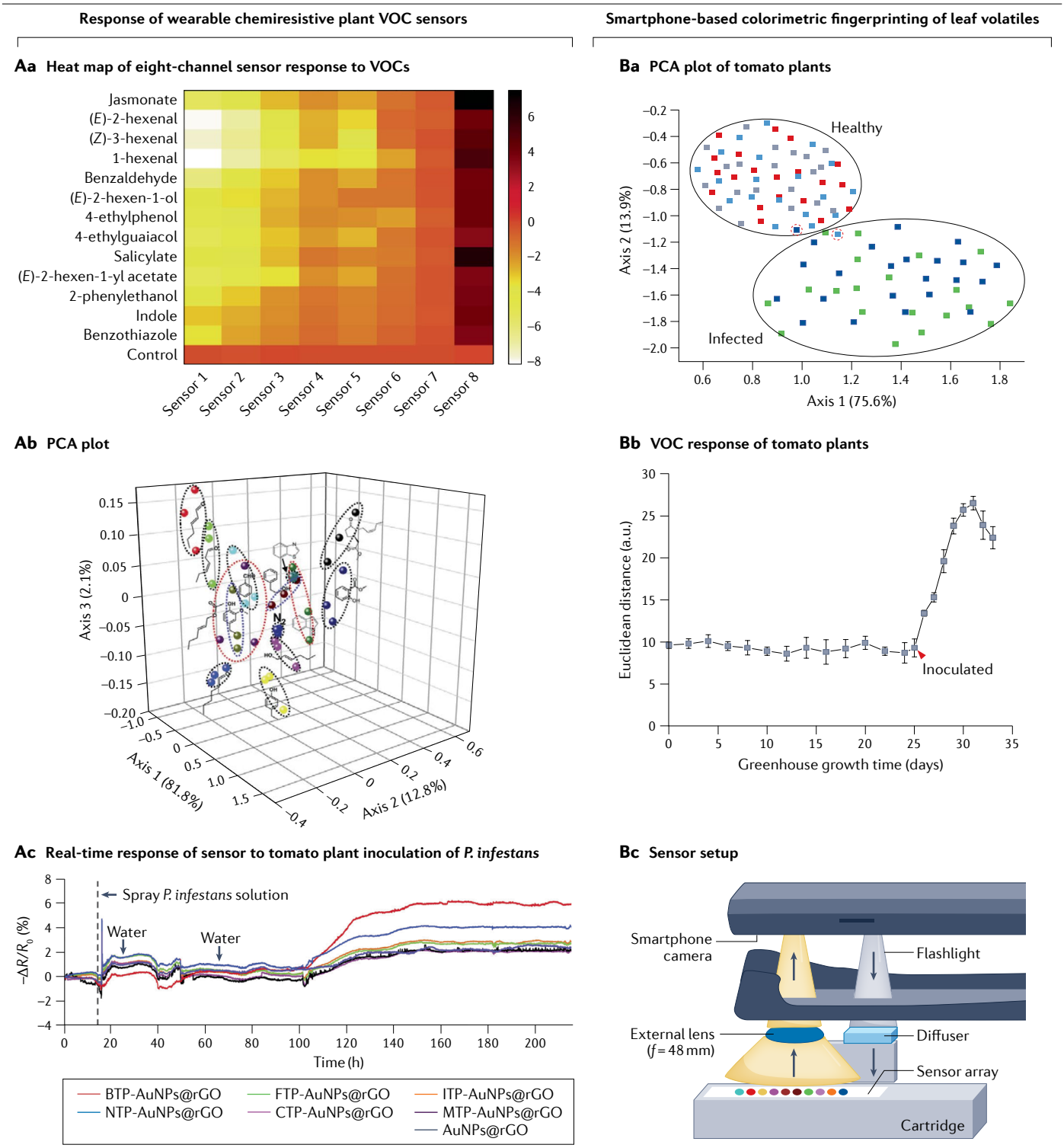


Fig. 7 | Modelling and analysis of plant stress. Aa, Heat map showing the response of an eight-channel sensor to 13 plant volatile organic compounds (VOCs) and N_2 control. **Ab**, The corresponding principal component analysis (PCA) plot. **Ac**, Real-time response curves of the sensor array to inoculation of tomato plants with *Phytophthora infestans*. **B**, Smartphone-based colorimetric sensor for VOC analysis. **Ba**, PCA plot of tomato leaves: red, healthy control; cyan, laboratory healthy; green, laboratory diseased; violet, field healthy; blue, field diseased. The dashed red circles indicate misdiagnosed samples. **Bb**, VOC response curves of tomato plants monitored in greenhouse. **Bc**, Sensor setup. AuNP, gold nanoparticle; rGO, reduced graphene oxide. Part **A** reprinted with permission from ref.[81], Elsevier. Part **B** adapted from ref.[151], Springer Nature Limited.

Glossary

Avirulent
Not capable of causing disease.

Bioimpedance spectroscopy
A non-invasive electrochemical spectroscopic technique for the measurement of electrical impedance of biological samples.

Effector-triggered immunity
(ETI). A stronger immune response triggered upon detection of effector proteins released by the pathogen.

Electrical impedance
The opposition to electrical flow.

Electrodic technique
A technique measuring properties at the electrode–electrolyte interface.

Genetic transformations
Insertion and incorporation of exogenous genetic material into a host organism.

Oomycetes
Fungus-like filamentous microorganisms.

PAMP-triggered immunity
(PTI). The primary plant immunity response, triggered when PAMPs are detected by recognition receptors in plants.

Pathogen-associated molecular patterns
(PAMPs). Structural molecular components of pathogens that are recognized by receptors in plants, triggering an immune response.

Phloem
Living tissue that transports soluble organic compounds (especially sugars) produced during photosynthesis around the plant.

Protease inhibitors
Large variety of antiherbivore molecules (mostly proteins) that inhibit protease enzyme function to reduce herbivore digestion.

Stomata
Pores on the epidermis of leaves that control exchange of CO_2 and water vapour with the environment.

Stomatal aperture
The width of the pore size of a stoma as controlled by the two guard cells.

Synthetic-aperture radar
A remote imaging technique involving the transmission and reception of sequential electromagnetic waves by a device on a moving platform.

Virulent
Capable of causing disease.

Xylem
Vascular tissue that transports water and dissolved nutrients up from the roots to other organs.

machine learning to predict drought resistance in potato cultivars with high accuracy using a random forest model[147–150]. Due to the large number of chemical sensors required to build such a fingerprint, real-time measurement techniques have not yet been fully investigated internally in plants. However, both optical and electrochemical techniques have been used to detect and predict stress through fingerprinting of VOCs. Alongside the wearable VOC sensor discussed previously, which predicted infection of *P. infestans* (late blight) in a tomato plant by detecting a significant change in the VOC profile emitted from the plant (Fig. 7A), an optical VOC fingerprinting method has been investigated[81]: a smartphone-based system used an array of colorimetric sensors (chemo-responsive Au nanorods and organic dyes) and the camera of the phone to fingerprint VOC release. The visual appearance of the colorimetric sensors was able to be recorded continuously or on demand and the device was able to accurately detect and differentiate late blight in tomato leaves using unsupervised pattern recognition with an accuracy of 97.5%[151] (Fig. 7B). The device used the gas in the headspace from a container with a leaf sample, rather than the air surrounding a plant, but it is possible that the setup could be altered to better facilitate whole plants.

Challenges and outlook

Although a wide variety of chemical signals have been measured in whole plants, the field of continuous or real-time sensing of the chemistry of plants is still in its early years and has largely been limited to laboratory prototypes. Significant advances are, therefore, required to commercialize sensing technologies capable of capturing chemical data with high temporal resolution, especially at the level of an individual plant under field conditions. Challenges in continuous chemical sensing technologies are common between plants and medicine, where these include sensor selectivity and sensitivity, system integration, ease of use and high-volume manufacturability[92,94,95]; these challenges will need to be addressed to move plant sensors up the technology readiness level.

Electrochemical devices for continuous monitoring of chemical signals in plants have additional inherent challenges that currently prevent their translation into commercial products[152]. For sensing devices that require direct contact with the plant, one of the main concerns is the location of the sensors. Many parts of plants (such as the leaves), smaller plant species and seedlings are fragile and may not be able to carry the weight of a sensor module, where wounding by excessive weight could lead to stress response[81,153,154]. To address this, either the sensors must be miniaturized by decoupling the electrodes from the rest of the measurement unit or placed in alternative locations, such as the roots. Insertable sensors also wound the tissue, leading to a temporary stress response that introduces chemical noise to the measurement, and the measurement system must therefore be stable enough to monitor chemical signals beyond this initial response[30,81,155]. Electrode geometries that reduce cell damage in plants may also need to be further explored; for example, microneedle-type sensors used in medical diagnostics have optimized needle geometries to push cells away without killing them during insertion[156]. Furthermore, in situ fabrication of organic electrodes within the plants may also be a viable approach for continuous monitoring of chemical signals[157]. The impact of in situ fabrication of electrodes on chemical signalling is not yet fully known, however. Minimally invasive advances successfully implemented in animals might also be adopted in plants, such as injectable nanoelectronic devices to monitor stress signalling molecules such as Ca^{2+} and ROS[10,158]. Injectable sensors can be combined with highly size-scalable passive wireless technologies (such as near-field communication technology) to continuously collect chemical data within the plants without wires[159]. Biorecognition elements, such as enzymes and antibodies, can denature over time, leading to reduced signal intensity, loss of selectivity and errors in sensor response. Such sensing elements may be inappropriate for use in real-time detection when placed in constant contact with a plant organ[75]. Biomimetic materials may provide a suitable solution however, where they attempt to replicate enzymatic behaviour through

the use of surface atoms (nanozymes) or intricate structures with cavities and functional groups (synzymes). While these materials often have superior kinetic performance (reaction rates) compared to their natural counterparts, their current shortcomings include often poor selectivity and specificity[160]. Another issue with electrochemical sensors is that sensor drift can occur due to water layer formation between the ISM and electrode material or fouling of the electrode through electro-oxidation and deposition of undesirable compounds[161–163]. Encapsulation or protection of the electrode with a material, such as Nafion (a sulfonated-tetrafluoroethylene-based fluoropolymer-copolymer), can protect electrodes from chemical attack whilst allowing detection of ionic analytes[164].

Optical techniques for continuous monitoring that require the addition of nanomaterials or genetically encoded sensors are already widely used in research, and further developments into non-species-specific techniques (for example, DNA transfer via SWCNTs) and sensors for new analytes will improve our understanding of chemical signalling in plants[66]. These techniques may even cross over into agriculture through genetically encoded sensors that induce change in plant pigments rather than fluorescence, negating the requirement for specialist equipment and enabling continuous visual analysis by typical camera equipment or the naked eye[165,166]. Satellite imagery is becoming more commercially available for agriculture, with an increasing number of private companies offering imaging with spatial resolution down to 30 cm and temporal frequency of 'a few hours or less'[70].

One of the most exciting possibilities concerning the continuous monitoring of chemical signals in plants is that it will likely lead to the discovery of new biological processes and pathways. These discoveries will eventually lead to the development of more robust and high-yielding varieties of crops or improved agricultural practices. The possibility of studying systemic signalling across entire whole plants (including root to shoot) with high spatiotemporal resolution will especially deepen our understanding of stress responses in plants that cannot be captured with current traditional, analytical methods with low temporal frequency[8,30,167]. Simultaneous measurement of both plant and soil properties could also elucidate how the condition of the growth medium affects plant signalling and physiology. Beyond basic research in the laboratory, continuous monitoring of chemical signals in crops has incredible potential for optimizing the use of agricultural inputs (such as water, fertilizer, pesticides), managing soil health and early detection of diseases or other stressors. Distributed networks of Internet of Things sensors capable of continuous monitoring of chemical signals within plants will also prevent blanket treatment of farmlands and allow precision agriculture at an unprecedented scale[83]. These new technologies, when combined with advanced analytical techniques such as artificial intelligence, can reduce the environmental impact of agriculture while increasing its economic viability. Reduced human intervention, increased automation and improved productivity will eventually lead to lower cost of production of food with a smaller environmental footprint, important for making food more equitable for the growing population.

Published online: 12 December 2022

References

1. Geilfus, C. M. The pH of the apoplast: dynamic factor with functional impact under stress. *Mol. Plant* **10**, 1371–1386 (2017).
2. Zhang, J. & Zhou, J.-M. Plant immunity triggered by microbial molecular signatures. *Mol. Plant* **3**, 783–793 (2010).
3. Chisholm, S. T., Coaker, G., Day, B. & Staskawicz, B. J. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* **124**, 803–814 (2006).
4. Lam, E., Kato, N. & Lawton, M. Programmed cell death, mitochondria and the plant hypersensitive response. *Nature* **411**, 848–853 (2001).
5. Felle, H. H., Waller, F., Molitor, A. & Kogel, K. H. The mycorrhiza fungus *Piriformospora indica* induces fast root-surface pH signaling and primes systemic alkalinization of the leaf apoplast upon powdery mildew infection. *Mol. Plant Microbe Interact.* **22**, 1179–1185 (2009).
6. Hussain, M., Malik, M. A., Farooq, M., Ashraf, M. Y. & Cheema, M. A. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* **194**, 193–199 (2008).
7. Munné-Bosch, S. & Peñuelas, J. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* **217**, 758–766 (2003).
8. Fichman, Y., Miller, G. & Mittler, R. Whole-plant live imaging of reactive oxygen species. *Mol. Plant* **12**, 1203–1210 (2019).
9. Albert, M. & Fürst, U. in *Plant Receptor Kinases. Methods in Molecular Biology* Vol. 1621 (ed. Aalen, R.) 69–76 (Humana, 2017).
10. Giraldo, J. P., Wu, H., Newkirk, G. M. & Kruss, S. Nanobiotechnology approaches for engineering smart plant sensors. *Nat. Nanotechnol.* **14**, 541–553 (2019).
11. Hill, O. The ultimate guide to crop disease and pest forecasting tools. *Farmers Weekly* https://www.fwi.co.uk/arable/find-latest-crop-disease-pest-forecasts-season (2016).
12. Liu, J. & Wang, X. Plant diseases and pests detection based on deep learning: a review. *Plant Methods* **17**, 22 (2021).
13. Harakannanavar, S. S., Rudagi, J. M., Puranikmath, V. I., Siddiqua, A. & Pramodhini, R. Plant leaf disease detection using computer vision and machine learning algorithms. *Glob. Transit. Proc.* **3**, 305–310 (2022).
14. Mohanty, S. P., Hughes, D. P. & Salathé, M. Using deep learning for image-based plant disease detection. *Front. Plant Sci.* **7**, 1419 (2016).
15. Flynn, P. Biotic vs. abiotic - distinguishing disease problems. *Iowa State University* https://hortnews.extension.iastate.edu/biotic-vs-abiotic-distinguishing-disease-problems (2003).
16. Basu, S., Varsani, S. & Louis, J. Altering plant defenses: herbivore-associated molecular patterns and effector arsenal of chewing herbivores. *Mol. Plant Microbe Interact.* **31**, 13–21 (2018).
17. Bi, G. et al. The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. *Cell* **184**, 3528–3541.e12 (2021).
18. Ngou, B. P. M., Ahn, H. K., Ding, P. & Jones, J. D. G. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* **592**, 110–115 (2021).
19. Barker, A. V & Pilbeam, D. J. *Handbook of Plant Nutrition* (CRC, 2015).
20. John Cram, B. W. et al. Handbook of reference methods for plant analysis. *Crop Sci.* **38**, 1710–1711 (1998).
21. Crawford, N. M. Nitrate: nutrient and signal for plant growth. *Plant Cell* **7**, 859–868 (1995).
22. Naveed, Z. A., Wei, X., Chen, J., Mubeen, H. & Ali, G. S. The PTI to ETI continuum in *Phytophthora*-plant interactions. *Front. Plant Sci.* **11**, 593905 (2020).
23. Flowers, T. J. & Colmer, T. D. Salinity tolerance in halophytes. *New Phytol.* **179**, 945–963 (2008).
24. He, M., He, C.-Q. & Ding, N.-Z. Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. *Front. Plant Sci.* **9**, 1771 (2018).
25. Colmenero-Flores, J. M., Franco-Navarro, J. D., Cubero-Font, P., Peinado-Torrubia, P. & Rosales, M. A. Chloride as a beneficial macronutrient in higher plants: new roles and regulation. *Int. J. Mol. Sci.* **20**, 4686 (2019).
26. Broyer, T. C., Carlton, A. B., Johnson, C. M. & Stout, P. R. Chlorine — a micronutrient element for higher plants. *Plant Physiol.* **29**, 526–532 (1954).
27. Yuan, P., Yang, T. & Poovaiah, B. W. Calcium signaling-mediated plant response to cold stress. *Int. J. Mol. Sci.* **19**, 3896 (2018).
28. Tuteja, N. & Mahajan, S. Calcium signaling network in plants: an overview. *Plant Signal. Behav.* **2**, 79–85 (2007).
29. Muday, G. K. & Brown-Harding, H. Nervous system-like signaling in plant defense. *Science* **361**, 1068–1069 (2018).
30. Toyota, M. et al. Glutamate triggers long-distance, calcium-based plant defense signaling. *Science* **361**, 1112–1115 (2018).
Revelation of a hormone-like role for glutamate in long-distance, time-dependent calcium signalling resulting from herbivore feeding and mechanical wounding.
31. Hanstein, S., de Beer, D. & Felle, H. H. Miniaturised carbon dioxide sensor designed for measurements within plant leaves. *Sens. Actuators B Chem.* **81**, 107–114 (2001).
Measurement of CO_2 inside the stomatal pore enabled using a miniaturized glass-capillary electrochemical sensor.
32. Hu, S., Ding, Y. & Zhu, C. Sensitivity and responses of chloroplasts to heat stress in plants. *Front. Plant Sci.* **11**, 375 (2020).
33. Im, H., Lee, S., Naqi, M., Lee, C. & Kim, S. Flexible PI-based plant drought stress sensor for real-time monitoring system in smart farm. *Electronics* **7**, 114 (2018).
34. Ali, M., Cheng, Z., Ahmad, H. & Hayat, S. Reactive oxygen species (ROS) as defenses against a broad range of plant fungal infections and case study on ROS employed by crops against *Verticillium dahliae* wilts. *J. Plant Interact.* **13**, 353–363 (2018).
35. Torres, M. A., Jones, J. D. G. & Dangl, J. L. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* **141**, 373–378 (2006).
36. Lamb, C. & Dixon, R. A. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 251–275 (1997).
37. Xu, Q. et al. In vivo monitoring of oxidative burst induced by ultraviolet A and C stress for oilseed rape by microbiosensor. *Sens. Actuators B Chem.* **141**, 599–603 (2009).

38. Ding, Y., Shi, Y. & Yang, S. Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. *New Phytol.* **222**, 1690–1704 (2019).
39. Iovine, N. M. et al. Reactive nitrogen species contribute to innate host defense against *Campylobacter jejuni*. *Infect. Immun.* **76**, 986–993 (2008).
40. Pauly, N. et al. Reactive oxygen and nitrogen species and glutathione: key players in the legume–*Rhizobium* symbiosis. *J. Exp. Bot.* **57**, 1769–1776 (2006).
41. Parankusam, S., Adimulam, S. S., Bhatnagar-Mathur, P. & Sharma, K. K. Nitric oxide (NO) in plant heat stress tolerance: current knowledge and perspectives. *Front. Plant Sci.* **8**, 1582 (2017).
42. Šimura, J. et al. Plant hormonomics: multiple phytohormone profiling by targeted metabolomics. *Plant Physiol.* **177**, 476–489 (2018).
43. Park, J., Lee, Y., Martinoia, E. & Geisler, M. Plant hormone transporters: what we know and what we would like to know. *BMC Biol.* **15**, 93 (2017).
44. Shah, J. & Zeier, J. Long-distance communication and signal amplification in systemic acquired resistance. *Front. Plant Sci.* **4**, 30 (2013).
45. McConn, M., Creelman, R. A., Bell, E., Mullet, J. E. & Browse, J. Jasmonate is essential for insect defense in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **94**, 5473–5477 (1997).
46. Snoeren, T. A. L. et al. The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. *J. Chem. Ecol.* **36**, 479–489 (2010).
47. Fong, D., Luo, S.-X., Andre, R. S. & Swager, T. M. Trace ethylene sensing via Wacker oxidation. *ACS Cent. Sci.* **6**, 507–512 (2020).
48. Dudareva, N., Negre, F., Nagegowda, D. A. & Orlova, I. Plant volatiles: recent advances and future perspectives. *Crit. Rev. Plant Sci.* **25**, 417–440 (2006).
49. Raghava, T., Ravikumar, P., Hegde, R. & Kush, A. Spatial and temporal volatile organic compound response of select tomato cultivars to herbivory and mechanical injury. *Plant Sci.* **179**, 520–526 (2010).
50. Taiz, L., Zeiger, E., Moller, I. M. & Mirphy, A. *Plant Physiology and Development* (Sinauer Associates, 2015).
51. Farmer, E. E. & Ryan, C. A. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc. Natl Acad. Sci. USA* **87**, 7713–7716 (1990).
52. Altangerel, N. et al. In vivo diagnostics of early abiotic plant stress response via Raman spectroscopy. *Proc. Natl Acad. Sci. USA* **114**, 3393–3396 (2017).
53. Chalker-Scott, L. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* **70**, 1–9 (1999).
54. Kovinich, N. et al. Not all anthocyanins are born equal: distinct patterns induced by stress in *Arabidopsis*. *Planta* **240**, 931–940 (2014).
55. Wang, M. et al. Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. *Nat. Plants* **2**, 16151 (2016).
56. Kim, G., LeBlanc, M. L., Wafula, E. K., DePamphilis, C. W. & Westwood, J. H. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. *Science* **345**, 808–811 (2014).
57. Chen, Y. et al. An aphid RNA transcript migrates systemically within plants and is a virulence factor. *Proc. Natl Acad. Sci. USA* **117**, 12763–12771 (2020).
58. Qiao, Y. et al. Oomycete pathogens encode RNA silencing suppressors. *Nat. Genet.* **45**, 330–333 (2013).
59. Hou, Y. et al. A *Phytophthora* effector suppresses trans-kingdom RNAi to promote disease susceptibility. *Cell Host Microbe* **25**, 153–165.e5 (2019).
60. Yin, C. et al. A novel fungal effector from *Puccinia graminis* suppressing RNA silencing and plant defense responses. *New Phytol.* **222**, 1561–1572 (2019).
61. Moroz, N. et al. Extracellular alkalinization as a defense response in potato cells. *Front. Plant Sci.* **8**, 32 (2017).
62. González-Sánchez, M. I. et al. Electrochemical detection of extracellular hydrogen peroxide in *Arabidopsis thaliana*: a real-time marker of oxidative stress. *Plant Cell Environ.* **36**, 869–878 (2013).
63. Roper, J. M., Garcia, J. F. & Tsutsui, H. Emerging technologies for monitoring plant health in vivo. *ACS Omega* **6**, 5101–5107 (2021).
64. Schornack, S. et al. Ancient class of translocated oomycete effectors targets the host nucleus. *Proc. Natl Acad. Sci. USA* **107**, 17421–17426 (2010).
65. Kikkert, J. R., Vidal, J. R. & Reisch, B. I. in *Transgenic Plants: Methods and Protocols* (ed. Peña, L.) 61–78 (Humana, 2004).
66. Wong, M. H. et al. Lipid exchange envelope penetration (LEEP) of nanoparticles for plant engineering: a universal localization mechanism. *Nano Lett.* **16**, 1161–1172 (2016).
67. Gupta, S. et al. Portable Raman leaf-clip sensor for rapid detection of plant stress. *Sci. Rep.* **10**, 20206 (2020).
A portable, handheld Raman sensor designed to clip onto leaves was used for rapid detection of nutrient deficiency in a range of crops.
68. Montanha, G. S. et al. X-ray fluorescence spectroscopy (XRF) applied to plant science: challenges towards in vivo analysis of plants. *Metallomics* **12**, 183–192 (2020).
69. Sadoine, M. et al. Designs, applications, and limitations of genetically encoded fluorescent sensors to explore plant biology. *Plant Physiol.* **187**, 485–503 (2021).
70. Dakir, A., Zahra, B. F. & Omar, A. B. Optical satellite images services for precision agricultural use: a review. *Adv. Sci. Technol. Eng. Syst. J.* **6**, 326–331 (2021).
71. Ruwanpathirana, G. P. et al. Continuous monitoring of plant sodium transport dynamics using clinical PET. *Plant Methods* **17**, 8 (2021).
PET enables continuous, whole-plant, 3D visualization of long-distance Na^+ transport dynamics.
72. Sophocleous, M. & Atkinson, J. K. A review of screen-printed silver/silver chloride (Ag/AgCl) reference electrodes potentially suitable for environmental potentiometric sensors. *Sens. Actuators A Phys.* **267**, 106–120 (2017).
73. Inzelt, G. in *Handbook of Reference Electrodes* (eds Inzelt, G., Lewenstam, A. & Scholz, F.) 331–332 (Springer, 2013).
74. Waleed Shinwari, M. et al. Microfabricated reference electrodes and their biosensing applications. *Sensors* **10**, 1679–1715 (2010).
75. Morales, M. A. & Halpern, J. M. Guide to selecting a biorecognition element for biosensors. *Bioconjug. Chem.* **29**, 3231–3239 (2018).
76. Westbroek, P. in *Analytical Electrochemistry in Textiles* (eds Westbroek, P., Priniotakis, G. & Kiekens, P.) 3–36 (2005).
77. Søpstad, S., Johannessen, E. A. & Imenes, K. Analytical errors in biosensors employing combined counter/pseudo-reference electrodes. *Results Chem.* **2**, 100028 (2020).
78. Cao, S. et al. ISFET-based sensors for (bio)chemical applications: a review. *Electrochem. Sci. Adv.* https://doi.org/10.1002/ELSA.202100207 (2022).
79. Srinivasan, P., Ezhilan, M., Kulandaisamy, A. J., Babu, K. J. & Rayappan, J. B. B. Room temperature chemiresistive gas sensors: challenges and strategies — a mini review. *J. Mater. Sci. Mater. Electron.* **30**, 15825–15847 (2019).
80. Esser, B., Schnorr, J. M. & Swager, T. M. Selective detection of ethylene gas using carbon nanotube-based devices: utility in determination of fruit ripeness. *Angew. Chem. Int. Ed.* **51**, 5752–5756 (2012).
81. Li, Z. et al. Real-time monitoring of plant stresses via chemiresistive profiling of leaf volatiles by a wearable sensor. *Matter* **4**, 2553–2570 (2021).
Kirigami-inspired, lightweight, wearable, e-nose-style sensor for the detection and prediction of pathogenic infection by VOC release.
82. Cui, S., Ling, P., Zhu, H. & Keener, H. M. Plant pest detection using an artificial nose system: a review. *Sensors* **18**, 378 (2018).
83. Yin, H. et al. Soil sensors and plant wearables for smart and precision agriculture. *Adv. Mater.* **33**, 2007764 (2021).
84. Calisgan, S. D. et al. Micromechanical switch-based zero-power chemical detectors for plant health monitoring. *J. Microelectromech. Syst.* **29**, 755–761 (2020).
85. Felle, H. Proton transport and pH control in *Sinapis alba* root hairs: a study carried out with double-barrelled pH micro-electrodes. *J. Exp. Bot.* **38**, 340–354 (1987).
86. Felle, H. H. The apoplastic pH of the *Zea mays* root cortex as measured with pH-sensitive microelectrodes: aspects of regulation. *J. Exp. Bot.* **49**, 987–995 (1998).
87. Izumi, R. et al. in *Proc. 2017 IEEE Sensors* (IEEE, 2017).
88. Martinière, A., Desbrosses, G., Sentenac, H. & Paris, N. Development and properties of genetically encoded pH sensors in plants. *Front. Plant Sci.* **4**, 523 (2013).
89. Gjetting, S. K., Ytting, C. K., Schulz, A. & Fuglsang, A. T. Live imaging of intra- and extracellular pH in plants using pHusion, a novel genetically encoded biosensor. *J. Exp. Bot.* **63**, 3207–3218 (2012).
90. Tantama, M., Hung, Y. P. & Yellen, G. Imaging intracellular pH in live cells with a genetically encoded red fluorescent protein sensor. *J. Am. Chem. Soc.* **133**, 10034–10037 (2011).
91. Fujimaki, S. et al. Base to tip and long-distance transport of sodium in the root of common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] at steady state under constant high-salt conditions. *Plant Cell Physiol.* **56**, 943–950 (2015).
92. Nyein, H. Y. Y. et al. A wearable electrochemical platform for noninvasive simultaneous monitoring of Ca^{2+} and pH. *ACS Nano* **10**, 7216–7224 (2016).
93. Keene, S. T. et al. Wearable organic electrochemical transistor patch for multiplexed sensing of calcium and ammonium ions from human perspiration. *Adv. Healthc. Mater.* **8**, 1901321 (2019).
94. Pirovano, P. et al. A wearable sensor for the detection of sodium and potassium in human sweat during exercise. *Talanta* **219**, 121145 (2020).
95. Gao, W. et al. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. *Nature* **529**, 509–514 (2016).
96. Bandodkar, A. J. et al. Epidermal tattoo potentiometric sodium sensors with wireless signal transduction for continuous non-invasive sweat monitoring. *Biosens. Bioelectron.* **54**, 603–609 (2014).
97. Schazmann, B. et al. A wearable electrochemical sensor for the real-time measurement of sweat sodium concentration. *Anal. Methods* **2**, 342–348 (2010).
98. Garcia-Cordero, E. et al. Three-dimensional integrated ultra-low-volume passive microfluidics with ion-sensitive field-effect transistors for multiparameter wearable sweat analyzers. *ACS Nano* **12**, 12646–12656 (2018).
99. Parrilla, M. et al. Wearable potentiometric ion patch for on-body electrolyte monitoring in sweat: toward a validation strategy to ensure physiological relevance. *Anal. Chem.* **91**, 8644–8651 (2019).
100. Demuru, S., Kunnel, B. P. & Briand, D. Real-time multi-ion detection in the sweat concentration range enabled by flexible, printed, and microfluidics-integrated organic transistor arrays. *Adv. Mater. Technol.* **5**, 2000328 (2020).
101. Zhai, Q. et al. Vertically aligned gold nanowires as stretchable and wearable epidermal ion-selective electrode for noninvasive multiplexed sweat analysis. *Anal. Chem.* **92**, 4647–4655 (2020).
102. Parrilla, M. et al. A textile-based stretchable multi-ion potentiometric sensor. *Adv. Healthc. Mater.* **5**, 996–1001 (2016).

103. Kim, M.-Y. et al. Highly stable potentiometric sensor with reduced graphene oxide aerogel as a solid contact for detection of nitrate and calcium ions. *J. Electroanal. Chem.* **897**, 115553 (2021).
104. Jiao, Y. et al. in *Int. Conf. Solid-State Sensors Actuators Microsystems Eurosensors XXXIII* 37–40 (IEEE, 2019).
105. Lu, Y. et al. Multimodal plant healthcare flexible sensor system. *ACS Nano* **14**, 10966–10975 (2020).
106. Oren, S., Ceylan, H., Schnable, P. S. & Dong, L. High-resolution patterning and transferring of graphene-based nanomaterials onto tape toward roll-to-roll production of tape-based wearable sensors. *Adv. Mater. Technol.* **2**, 1700223 (2017).
107. Kim, J. J., Allison, L. K. & Andrew, T. L. Vapor-printed polymer electrodes for long-term, on-demand health monitoring. *Sci. Adv.* **5**, eaaw0463 (2019).
108. Lee, K. et al. In-situ synthesis of carbon nanotube–graphite electronic devices and their integrations onto surfaces of live plants and insects. *Nano Lett.* **14**, 2647–2654 (2014).
Direct application of electrochemical sensors onto the leaf surface for the detection of gaseous analytes.
109. Koman, V. B. et al. Persistent drought monitoring using a microfluidic-printed electro-mechanical sensor of stomata: in planta. *Lab Chip* **17**, 4015–4024 (2017).
110. Chauhan, S., Srivastava, H. S. & Patel, P. Wheat crop biophysical parameters retrieval using hybrid-polarized RISAT-1 SAR data. *Remote Sens. Environ.* **216**, 28–43 (2018).
111. Grimnes, S. & Martinsen, Ø. G. *Bioimpedance and Bioelectricity Basics* 179–254 (Elsevier, 2015).
112. Xu, Q. et al. Microsensor *in vivo* monitoring of oxidative burst in oilseed rape (*Brassica napus* L.) leaves infected by *Sclerotinia sclerotiorum*. *Anal. Chim. Acta* **632**, 21–25 (2009).
Insertable Pt-based sensor for detecting successive bursts of ROS in plants with fungal infection.
113. Tangkuaram, T., Ponchio, C., Kangkasomboon, T., Katikawong, P. & Veerasai, W. Design and development of a highly stable hydrogen peroxide biosensor on screen printed carbon electrode based on horseradish peroxidase bound with gold nanoparticles in the matrix of chitosan. *Biosens. Bioelectron.* **22**, 2071–2078 (2007).
114. Lu, S.-Y., Chen, Y., Fang, X. & Feng, X. Hydrogen peroxide sensor based on electrodeposited Prussian blue film. *J. Appl. Electrochem.* **47**, 1261–1271 (2017).
115. Niemeyer, J., Scheuring, D., Oestreicher, J., Morgan, B. & Schroda, M. Real-time monitoring of subcellular H_2O_2 distribution in *Chlamydomonas reinhardtii*. *Plant Cell* **33**, 2935–2949 (2021).
116. Hernández-Barrera, A. et al. Using hyper as a molecular probe to visualize hydrogen peroxide in living plant cells: a method with virtually unlimited potential in plant biology. *Methods Enzymol.* **527**, 275–290 (2013).
117. Ugalde, J. M., Schlößer, M., Dongois, A., Martinière, A. & Meyer, A. J. The latest HyPe(r) in plant H_2O_2 biosensing. *Plant Physiol.* **187**, 480–484 (2021).
118. Lew, T. T. S. et al. Real-time detection of wound-induced H_2O_2 signalling waves in plants with optical nanosensors. *Nat. Plants* **6**, 404–415 (2020).
119. Giraldo, J. P. et al. Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nat. Mater.* **13**, 400–408 (2014).
120. Giraldo, J. P. et al. A ratiometric sensor using single chirality near-infrared fluorescent carbon nanotubes: application to in vivo monitoring. *Small* **11**, 3973–3984 (2015).
121. Gan, T., Hu, C., Chen, Z. & Hu, S. A disposable electrochemical sensor for the determination of indole-3-acetic acid based on poly(safranine T)-reduced graphene oxide nanocomposite. *Talanta* **85**, 310–316 (2011).
122. Li, H. et al. A highly sensitive electrochemical impedance immunosensor for indole-3-acetic acid and its determination in sunflowers under salt stress. *RSC Adv.* **7**, 54416–54421 (2017).
123. Sun, L. J. et al. Paper-based electroanalytical devices for in situ determination of salicylic acid in living tomato leaves. *Biosens. Bioelectron.* **60**, 154–160 (2014).
124. Yang, L. et al. Ratiometric electrochemical sensor for accurate detection of salicylic acid in leaves of living plants. *RSC Adv.* **10**, 38841–38846 (2020).
125. Li, Y.-W., Xia, K., Wang, R.-Z., Jiang, J.-H. & Xiao, L.-T. An impedance immunosensor for the detection of the phytohormone abscisic acid. *Anal. Bioanal. Chem.* **391**, 2869–2874 (2008).
126. Brunoud, G. et al. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* **482**, 103–106 (2012).
127. Waadt, R. et al. FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis. *eLife* **3**, e01739 (2014).
128. Larrieu, A. et al. A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants. *Nat. Commun.* **6**, 6043 (2015).
129. Zhang, H. & Wang, J. Detection of age and insect damage incurred by wheat, with an electronic nose. *J. Stored Prod. Res.* **43**, 489–495 (2007).
130. Li, C., Krewer, G. & Kays, S. J. in *2009 Reno, Nevada, June 21–June 24* Vol. 8 5289–5301 (American Society of Agricultural and Biological Engineers, 2009).
131. Sankaran, S., Mishra, A., Ehsani, R. & Davis, C. A review of advanced techniques for detecting plant diseases. *Comput. Electron. Agric.* **72**, 1–13 (2010).
132. Baker, M. J., Hughes, C. S. & Hollywood, K. A. *Biophotonics: Vibrational Spectroscopic Diagnostics* Ch. 3 (Morgan & Claypool, 2016).
133. Nißler, R. et al. Detection and imaging of the plant pathogen response by near-infrared fluorescent polyphenol sensors. *Angew. Chem. Int. Ed.* **61**, e202108373 (2022).
134. Galieni, A. et al. Past and future of plant stress detection: an overview from remote sensing to positron emission tomography. *Front. Plant Sci.* **11**, 609155 (2021).
135. Weiss, M., Jacob, F. & Duveiller, G. Remote sensing for agricultural applications: a meta-review. *Remote Sens. Environ.* **236**, 111402 (2020).
136. Wójtowicz, M., Wójtowicz, A. & Piekarczyk, J. Application of remote sensing methods in agriculture. *Commun. Biometry Crop Sci.* **11**, 31–50 (2016).
137. Huang, S., Tang, L., Hupy, J. P., Wang, Y. & Shao, G. A commentary review on the use of normalized difference vegetation index (NDVI) in the era of popular remote sensing. *J. For. Res.* **32**, 1–6 (2021).
138. Beisel, N. S. et al. Utilization of single-image normalized difference vegetation index (SI-NDVI) for early plant stress detection. *Appl. Plant Sci.* **6**, e01186 (2018).
139. Zubler, A. V. & Yoon, J. Y. Proximal methods for plant stress detection using optical sensors and machine learning. *Biosensors* **10**, 193 (2020).
140. Gao, Z., Luo, Z., Zhang, W., Lv, Z. & Xu, Y. Deep learning application in plant stress imaging: a review. *AgriEngineering* **2**, 430–446 (2020).
141. Ma, J. et al. A recognition method for cucumber diseases using leaf symptom images based on deep convolutional neural network. *Comput. Electron. Agric.* **154**, 18–24 (2018).
142. Esgario, J. G. M., Krohling, R. A. & Ventura, J. A. Deep learning for classification and severity estimation of coffee leaf biotic stress. *Comput. Electron. Agric.* **169**, 105162 (2020).
143. Pérez-Clemente, R. M. et al. Biotechnological approaches to study plant responses to stress. *BioMed. Res. Int.* **2013**, 654120 (2013).
144. Arbona, V., Manzi, M., Ollas, Cde & Gómez-Cadenas, A. Metabolomics as a tool to investigate abiotic stress tolerance in plants. *Int. J. Mol. Sci.* **14**, 4885–4911 (2013).
145. Shulaev, V., Cortes, D., Miller, G. & Mittler, R. Metabolomics for plant stress response. *Physiol. Plant* **132**, 199–208 (2008).
146. Krishnan, P., Kruger, N. J. & Ratcliffe, R. G. Metabolite fingerprinting and profiling in plants using NMR. *J. Exp. Bot.* **56**, 255–265 (2005).
147. Widodo, W. et al. Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J. Exp. Bot.* **60**, 4089–4103 (2009).
148. Michaletti, A., Naghavi, M. R., Toorchi, M., Zolla, L. & Rinalducci, S. Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. *Sci. Rep.* **8**, 5710 (2018).
149. Djoukeng, J. D., Arbona, V., Argamasilla, R. & Gomez-Cadenas, A. Flavonoid profiling in leaves of citrus genotypes under different environmental situations. *J. Agric. Food Chem.* **56**, 11087–11097 (2008).
150. Sprenger, H. et al. Metabolite and transcript markers for the prediction of potato drought tolerance. *Plant Biotechnol. J.* **16**, 939–950 (2018).
151. Li, Z. et al. Non-invasive plant disease diagnostics enabled by smartphone-based fingerprinting of leaf volatiles. *Nat. Plants* **5**, 856–866 (2019).
A colorimetric-based VOC fingerprinting smartphone sensing setup with diagnosis of infection through modelling.
152. Lee, G., Wei, Q. & Zhu, Y. Emerging wearable sensors for plant health monitoring. *Adv. Funct. Mater.* **31**, 2106475 (2021).
153. Nassar, J. M. et al. Compliant plant wearables for localized microclimate and plant growth monitoring. *npj Flex. Electron.* **2**, 24 (2018).
154. Tang, W., Yan, T., Ping, J., Wu, J. & Ying, Y. Rapid fabrication of flexible and stretchable strain sensor by chitosan-based water ink for plants growth monitoring. *Adv. Mater. Technol.* **2**, 1700021 (2017).
155. Diacci, C. et al. Diurnal *in vivo* xylem sap glucose and sucrose monitoring using implantable organic electrochemical transistor sensors. *iScience* **24**, 101966 (2021).
156. Rawson, T. M. et al. Microneedle biosensors for real-time, minimally invasive drug monitoring of phenoxymethylpenicillin: a first-in-human evaluation in healthy volunteers. *Lancet Digit. Health* **1**, e335–e343 (2019).
157. Stavrinidou, E. et al. In vivo polymerization and manufacturing of wires and supercapacitors in plants. *Proc. Natl Acad. Sci. USA* **114**, 2807–2812 (2017).
158. Liu, J. et al. Syringe-injectable electronics. *Nat. Nanotechnol.* **10**, 629–636 (2015).
159. Olenik, S., Lee, H. S. & Güder, F. The future of near-field communication-based wireless sensing. *Nat. Rev. Mater.* **6**, 286–288 (2021).
160. Romanholo, P. V. V. et al. Biomimetic electrochemical sensors: new horizons and challenges in biosensing applications. *Biosens. Bioelectron.* **185**, 113242 (2021).
161. Li, B. et al. Toward long-term accurate and continuous monitoring of nitrate in wastewater using poly(tetrafluoroethylene) (PTFE)–solid-state ion-selective electrodes (S-ISEs). *ACS Sens.* **5**, 3182–3193 (2020).
162. Liu, X., You, S., Ma, F. & Zhou, H. Characterization of electrode fouling during electrochemical oxidation of phenolic pollutant. *Front. Environ. Sci. Eng.* **15**, 53 (2021).
163. Hanssen, B. L., Siraj, S. & Wong, D. K. Y. Recent strategies to minimise fouling in electrochemical detection systems. *Rev. Anal. Chem.* **35**, 1–28 (2016).
164. Kusoglu, A. & Weber, A. Z. New insights into perfluorinated sulfonic-acid ionomers. *Chem. Rev.* **117**, 987–1104 (2017).
165. Kwak, S. Y. et al. Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers. *Nat. Nanotechnol.* **14**, 447–455 (2019).

166. Demirer, G. S. et al. High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nat. Nanotechnol.* **14**, 456–464 (2019).
167. Voke, E., Pinals, R. L., Goh, N. S. & Landry, M. P. In planta nanosensors: understanding biocorona formation for functional design. *ACS Sens.* **6**, 2802–2814 (2021).
168. Xu, Y., Zhang, P., Liu, X., Wang, Z. & Li, S. Preparation and irreversible inhibition mechanism insight into a recombinant Kunitz trypsin inhibitor from *Glycine max* L. seeds. *Appl. Biochem. Biotechnol.* **191**, 1207–1222 (2020).

Acknowledgements

F.G. thanks the Bill and Melinda Gates Foundation (Grand Challenges Explorations scheme under grant number OPP1212574) and the US Army (U.S. Army Foreign Technology (and Science) Assessment Support programme under grant number W911QY-20-R-0022) for their generous support. L.G.-M. acknowledges the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 101025390. F.G., P.C. and A.S.P.C. thank EPSRC (EP/L016702/1) and BBSRC DTP (reference 2177734). T.B. acknowledges BBSRC (BB/T006102/1) for their support. F.G. and P.C. thank the Imperial College Centre for Processable Electronics (CPE). F.G. also acknowledges Agri Futures Lab.

Author contributions

P.C., L.G.-M. and F.G. conceived the structure of the manuscript. P.C. led the writing of the manuscript. All authors contributed to the writing and reviewed and agreed on the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to Philip Coatsworth or Firat Güder.

Peer review information *Nature Reviews Chemistry* thanks M. Teresa Fernández Abedul, Ramses V. Martinez and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.