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Next generation chemical priming: with a little help from our nanocarrier friends

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Plants are exposed to multiple threats linked to climate change which can cause critical yield losses. Therefore, designing novel crop management tools is crucial. Chemical priming has recently emerged as an effective technology for improving tolerance to stress factors. Several compounds such as phytohormones, reactive species, and synthetic chimeras have been identified as promising priming agents. Following remarkable developments in nanotechnology, several unique nanocarriers (NCs) have been engineered that can act as smart delivery systems. These provide an eco-friendly, next-generation method for chemical priming, leading to increased efficiency and reduced overall chemical usage. We review novel engineered NCs (NENCs) as vehicles for chemical agents in advanced priming strategies, and address challenges and opportunities to be met towards achieving sustainable agriculture.

Highlights

-Nanocarriers (NCs) functionalized with chemical agents represent a novel approach for improved priming efficiency through targeted delivery.

Multifunctional priming through the combined used of different agents in novel engineered nanocarriers (NENCs) has the potential to achieve multiple benefits in plants.

The application of NENCs as seed coatings has the potential to improve crop yields, while achieving maximum cost-effectiveness compared with application at the plant level as it requires less time and labor.

Gene-editing techniques can be used to modify the expression of targeted genes involved in plant priming as identified by transcriptomic approaches, and can enhance the ability of plants to respond to priming treatments and improve their overall performance.

Essentials of plant chemical priming in a nutshell

Due to their sessile lifestyle, plants, including both crop and non-crop species, are continuously challenged by multiple types of biotic and abiotic stresses throughout their life cycle. Plants may be exposed to stress episodes sequentially or simultaneously. Crucially, a combination of biotic or abiotic stresses may exacerbate the devastating effects on crop productivity compared with the individual effects of the stressors. Recent advances in plant stress physiology have focused the questions of plant biologists on how plants prepare themselves for the possible recurrence of a stress that has passed, and on the type of responses that are generated following recurrence of the same stress factor. Another critical concern has arisen about the responses of plants exposed to different stresses at different stages of their life cycle.

Among the approaches used to address environmental constraints, seed and seedling priming has been receiving an increasing degree of attention, as evidenced by ~1957 documents in the agricultural and biological sciences according to the SCOPUS database, and more than half of these works have been published in the past 5 years. The main philosophy of priming is to enhance the tolerance of plants to stress factors by using priming agents, and this is achieved by activating multiple defense-related pathways. Plants can be primed to better tolerate the stressors through modifications in primary and secondary metabolism. The remarkable effects of priming have been demonstrated across a range of crop and non-crop species. Priming can be initiated naturally following exposure to an environmental stress (also known as physiological priming or hardening), and it can also be achieved by exogenous treatment with biotic (organismal) and abiotic (nonorganismal) priming agents [1]. The latter most commonly involve chemical agents such as natural metabolites or synthetic chemical compounds, and present exciting opportunities for more effective use of plant priming in crop stress management [2].

Changes in osmoregulation, detoxification of reactive oxygen species (ROS), and protein and ion homeostasis mediated by chemical priming agents have been associated with acquired tolerance

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in plants. Morphogenetic responses and the release of biologically active compounds are the result of such metabolic changes. Among the priming agents used, sodium nitroprusside [3,4], hydrogen peroxide [5], sodium hydrosulfide [6,7], melatonin [8,9], polyamines [10,11], amino acids [12], and volatile organic compounds [13,14] have been shown to confer remarkable increases in stress tolerance. Recently, Antoniou *et al.* [15] demonstrated the potential of a newly synthesized aspirin variant (**NOSH-A**, see Glossary) as a highly promising priming agent. NOSH-A acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H_2S), and aspirin (acetylsalicylic acid) concurrently, and NOSH specifically donates NO and H_2S. The simultaneous donation of multiple signal/hormonal molecules by NOSH compounds makes them highly attractive candidates for priming agents through the concept of multifunctional priming whereby multiple benefits can be achieved through the synergistic activity of different agents.

Mode of action

Priming results in a modified physiological state that causes the plants to respond in a more robust way after they have been exposed to a stressor. Compared with nonprimed plants, such an acquired primed state manifests itself as faster and/or more efficient defense responses to the stressors, leading to improved plant phenotypes. To survive and reproduce, plants rely on highly sophisticated and elaborate systems to defend themselves. Priming triggers an array of complex biochemical and molecular changes via molecular signaling [2,16]. ROS, which act as second messengers in plant signaling pathways, accumulate during eustress and/or distress [16]. The acquisition of tolerance to stressors also depends on signaling by calcium ions which act as a second messengers. Ca^{2+} is a key cellular component that plays a role in coordinating cellular signaling responses to environmental stimuli [17]. Such signaling molecules can then trigger the induction of defense-related genes. Furthermore, enhancement of plant tolerance through the exogenous application of NO, H_2S, and H_2O_2 involves the regulation of various defense-related genes. The expression of these genes is not only influenced before the imposition of abiotic stress but is also increased during stress conditions. These include not only genes encoding proteins directly engaged in safeguarding plants from stress but also genes related to gene regulation, such as transcription factor (TF) genes, and those involved in signal transduction pathways [18,19]. For instance, ethylene priming increased the expression levels of antioxidant enzymes and ethylene biosynthesis genes, resulting in tolerance to waterlogging stress [20]. Sucrose-mediated priming can induce the expression of specific genes involved in the synthesis of pathogenesis-related proteins (i.e., *PRs*) [21]. In addition to second messenger signaling and gene regulation, hormonal signaling is also a critical player involved in the primed state [22]. Fang *et al.* [22] reported that priming with salicylic acid (SA) increased the concentration of hormones such as SA, abscisic acid, and dihydrozeatin, while it decreased the levels of gibberellins GA_4 and GA_7. Such changes in hormonal status may act as signals to activate defense pathways and modulate subsequent responses of plants to stressors. Furthermore, induced epigenetic modifications are major contributors to maintaining the primed state in subsequent plant generations, and thus contribute to the concept of priming memory, discussed later.

Priming memory

Priming memory can be simply defined as the phenomenon whereby plants, including seeds and seedlings, are exposed to a mild stress event which in turn boosts their ability to cope with future stressors [23,24]. In other words, the ability to retain the information/experience of past stressors is referred to as the memory of primed seeds or seedlings. Both the memory of initial (priming) stress and the retrieval of stored information are essential when faced with subsequent stress, particularly when no stress occurs between the two stressful events [25]. This acquired ability primes plants to respond more quickly or strongly to recurrent stressors [26]. Such acquired information in the primed state can prime plants in the same generation and pass on the 'information' to the next generation [27].

Glossary

Nanocarriers (NCs): small, practical transporters that can change their physical properties such as charge and shape to deliver relevant chemicals to plant tissues.

Nanoparticles (NPs): particles that range in size from 1 to 100 nm and feature two or three exterior dimensions or internal surface structures. In contrast to their molecular counterparts, NPs exhibit unique physicochemical characteristics such as a high surface-to-volume ratio, an unusual surface structure, and increased reactivity. These characteristics are a result of NP cohesiveness, chemical composition, stability, surface structure, shape, and minor size. The capabilities of NPs can be time-controlled, target-specific, self-regulated, programmable, and multifunctional.

Nanoparticle-based smart delivery systems (NSDS): systems that communicate with various organs, tissues, cells, or chemicals as they move through plant tissues. Nanoparticle–biological (nano-bio) interactions relate to the interaction between man-made nanomaterials and a biological system.

Nanotubes (NTs): cylinder-shaped objects with sizes ranging from 1 to 100 nm.

NOSH-A: also known as NBS-1120, NOSH-A is a novel nitric oxide- and hydrogen sulfide-releasing hybrid, which was initially formulated as an anticancer drug but also displays protective effects against abiotic stress conditions in plants.

Novel engineered nanocarriers (NENCs): to effectively convey the substance, loaded NENCs are delivered to the target plant tissues. NENCs provide the opportunity for surface functionalization with targeting ligands. Consequently, NENCs may be made to release the materials they are laden with in a regulated manner to maintain the level of delivery to target areas for a longer time.

Facilitated quicker and stronger responses may be associated with changes in chromatin structure owing to DNA methylation and/or histone modifications [28,29]. As has been well reviewed by Rapp and Wendel [30], the term 'epigenetics' refers to a class of heritable molecular changes that do not involve alteration in the sequence of the DNA [31]. These epigenetic modifications generally cause critical changes in chromatin structure. They can contribute to stress memory and plant resistance to stressors by influencing the expression levels of relevant genes [29]. For example, Laura *et al.* [28] demonstrated epigenetic control of defense genes during methyl jasmonate (MeJA)-induced priming in rice (*Oryza sativa)*. In this work the expression levels of *OsBBPI* and *OsPOX*-like defense-related genes were upregulated by MeJA priming in response to wounding. Histone modifications (H3K9ac and H3K4me3) and DNA methylation-like epigenetic markers were also used to link gene upregulation to epigenetic regulation. The authors demonstrated a correlation between chromatin modifications and the level of expression of the *OsBBPI* gene. Following MeJa priming, acetylation and trimethylation of lysine residues in the N-terminus of histone H3 in the promoter region of the *OsBBPI* gene were observed in rice leaves in response to wounding. In addition, critical changes in genome-wide DNA methylation were modulated by MeJa priming upon wounding. Similarly, Kim *et al.* [32] reported that exogenously applied acetic acid promoted *de novo* jasmonic acid (JA) synthesis and histone H4 acetylation which primes the JA signaling pathway toward increased tolerance to drought stress. The priming process initiates a phase of stress memory. This involves a modified transcriptional regulatory event in which the priming stimulus induces lasting alterations in gene expression or a changed transcriptional reaction to a subsequent stimulus – indicative of memory. The discovery of the role of chromatin changes in stress priming can be traced back to a study investigating the impact of a secondary exposure to bacterial pathogens in relation to systemic acquired resistance (SAR) responses. This priming is linked to enduring alterations in histone modifications at various loci, demonstrating priming-dependent transcriptional memory after a delay of several days [33]. Epigenetic processes, including DNA methylation and histone modifications, are currently under examination as crucial elements in promoting broad-spectrum resistance to both abiotic and biotic stresses. These mechanisms are being investigated for their potential roles as carriers of stress memory that are capable of activating immune responses [34].

Regarding transcriptional priming, Holness *et al.* [35] recently investigated H3K4me3 as a potential priming/memory epigenetic mark in arabidopsis (*Arabidopsis thaliana*) plants subjected to high light stress followed by drought stress. In this report it was shown that there is a memory that enables the plants to store and apply the acquired information at a later time in cases where they have been subjected to priming. In comparison to the plants that were not primed or subjected to drought stress, H3K4me3 enrichment was observed in plants subjected to drought and high light stress, suggesting that this mark may be a target for stress memory in plants [35].

Priming stands out as an effective strategy to fortify plant resistance against biotic stresses and pathogens. During this process, plants implement defensive measures against potential threats, and concurrently ready their defense systems for swifter and/or more robust responses in the future. Notably, the effectiveness of priming goes beyond pathogens, and extends to resistance against arthropods [36]. Chemical priming, specifically the induction of SAR, can be achieved by the direct application of substances such as SA and 2,6-dichloroisonicotinic acid (NHP), or by the use of artificial compounds such as the SA structural analog benzothiadiazole (BTH). For instance, activation of SAR by priming compounds such as BTH is often associated with a primed state that allows plants to 'recall' prior infections or stress exposures. Treating arabidopsis with BTH resulted in priming marked by the accumulation of mRNAs and inactive proteins of mitogen-activated protein kinase 3 (MPK3) and MPK6 [26]. Primed plants through SAR equip themselves with pattern recognition receptors (PRRs) and pathogen-responsive MPKs, and these are activated upon a second infection by elicitors such as the flagellin-derived peptide flg22 through pathways dependent on NPR1. Chemical priming agents utilizing SAR were found to boost the transcription of genes encoding PRRs and accumulate MPK3 and MPK6 in the form of corresponding mRNAs and inactive proteins that can be activated during subsequent stress. Furthermore, epigenetic changes facilitate the rapid activation of stress response-related TFs, enabling plants to enhance and expedite their response to pathogens [26,37].

Nanotechnology-assisted improvement of priming approaches

In addition to the use of natural metabolites and synthetic chemical compounds, recent advances in the field of plant priming include the employment of nanotechnology and its tools as innovative solutions. For example, **nanoparticles (NPs)** have been shown to play a significant role in the protection of plants against adverse environmental conditions, and NPs have been shown to scavenge ROS [38] and improve photosynthetic efficiency by attenuating osmotic and oxidative stress [39–41]. This is largely linked with their nano-size (1–100 nm in at least one dimension), thus giving them diverse physicochemical properties such as higher solubility, reactivity, and biochemical activity depending on their high surface-to-volume ratio and high surface energy [42]. However, these properties also make them ideal candidates for acting as smart **nanocarriers (NCs)** for chemical priming agents, thus achieving targeted delivery and optimal priming activity while lowering their potential environmental impact [43]. This review summarizes current information on advanced NC systems that could be functionalized with chemical agents for enhanced priming efficiency, and concludes that further research will be necessary to address environmental impact and health and safety concerns (Box 1) to achieve optimal usage and exploitation of this technology in crop stress management.

Smart phyto-nanotechnology

More unexpected dangers are posing a threat to agricultural systems all around the world. The sophisticated agronomic use of nanotechnology in plants, known as phyto-nanotechnology, is crucial for preserving food supply and sustainable agriculture, and even economic stability

Box 1. Environmental and/or health and safety concerns

The public perception and acceptance of nanotechnology in food and agriculture also pose a challenge. Concerns about the unknown health effects of nanomaterials may influence consumer attitudes. Transparent communication and education about the safety assessments and benefits of nanomaterial applications are essential to build trust. Nanomaterials, like any other substances, must be handled safely and sustainably to contribute positively to society. They fall under the EU regulatory frameworks of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, the Classification, Labeling and Packaging (CLP) legislation, and the EU Observatory for Nanomaterials (EUON). Manufacturers, importers, and downstream users are obliged under REACH to ensure that nanomaterials do not pose risks to human health or the environment. In 2018, the European Commission made amendments to the REACH Annexes that enhance registration requirements for nanomaterials. Starting in 2020, REACH registration dossiers are required to provide more detailed information about nanomaterials to further ensure safety and compliance.

Assessment of the fate and transport of nanomaterials in the environment is important as they can enter soil, water, and air through various routes. It is critical to assess the potential adverse effects of nanomaterials on non-target organisms such as soil microorganisms, aquatic organisms, and beneficial insects. Understanding how nanomaterials can accumulate in organisms and move through food chains is vital for evaluating their long-term impacts. Regarding health and safety concerns, individuals involved in the production, handling, and application of nanomaterials should be aware of potential occupational exposure risks and take appropriate safety measures.

Health risks can arise from inhalation of NPs or direct contact with the skin, especially if NPs can penetrate biological barriers or cause toxic effects. Assessing the potential toxicity of nanomaterials to humans, including their effects on cells, genes, and specific organs, is crucial for understanding their safety profiles. Comprehensive risk assessments and the implementation of appropriate risk management strategies are essential to address these concerns. These strategies may include the use of safe-by-design approaches in the synthesis of nanomaterials, the application of protective measures during handling and use, and consideration of relevant regulations and guidelines related to nanomaterials.

[44,45]. The use of phyto-nanotechnology (such as new materials, novel methods, or advanced technologies) provides a wide range of potential applications and research areas, such as agrochemicals, nutrients, plant genetic breeding, and biosensors, compared with the production materials utilized in conventional agricultural methods.

Phyto-nanotechnology has the potential to change current agriculture practices by enabling the targeted transport of nutrients (such as proteins or nucleotides) and the planned release of agrochemicals (such as pesticides or fertilizers) [46,47]. By maximizing nutrient uptake and increasing the tolerance for environmental challenges, a revised consideration of the communications between crops and nanomaterials can increase agricultural productivity [48]. A high transformation efficiency for delivering genes using phyto-nanotechnology can be attained in plant cells without the use of external chemical and physical methods, demonstrating its remarkable applicability in crop breeding, especially plant genetic engineering [49,50]. Notably, phyto-nanotechnology can use nanomaterials with high tensile strength, high throughput, different charges, and small sizes to make it more accurate and effective [51]. Agriculture, biotechnology, and even the food industry have all employed phyto-nanotechnology to create biosensors or to act as 'sensing materials' [52,53]. Diverse categories of nanomaterials such as antibody nanosensors, carbon-based electrochemical nanosensors, nanowire nanosensors, plasmonic nanosensors, and fluorescence resonance energy transfer (FRET)-based nanosensors have been reported as instruments for measuring and detecting fungal pathogens, viruses, bacteria, plant metabolic flux, and residual of pesticides in food [54]. The use of smart phyto-nanotechnology in agricultural systems has supported traditional agricultural methods and practices by providing improved management and resource-efficient advanced 'smart' cropping system.

NP-based smart delivery systems: applications and advantages

Nanoparticle-based smart delivery systems (NSDS) comprise a series of nanomaterials with passive or active targeted transport, or physical and chemical targeted transport, which may achieve targeted release by monitoring endogenous stimuli (e.g., redox and pH value variations) or exogenous stimuli (e.g., electric pulses, light, magnetic fields, temperature variations), thereby improving the entire plant life cycle including seed germination and seedling establishment [55]. Compared with all other priming techniques, NSDS are markedly more efficient. The most important properties of NSDS in priming are to promote electron exchange and increase surface response capabilities related to different components of tissues and cells in plants [56].

NCs are nano-sized carriers based on the concept of NSDS. NCs can promote smart delivery and have favorable impacts on crops, including boosting mineral absorption, enhancing photosynthesis, inducing seed germination, improving crop yield and quality, and expediting crop breeding [57]. The contribution of NCs to plant growth and development is principally influenced by the size, composition, concentration, and physical and chemical properties of NCs [58]. The use of NCs as biosensors, agrochemicals, and nutrients for the protection or production of crops under regulated circumstances is presently a major topic in plant science (Figure 1). Although novel nanotechnology methods addressing technical issues in plant genetic breeding or genome-editing techniques in high demand, new developments in siRNA/miRNA/DNA delivery have only recently found applicability in plants.

NCs can be divided into two categories according to their material properties: organic NCs (ONCs) and inorganic NCs (INCs) (Figure 1). For instance, Santana *et al.* [59] developed targeted carbon-based nanomaterials, TP-β-CDs, for transporting chemical cargoes, and TP-pATV1-SWCNTs for plasmid DNA delivery to chloroplasts, and employed innovative plant biorecognition techniques. Evaluating the impact on cell viability, plants treated with TP-β-CDs (20 $mg.l^{-1}$) and

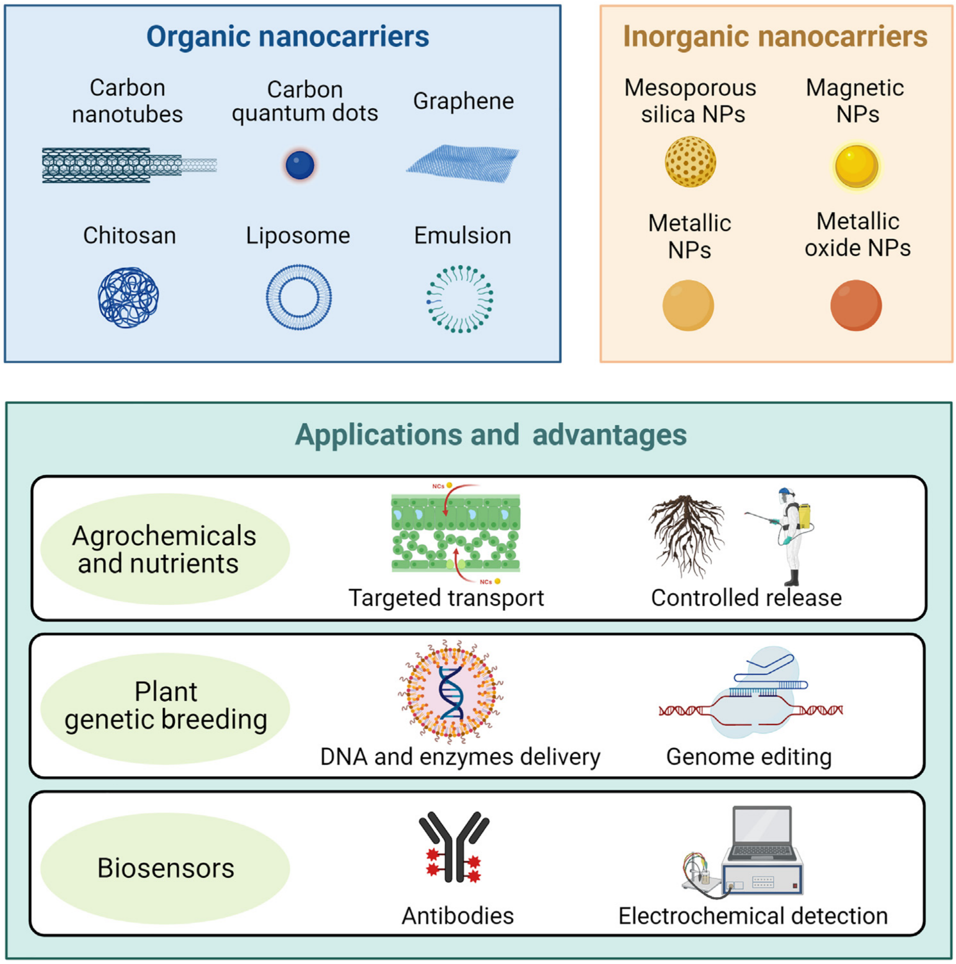

Figure 1. Nanoparticle (NP)-based smart delivery systems: applications and advantages. Organic nanocarriers (ONCs) have been extensively utilized in phyto-nanotechnology, including carbon-based ONCs [carbon nanotubes (CNTs), carbon quantum dots (CQDs), and graphene] and polymeric ONCs (chitosan, liposome, and emulsion). Inorganic nanocarriers (INCs) have been broadly applied in phyto-nanotechnology, such as mesoporous silica NCs (MSNCs), magnetic NCs (MNCs), metallic NCs, and metallic oxide NCs. The applications and advantages of nanoparticle-based smart delivery systems can be divided into three aspects: agrochemicals and nutrients (targeted transport, controlled release), plant genetic breeding (DNA and enzyme delivery, genome editing), and biosensors (antibodies, electrochemical detection).

TP-pATV1-SWCNTs (2 mg.l^{-1}) displayed no significant differences in the percentage of dead cells compared with the control group. This research highlights the heightened efficiency in delivering chemical and plasmid DNA cargoes into chloroplasts through the topical application of carbon nanomaterials engineered with targeting peptides.

Organic nanocarriers

ONCs are created in the context of agricultural systems from carbohydrates, proteins, lipids, and other organic molecules up to a specified size, for example, a radius of <100 nm [60]. Based on the fundamental laws of materials science, physical chemistry, and polymer sciences, as well as the physicochemical characteristics of the source materials, ONCs can be produced using top-down and bottom-up methods, or a mixture of both. To explore their applications and the structure–function correlations, ONCs are characterized by their biological properties, dimensions, internal structures, morphology, and surface properties. ONCs have been extensively applied in phyto-nanotechnology, for example, carbon-based ONCs [**nanotubes (NTs) such as** carbon nanotubes (CNTs), carbon quantum dots (CQDs), and graphene] and polymeric ONCs (chitosan NCs, liposomes, and emulsions).

Carbon-based ONCs

Carbon allotropes known as CNTs feature cylinder-shaped nanostructures with diameters of 1–50 nm [61]. They are characterized as multiwalled nanotubes (MWNTs) or single-walled nanotubes (SWNTs). CNTs are regarded as cutting-edge fertilizers that act as either growth promoters or slow-release fertilizers [62]. CQDs are semiconductor nanocrystals with diameters of 2–10 nm [63]. CQDs are able to enhance the effects of quantum yield or light coverage, and can act as light converters for photosynthesis in plants [64]. A nano-graphene coating made of carbon can lengthen the duration of KNO_3 release while minimizing loss from runoff and leaching [65].

Polymeric ONCs

By altering their physical or chemical properties, ONCs such as chitosan NCs help NCs to more easily enter the epidermal cells of plant leaves, enhance their stability, and reduce their tendency to aggregate [66]. The effects of chitosan NCs and their modified forms on defense-associated systems in crops under abiotic stress have recently been revealed in pertinent research [67]. siRNA transport systems with chitosan NCs incorporated have provided a new approach to crop improvement by allowing the target pest to dominate in a specific way because chitosan has the capacity to permeate cell membranes and interact with RNA [68]. Nano-liposomes help nutrient absorption and transport in a variety of plants because NCs enable the assimilation and transportation of nutrients [69]. Owing to their tiny size and increased surface contact area, nano-liposomes have been approved for the targeted delivery of vitamins, minerals, nutraceuticals, and antibacterial agents [70]. Nonspecific receptors encourage the introduction of negatively charged nano-liposomes into plant cells, which is then followed by adhesion, particle recognition, and eventually endocytosis [71]. A surfactant (such as proteins, lipids, or modified starches) with an average droplet size of 20–200 nm stabilizes a combination of several immiscible liquids to form nano-emulsions [72]. Because of their unique properties, nano-emulsions are ideal intermediates for the transport of nutraceuticals, hydrophobic medicines, and bioactive compounds, and can also encapsulate hydrophobic antioxidant components [73].

Inorganic nanocarriers

INCs such as mesoporous silica NCs (MSNCs), magnetic NCs (MNCs), metallic NCs, and metallic oxide NCs have been broadly applied in phyto-nanotechnology. MSNCs have porous structures resembling honeycombs, and have an adjustable pore size or outside particle diameter in the nm range [74]. They have a large number of unfilled channels that can enclose and absorb various agricultural chemicals and bioactive compounds. MSNCs have been used to transport plasmids carrying the *GFP* gene into plant cells for gene expression. Biochemical analysis and genome modifications in plants can take advantage of proteins and enzymes loaded into MSNCs [75]. By incorporating the transgene into the genome, this method prevents the transmission of the corrected traits to the next generation.

Diverse magnetic materials such as iron (Fe), cobalt (Co), and nickel (Ni), as well as the derived chemical compounds, can be included in MNCs. They can be classified as carbon-coated MNCs [76], magnetic virus-like NCs (VNCs) [77], and other magnetic NCs. For targeted delivery, they can be controlled by magnetic field gradients.

Metallic NCs such as Au and Ag NCs, and metallic oxide NCs such as titanium dioxide (TiO_2), copper oxide (CuO), and zinc oxide (ZnO) NCs, have been widely used as delivery carriers in plant systems because of their superior catalytic, electrical, and light-absorbing properties and high efficiency in the delivery of biomolecules to plants [78,79]. Many metallic oxides and metallic NCs have been utilized in various crop management practices including crop protection and fertilization [48]. Metallic oxides and metallic NCs can improve plant development and growth from the initial stage of germination through to senescence and death in many plant species [45].

Novel engineered nanocarriers

Promising **novel engineered nanocarriers (NENCs)** for chemical priming agents have been the subject of significant research and development, as illustrated in Figure 1. These carriers can be broadly categorized into two main types: organic and inorganic carriers. Within this diverse landscape, various reports have highlighted their versatile applications in delivering a range of essential elements to plants [80]. These elements include nutrients, phytohormones, plant osmolytes, polyamines, and amino acids, all of which play crucial roles in enhancing plant growth and stress tolerance and the quality of agricultural crops.

Among the array of NCs, chitosan stands out as one of the most effective and sustainable options. Chitosan is a biobased, biologically safe, and biodegradable polymer that holds immense potential for delivering various compounds such as SA [81], melatonin [82], putrescine [83], selenium [84], and gibberellic acid [85]. Moreover, chitosan-coated fertilizers can be considered to be an efficient means of delivering micronutrients to plants, particularly elements such as Fe [86]. This approach has proved to be effective in enhancing nutrient uptake by plants. Furthermore, chitosan holds significant promise as a carrier for postharvest treatments aimed at enhancing the quality and extending the shelf life of various agricultural crops. This versatile compound has demonstrated its effectiveness in delivering substances such as polyamines and amino acids, benefiting fruits like grapes [87], plums [88,89], strawberries [90], and persimmons [91].

Among carbon-based NCs, graphene oxide (GO) emerges as a promising candidate for delivering specific compounds to crops such as grapevine [92] and sweet basil [93] under salinity stress conditions. Furthermore, CQDs, characterized by their small size (<10 nm), have shown potential to transport biomolecules into grapevine leaves following foliar application under salinity stress. Notably, functionalized CQDs have been found to bolster enzymatic and nonenzymatic antioxidant systems, thus aiding plants in combating abiotic stresses such as salinity [41,94] and heavy metal stress [95]. In addition, the autofluorescent nature of CQDs enables real-time monitoring of carrier translocation within plants, facilitating precise tracking [96].

Recently, clay-based carriers have also gained attention as promising vehicles for delivering various beneficial compounds to plants. For instance, Masoudniaragh *et al.* [97] reported that the application of functionalized halloysite clay with proline not only improved agronomic parameters but also significantly increased the production of essential oil compounds such as germacrene D and methyl chavicol in sweet basil under salinity stress. This underscores the potential of clay-based carriers to enhance crop quality and yield through controlled compound delivery. The field of NCs for chemical priming agents is in constant evolution, and they present unique advantages through organic, inorganic, and carbon-based carriers. These have the potential to revolutionize agriculture by improving nutrient delivery, stress tolerance, and overall crop health. Future research will likely unveil even more innovative applications, further contributing to sustainable and efficient agricultural practices.

Controlled release mechanisms of NENCs

In agriculture there is growing interest in the use of nanotechnology to deliver biologically active ingredients via NCs because of its potential to address the challenges posed by adverse environmental conditions and the increasing demand for biologically active ingredients in food [42]. Previous reports have shown that excess application of traditional agrochemical application methods has reduced bioavailability and consequently has had a negative impact on the environment. These findings highlight the need for controlled delivery systems to improve efficacy [98]. Controlled release mechanisms using NCs have therefore been explored as part of crop improvement for the delivery of pesticides/biopesticides and slow-release fertilizers/biofertilizers, as well as for micronutrient encapsulation, stabilization of plant growth regulators, and targeted delivery of genetic material [99]. In addition, the use of nanomaterials to encapsulate agrochemicals allows improved storage and controlled release directly at the site of application, thus improving efficacy and reducing environmental impact. Several nano-delivery systems have been developed for agricultural applications, including emulsions, hydrogels, vesicular carriers (liposomes, noisomes, and transferosomes), and polymeric carriers (chitosan, chitin, polyhydroxybutyrate, cellulose, and starch), which offer advantages such as high surface area, increased activity, and rapid mass transfer [100].

From a chemical point of view, several different strategies can be used to achieve controlled release (Figure 2). Nanomaterials can be produced in several ways, including coating them with thin polymer films, encapsulating them in nanoporous materials, or creating nanoemulsions [101]. By using these approaches, the encapsulated compounds can be protected from loss through evaporation and leaching, thus ensuring gradual release of the encapsulated compounds. NCs are selected based on several factors, including the physicochemical properties of the chemical to be delivered, the desired release kinetics, and the biology of the target plant [102]. Carriers can be designed to provide controlled release profiles and protect compounds from degradation. They can also improve solubility and enhance cellular uptake [103]. To achieve controlled and slow release of compounds, NCs use various mechanisms. NCs can release compounds by diffusion, where the payload molecules move from regions of high concentration within the carrier to regions of lower concentration in the surrounding environment. By modifying the composition of the carrier, its surface properties, and the size of the carrier itself, the release rate can be controlled. In addition, these small vehicles can be designed to degrade over time and release the encapsulated compounds. Environmental factors such as temperature and pH, and the presence of enzymes such as proteases, phospholipases, and oxidoreductases, can trigger degradation [104]. The release of compounds can be tailored to specific requirements by controlling the degradation rate. NCs can be designed to respond to external stimuli such as light, temperature, pH, or magnetic fields. These stimuli can lead to the release of the encapsulated compounds by inducing changes in the structure or properties of the carrier [105]. External magnetic fields can also be used to guide and localize magnetic NCs. The release of compounds can be targeted to specific areas by directing the carriers to the desired location. This improves their efficiency and reduces the amount of compound required [105].

By providing a new delivery system for phytohormones, chemical priming agents, nutrients, and pesticides, nanomaterials can improve the efficiency of nutrient use. Various nanomaterials have the potential to effectively deliver plant growth-promoting compounds via roots, leaves, and seed coats, and these include carbon-based nanomaterials, mineral NPs, metal materials, and metal oxide materials. This delivery system can enhance uptake and translocation within the plant. This improves yields and reduces environmental impact [83,106]. In addition, nanomaterials have the potential to improve soil health and manipulate rhizosphere interactions, including root–soil–microbiome interactions, which can improve crop yields [106]. Smart delivery of

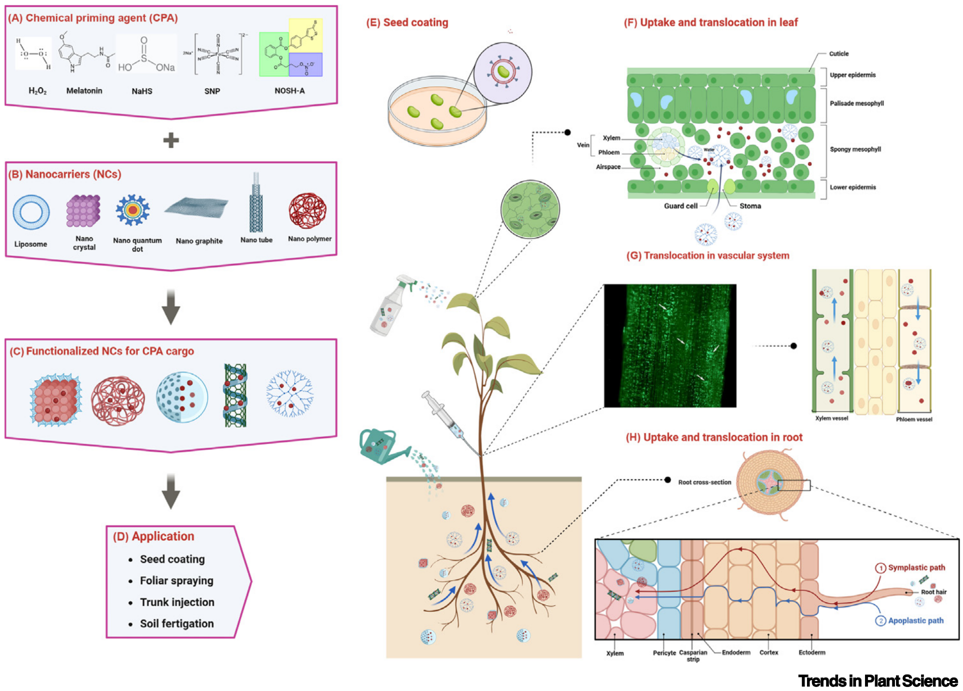

Figure 2. Schematic pathway of utilizing novel engineered nanocarriers (NENCs) for delivery of chemical priming agents in plants. (A) Various chemical priming agents (CPAs) have been explored for enhancing plant stress tolerance. Notable agents include sodium nitroprusside, hydrogen peroxide, sodium hydrosulfide, melatonin, and polyamines. In addition, a novel aspirin (acetylsalicylic acid) variant, NOSH-A, has shown promise by releasing nitric oxide (NO), hydrogen sulfide ($H_{2S)}$, and aspirin simultaneously, offering potential benefits in stress mitigation. (B) Nanocarriers (NCs) used in plant applications encompass a diverse range of materials, including liposomes, nanocrystals, nanotubes, graphene oxide, nanopolymers, nanosheets, and more. (C) These nanoparticles (NPs) undergo surface modifications to introduce specific functional groups or molecules, thereby enhancing their properties. When coupled with priming agents, these functionalized NCs give rise to novel engineered NPs. (D) Several modes of application of functionalized nanomaterials in plants can take place, such as through seed coating, foliar application, trunk injection, and soil fertigation. (E) Seed coating with NPs has emerged as a promising technique in seed priming technology. This innovative approach holds great potential for enhancing seed germination, promoting robust seedling growth, and ultimately improving overall plant performance, making it an increasingly important tool in modern agriculture. (F) Some NPs can also penetrate leaf tissues, facilitated by the relatively large size of stomatal openings. NPs <5 nm in size can directly penetrate the leaf cuticle. (G) One key process is the apoplastic transport of NPs through the endodermis, facilitating their movement towards the aerial parts of the plant. Within the plant vascular system, notably the xylem, NPs find a significant conduit for distribution and translocation. (H) NPs smaller than the pore size of root epidermal cell walls, typically in the range of 5–20 nm, can traverse these pores and enter the root tissues, illustrating the significance of the apoplastic pathway in root uptake. Figure created with BioRender.

nanomaterials in low doses to crops can alter their responses to biotic and abiotic stresses, thereby improving their resilience to drought, extreme heat, heavy metals, and salinity [42]. It remains a challenge to efficiently and effectively deliver nanomaterials to plant roots. Targeted approaches such as foliar application and seed coatings are promising, although soil application of nanomaterials is not energy-efficient [107]. Foliar applications face challenges such as degradation, low uptake, and weathering, although they can be applied using existing infrastructure. The development of nanomaterials with improved leaf adhesion and uptake will be necessary to overcome these challenges.

NENC uptake, translocation, and biological impact in plants

Although the mechanisms by which NENCs are taken up and transported in plants remain poorly understood, it is generally agreed that these processes depend on various factors such as the type of NP, its physicochemical properties, the plant species, and the plant substrate (soil, hydroponics, or culture medium). In general, active transport mechanisms including signaling, recycling, and plasma membrane regulation are involved in the uptake of NENCs by plants [108,109]. Although endocytic pathways are well characterized for the uptake of NPs in animals, plant cells also follow endocytic pathways to take up engineered NPs [108]. Only selected particles can pass through the pores of the plant cell wall, which acts as a semipermeable barrier [109,110].

By forming complexes with membrane transporter proteins or root exudates, plants translocate NPs [111,112]. The interaction between NPs and plants is influenced by NP properties including size, porosity, hydrophobicity, and surface characteristics [43]. There are several ways in which plants can absorb NPs. First, small NPs (<10 nm) can be taken up by roots through pores in the root epidermal cell walls, known as the apoplastic pathway, but larger particles are prevented from entering [111]. The diffusion of small NPs through the apoplast and into the endodermis can be driven by osmotic pressure and capillary forces [109]. Another way in which NPs can be taken up by plants is by crossing the inner side of the plasma membrane via the symplastic pathway [109]. NPs can cross the porous matrix of the cell wall by binding to protein carriers, passing through aquaporins and ion channels, or by penetrating the membrane and creating new channels [108,109]. NPs can also migrate to neighboring cells through plasmodesmata, which are channels 20–50 nm in diameter [111,112]. Stomatal pores are another route for NPs to enter plants [109]. In addition to entering the leaves, NPs can also be transported to the plant roots [113]. Recent advances have improved our understanding of the uptake, translocation, and agglomeration kinetics of NPs, including their dependence on shape, size, and composition [109,111]. Plants take up NPs through their cell walls and the cell membrane of the root epidermis, and then undergo a series of complex events that enable them to be translocated from the roots to the leaves via the vascular bundle (xylem). The uptake of nanomaterials is size-specific because NPs need to pass through pores on the cell membrane to cross intact cell membranes. Before they reach the xylem, the NPs must be passively integrated through the apoplast of the endodermis. The xylem plays a crucial role in the distribution and translocation of NPs. The cell wall is made up of a porous network of polysaccharide fibers which allows the cells to take up water molecules and other dissolved substances [108,109].

Foliar application dynamics

Entry of NPs through stomatal pores on the leaf surface is the main mechanism of NP uptake in plants following foliar application (Figure 2). Some NPs can penetrate the leaf tissue because of the relatively large size of the stomatal openings. Several studies have shown that NPs taken up by leaves are translocated to other parts of the plant [114,115]. Translocation of TiO_2 NPs to the roots via foliar uptake was observed in soybean plants by Hong *et al.* [115]. Furthermore, Gohari *et al.* [39] reported that TiO_2 NPs could penetrate from leaves and translocate in the xylem in sweet basil (*Ocimum basilicum* L.). Several factors such as NP size, surface properties, and leaf characteristics influence the foliar uptake of NPs. The size of the NPs is critical as smaller particles are more likely to enter the stomata. Hydrophilic NPs were shown to be able to pass through stomatal pores when their size was ~40 nm [116]. The cuticle acts as the main natural barrier against NPs entering plant tissues, and protects leaves from water loss and uncontrolled solute exchange. There are two pathways for solute uptake: diffusion and permeation for non-polar solutes via the lipophilic pathway, and passage of polar solutes through polar aqueous pores via the hydrophilic pathway. NPs <4.8 nm can penetrate directly into the cuticle, and larger NPs (>5 nm) can accumulate in leaves through an unclear mechanism [108]. In addition to size, the surface properties of the NPs and the characteristics of the leaf surface also play an important role in foliar uptake. Surface modifications or coatings on NPs can affect their interaction with the leaf surface and subsequent uptake. Moreover, as widely known, the inclusion of surfactants such as Tween during the spraying of nanomaterials can enhance the efficacy of foliar uptake. This improvement is realized by increasing cuticular and subcuticular penetration, thereby facilitating the transport of nanomaterials following foliar spraying. It is worth highlighting that the incorporation of Tween 20 or Tween 80 can be beneficial for the foliar delivery of NCs [39–41]. The concentration of NPs is a critical factor to consider in their relocation and aggregation when applied by foliar spraying. This factor is evident in the application of multiwall carbon nanotubes (MWCNTs) to sweet basil plants, where a dose-dependent relationship was observed. Lower doses of MWCNTs (25 and 50 $mg.l^{-1}$) exhibited positive effects by alleviating salt stress-induced damage. These effects included enhancements of antioxidant enzymatic activities, phenolic compounds, physiological parameters such as chlorophyll and carotenoid content, nonenzymatic (i.e., phenolic content) and enzymatic antioxidant components [i.e., ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GP) activities], and essential oil content and composition. However, higher concentrations (100 $mg.l^{-1}$) of MWCNTs-COOH resulted in aggregation within plant tissues, leading to toxicity symptoms [117]. Furthermore, increasing the concentration of TiO_2 and ZnO NPs from 20 $mg.l^{-1}$ to 40 $mg.l^{-1}$ resulted in a significant reduction in germination of both onion and fennel seeds [45]. Therefore, the concentration of NPs plays a crucial role in determining their effectiveness and potential adverse effects on plants following foliar application.

Soil application dynamics

The uptake mechanism of NPs in plants by soil application involves the interaction of NPs with the roots and their subsequent uptake into vascular system of the plants (Figure 2). NPs come into contact with the root surface when they are applied to the soil. The uptake mechanisms and behaviors of NPs in plant roots have been investigated in several studies [118]. Among the mechanisms, the apoplastic pathway is one of the major routes of NP uptake. NPs smaller than the pore size of root epidermal cell walls (typically ~5–20 nm) can enter the roots through these pores [112]. However, particles larger than the pore size are typically blocked and cannot enter. Once NPs enter the root, they may undergo several processes to reach the plant vascular system. These include diffusion through the apoplast, where NPs move through the spaces between cells, and eventually reach the endodermis [119]. NPs must passively integrate into the apoplast to cross the endodermis and enter the stele [119]. An important step in the translocation of NPs to the aerial parts of the plant is the apoplastic transport of NPs through the endodermis. The vascular system of plants, particularly the xylem, plays a significant role in the distribution and translocation of NPs within the plant. Xylem vessels serve as the main pathway for the uptake of water and solutes, including NPs, from roots to shoots [120]. Wang *et al.* [121] demonstrated the uptake, translocation, and distribution of CuO NPs in maize plants through the xylem. The uptake of NPs by plant roots is influenced by several factors, including NP properties, root structure, and environmental conditions. The interaction with the root surface and subsequent uptake can be modulated by NP properties such as size, shape, and surface characteristics [38]. NP uptake can also be influenced by root characteristics such as root exudates, surface charge, and root hair density [110].

Seed priming dynamics

The mechanism of uptake of NPs into plants by seed coatings involves the application of NPs to the surface of seeds, which allows their penetration into the seed and subsequent transport to various plant tissues during germination and seedling development. Seed coating with NPs has gained interest in recent years, particularly in seed priming technology, because of its potential benefits in improving seed germination, seedling growth, and overall plant performance [55]. NPs can adhere to the seed surface or penetrate the seed tissue when applied as a seed coating. The mechanism of NP penetration into seeds is still not fully understood and may vary depending on the specific NP and seed characteristics. However, insights into the possible pathways and mechanisms involved have been provided by several studies. The seed coat, which consists of several layers that form a physical barrier, is one possible mechanism for NP penetration. NPs can interact with the seed coat and penetrate through microcracks, pores, or gaps on the surface of the seed coat [122,123]. These apertures may be the result of natural imperfections in the seed coat, mechanical damage, or chemical treatments applied during the processing of the seed. In the study by Yu *et al.* [123], machine learning was employed to assess NP uptake during seed priming. The findings revealed that the concentration of NPs and ionic strength played a crucial role in influencing shoot fresh weight, primarily by regulating NP uptake. Notably, the uptake of NPs experienced a significant deceleration when the NP concentration surpassed 50 $mg.l^{-1}$. Although factors including zeta potential and hydrodynamic diameter did not demonstrate noticeable effects on NP uptake, their biological impacts should not be disregarded. Natural openings such as stomata or lenticels in some seed coats are another mechanism for NP uptake in seeds. Stomata are tiny pores that are mainly found on the surface of leaves, but they can also be found on the seed coats of some species of plants. Through these openings, which provide direct access to the inside of the seed, NPs can enter the seed [124]. Seed priming involves the pre-soaking or pre-germination treatment of seeds in various solutions (e.g., 'NP suspensions') and is a very promising approach for improving seed performance. During the seed-priming process, NPs can penetrate into the tissues of the seed through the process of imbibition in which water and solutes are absorbed by the seed [2]. The NPs contained in the priming solution can be absorbed by water, allowing them to enter the seed [125]. Once inside the seed, NPs can interact with the seed components such as proteins, lipids, and cell membranes, and affect seed metabolism and germination processes.

Bioimaging and detection of NENCs in plant tissues

To understand the uptake, distribution, and potential effects of NENCs, bioimaging and detection of NENCs in plant tissues is crucial. Electron microscopy techniques, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM), have been instrumental in visualizing the internalization and localization of NENCs in plant tissues at high resolution. The SEM provides a detailed view of surface morphology, whereas the TEM enables the study of subcellular structures. Recent studies have used these techniques to investigate the interactions between NENCs and plant tissues, including uptake pathways and cellular localization [126,127]. For example, SEM and TEM were employed to visualize the accumulation of silver NPs in various plant tissues, revealing their internalization and localization within root cells [128]. Furthermore, electron microscopy techniques have aided in determining the size, shape, and aggregation state of NENCs within plant tissues, providing valuable insights into their behavior and potential impacts [128]. For example, a study by Proença *et al.* [129] investigated the uptake and distribution of TiO_2 NPs in lettuce leaves using SEM and TEM. The results showed that the TiO_2 NPs were mainly aggregated on the leaf surface in the form of larger agglomerates. TEM analysis revealed the presence of internalized NPs within the cells of the leaf, suggesting the possibility of their translocation through the tissues of the plant. To visualize and track NENCs in plant tissues, fluorescence microscopy has emerged as a valuable tool. Fluorescent labels attached to the NENCs allow real-time monitoring and localization. This technique enables the visualization of NENCs within plant cells and tissues, thus providing insights into their distribution patterns and cellular interactions. Recent advances in fluorescence microscopy, such as the use of quantum dots and other fluorophores, have improved the sensitivity and specificity of NENC detection in plants [128]. For example, fluorescence microscopy coupled with confocal imaging was used to study the intracellular fate of silica-based NCs in arabidopsis, and enabled the visualization of their uptake and distribution within different plant cell compartments [130].

Compared with conventional fluorescence microscopy, confocal laser scanning microscopy offers improved resolution and depth penetration. It enables 3D imaging of NENCs in plant tissues, making it easier to analyze how NENCs distribute at different depths in the tissue. The internalization and subcellular trafficking of NENCs in different plant species was visualized using confocal microscopy [114,129]. For example, a study by Sun *et al.* [130] used confocal laser scanning microscopy to investigate the uptake and intracellular localization of gold NPs in arabidopsis root cells. This revealed endocytic internalization of NPs and their accumulation within endosomal compartments, shedding light on the interactions between cells and the possible mechanisms of uptake. Super-resolution microscopy techniques, such as stimulated emission depletion (STED) microscopy and structured illumination microscopy (SIM), have revolutionized bioimaging by breaking the diffraction barrier. Detailed insights into the subcellular localization and interactions of NENCs are provided by these techniques. Super-resolution microscopy has been used to study the localization and movement of quantum dot-labeled NCs within plant cells, allowing visualization at the nm scale [129]. Wang et al. [131] used STED microscopy to visualize the uptake and intracellular trafficking of polymer-coated magnetic NPs in maize root cells. The results demonstrated the ability of STED microscopy to resolve the subcellular distribution of NENCs and their interactions with specific organelles.

Concluding remarks and future perspectives

In the past decade, nanotechnology has made great strides in the design, production, and use of NCs in agriculture. NCs for crops cannot be broadly applied in agricultural operations or activities because of high costs or other issues. By promoting interdisciplinary approaches to the design and synthesis of intelligent NCs, the challenges of phyto-nanotechnology can be overcome. To achieve this, a combined collaborative project combining the complementary professional strengths of geneticists, engineers, botanists, biochemists, and chemists may open up a new frontier in phyto-nanotechnology. Current applications suggest that further research is required in this area of plant development or growth to improve the sustainability of agricultural systems. Identifying the underlying mechanism of the influence of NCs on plants may further benefit from future studies utilizing open-field experiments.

Plant cell walls have a porosity of only 15 nm [132], indicating the need to manage the size of NCs for effective translocation. In addition, the effectiveness of NCs in entering the chloroplast membrane is strongly correlated with their zeta potential values [133]. Together with the information in the previous section, it is important to characterize or describe the cultivation method, concentration, size, and zeta potential of the NCs in a study so that the researchers can more easily follow the research and understand how the NCs improve plant growth and development.

Despite all the positive outcomes, design safety considerations must be taken into consideration to help the community to deal with the potential negative consequences of new NCs on the environment (e.g., NCs in a daily necessity product) [134]. These NCs must be properly formulated for usage in the agricultural system, taking the treatment techniques (foliar and soil) into account, to have a significant impact and guarantee superior crop quality. While using these NCs in plant systems, additional precautions must be taken because their excessive usage may harm the environment. However, it cannot be denied that the favorable effects of NCs have made significant contributions to many areas of agricultural systems from germination to postharvest (see Outstanding questions).

Outstanding questions

Is nano-priming more effective than traditional chemical priming in promoting plant growth and development under control and stress conditions? If so, what are the underlying mechanisms?

Which molecular pathways regulate the enhancement of plant growth, development, and stress tolerance by nano-priming? Which genes play a vital role in these regulatory processes?

What are the most effective NCs, and how can we improve the innovative methods for the controlled release of biomolecules in crop plants?

How can nano-priming with NPs affect the next generation of plants?

In terms of transgenerational changes in plants, are there any differences in seed priming or seedling priming approaches?

Once plants have been primed with NPs, what epigenetic mechanisms are involved? Is there a common epigenetic response to different stressors?

How will nano-primed plants respond when they are exposed to recurring stressors? Will there be any significant differences in comparison to nonprimed plants?

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Declaration of interests

V.F. is a coinventor of patent WO/2015/123273 dealing with the use of NOSH-A in plants. The remaining authors declare no conflicts of interest.

References

1. Westman, S.M. *et al.* (2019) Defence priming in *Arabidopsis* – a meta-analysis. *Sci. Rep.* 9, 13309
2. Savvides, A. *et al.* (2016) Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends Plant Sci.* 21, 329–340
3. Tanou, G. *et al.* (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *Plant J.* 72, 585–599
4. Mohammadi, M. *et al.* (2023) Stress memory in seedlings of primed seed chickpea (*Cicer arietinum* L.) using sodium nitroprusside under cold stress. *Plant Stress* 8, 100163
5. Gohari, G. *et al.* (2020) Interaction between hydrogen peroxide and sodium nitroprusside following chemical priming of *Ocimum basilicum* L. against salt stress. *Physiol. Plant.* 168, 361–373
6. Christou, A. *et al.* (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defense pathways. *J. Exp. Bot.* 64, 1953–1966
7. Ocvirk, D. *et al.* (2021) The effects of seed priming with sodium hydrosulphide on drought tolerance of sunflower (*Helianthus annuus* L.) in germination and early growth. *Ann. Appl. Biol.* 178, 400–413
8. Antoniou, C. *et al.* (2017) Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. *J. Pineal Res.* 62, e12401
9. Zhang, Y. *et al.* (2021) Seed priming with melatonin improves salt tolerance in cotton through regulating photosynthesis, scavenging reactive oxygen species and coordinating with phytohormone signal pathways. *Ind. Crop. Prod.* 169, 113671
10. Tanou, G. *et al.* (2014) Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. *Plant Cell Environ.* 37, 864–885
11. Alcázar, R. *et al.* (2020) Polyamines: small amines with large effects on plant abiotic stress tolerance. *Cells* 9, 2373
12. Cai, J. and Aharoni, A. (2022) Amino acids and their derivatives mediating defense priming and growth tradeoff. *Curr. Opin. Plant Biol.* 69, 102288
13. Tian, S. *et al.* (2019) Priming with the green leaf volatile (Z)-3-hexeny-1-yl acetate enhances salinity stress tolerance in peanut (*Arachis hypogaea* L.) seedlings. *Front. Plant Sci.* 10, 785
14. Li, X. *et al.* (2022) Priming with the green leaf volatile (Z)-3-hexeny-1-yl acetate enhances drought resistance in wheat seedlings. *Plant Growth Regul.* 98, 477–490
15. Antoniou, C. *et al.* (2020) Exploring the potential of nitric oxide and hydrogen sulfide (NOSH)-releasing synthetic compounds as novel priming agents against drought stress in *Medicago sativa* plants. *Biomolecules* 10, 120
16. Dildabek, A. *et al.* (2020) Crosstolerant effect of salt priming and viral infection on *Nicotiana benthamiana*. *Eurasian J. Appl. Biotechnol.* 2020, UDC578.24
17. Tuteja, N. and Sopory, S.K. (2008) Chemical signaling under abiotic stress environment in plants. *Plant Signal. Behav.* 3, 525–536
18. Antoniou, C. *et al.* (2016) Unravelling chemical priming machinery in plants: the role of reactive oxygen–nitrogen–sulfur species in abiotic stress tolerance enhancement. *Curr. Opin. Plant Biol.* 33, 101–107
19. Kerchev, P. *et al.* (2020) Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnol. Adv.* 40, 107503
20. Vwioko, E. *et al.* (2017) Comparative physiological, biochemical, and genetic responses to prolonged waterlogging stress in okra and maize given exogenous ethylene priming. *Front. Physiol.* 8, 632
21. Gómez-Ariza, J. *et al.* (2007) Sucrose-mediated priming of plant defense responses and broad-spectrum disease resistance by overexpression of the maize pathogenesis-related PRms protein in rice plants. *Mol. Plant-Microbe Interact.* 20, 832–842
22. Fang, S. *et al.* (2018) Chemical priming of seed alters cotton floral bud differentiation by inducing changes in hormones, metabolites and gene expression. *Plant Physiol. Biochem.* 130, 633–640
23. Hake, K. and Romeis, T. (2019) Protein kinase-mediated signalling in priming: Immune signal initiation, propagation, and establishment of long-term pathogen resistance in plants. *Plant Cell Environ.* 42, 904–917
24. Avramova, Z. (2019) Defence-related priming and responses to recurring drought: Two manifestations of plant transcriptional memory mediated by the ABA and JA signalling pathways. *Plant Cell Environ.* 42, 983–997
25. Hilker, M. and Schmülling, T. (2019) Stress priming, memory, and signalling in plants. *Plant Cell Environ.* 42, 753–761
26. Hönig, M. *et al.* (2023) Chemical priming of plant defense responses to pathogen attacks. *Front. Plant Sci.* 14, 1146577
27. Wang, X. *et al.* (2017) Priming: a promising strategy for crop production in response to future climate. *J. Integr. Agric.* 16, 2709–2716
28. Laura, B. *et al.* (2018) Epigenetic control of defense genes following MeJA-induced priming in rice (*O. sativa*). *J. Plant Physiol.* 228, 166–177
29. Nguyen, H.M. *et al.* (2020) Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. *Front. Plant Sci.* 11, 494
30. Rapp, R.A. and Wendel, J.F. (2005) Epigenetics and plant evolution. *New Phytol.* 168, 81–91
31. Bonasio, R. *et al.* (2010) Molecular signals of epigenetic states. *Science* 330, 612–616
32. Kim, J.M. *et al.* (2017) Acetate-mediated novel survival strategy against drought in plants. *Nat. Plants* 3, 17097
33. Oberkofler, V. *et al.* (2021) Epigenetic regulation of abiotic stress memory: Maintaining the good things while they last. *Curr. Opin. Plant Biol.* 61, 102007
34. Mladenov, V. *et al.* (2021) Deciphering the epigenetic alphabet involved in transgenerational stress memory in crops. *Int. J. Mol. Sci.* 22, 7118
35. Holness, S. *et al.* (2023) Highlight induced transcriptional priming against a subsequent drought stress in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* 24, 6608
36. Mauch-Mani, B. *et al.* (2017) Defense priming: an adaptive part of induced resistance. *Annu. Rev. Plant Biol.* 68, 485–512
37. Gully, K. *et al.* (2019) Biotic stress-induced priming and depriming of transcriptional memory in *Arabidopsis* and apple. *Epigenomes* 3, 3
38. Rico, C.M. *et al.* (2013) Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and in vivo fluorescence imaging. *Environ. Sci. Technol.* 47, 5635–5642
39. Gohari, G. *et al.* (2020) Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. *Sci. Rep.* 10, 912
40. Mohammadi, M.H.Z. *et al.* (2021) Cerium oxide nanoparticles (CeO_2 NPs) improve growth parameters and antioxidant defense system in Moldavian balm (*Dracocephalum moldavica* L.) under salinity stress. *Plant Stress* 1, 100006
41. Gohari, G. *et al.* (2021) Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticles effectively prime grapevine (*Vitis vinifera* cv.'Sultana') against salt stress. *BMC Plant Biol.* 21, 120
42. Ioannou, A. *et al.* (2020) Advanced nanomaterials in agriculture under a changing climate: the way to the future? *Environ. Exp. Bot.* 176, 104048

43. Khan, I. *et al.* (2019) Nanoparticles: properties, applications and toxicities. *Arab. J. Chem.* 12, 908–931
44. Shang, Y. *et al.* (2019) Applications of nanotechnology in plant growth and crop protection: a review. *Molecules* 24, 2558
45. Jiang, M. *et al.* (2021) Phytonanotechnology applications in modern agriculture. *J. Nanobiotechnol.* 19, 430
46. Acharya, A. and Pal, P.K. (2020) Agriculture nanotechnology: translating research outcome to field applications by influencing environmental sustainability. *NanoImpact* 19, 100232
47. Salama, D.M. *et al.* (2021) Applications of nanotechnology on vegetable crops. *Chemosphere* 266, 129026
48. Gogos, A. *et al.* (2012) Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J. Agric. Food Chem.* 60, 9781–9792
49. Yan, Y. *et al.* (2022) Nanotechnology strategies for plant genetic engineering. *Adv. Mater.* 34, 2106945
50. Agrawal, S. *et al.* (2022) Plant development and crop protection using phytonanotechnology: a new window for sustainable agriculture. *Chemosphere* 299, 134465
51. Demirer, G.S. *et al.* (2021) Nanotechnology to advance CRISPR–Cas genetic engineering of plants. *Nat. Nanotechnol.* 16, 243–250
52. Duhan, J.S. *et al.* (2017) Nanotechnology: the new perspective in precision agriculture. *Biotechnol. Rep.* 15, 11–23
53. Sanzari, I. *et al.* (2019) Nanotechnology in plant science: to make a long story short. *Front. Bioengin. Biotechnol.* 7, 120
54. Chaudhry, N. *et al.* (2018) Bio-inspired nanomaterials in agriculture and food: Current status, foreseen applications and challenges. *Microb. Pathog.* 123, 196–200
55. do Espirito Santo Pereira, A. *et al.* (2021) Nanotechnology potential in seed priming for sustainable agriculture. *Nanomaterials* 11, 267
56. Nile, S.H. *et al.* (2022) Nano-priming as emerging seed priming technology for sustainable agriculture – recent developments and future perspectives. *J. Nanobiotechnol.* 20, 254
57. Nair, R. (2016) Effects of nanoparticles on plant growth and development. *Plant Nanotechnol.* 5, 95–118
58. Siddiqi, K.S. and Husen, A. (2017) Plant response to engineered metal oxide nanoparticles. *Nanoscale Res. Lett.* 12, 92
59. Santana, I. *et al.* (2022) Targeted carbon nanostructures for chemical and gene delivery to plant chloroplasts. *ACS Nano* 16, 12156–12173
60. Pan, K. and Zhong, Q. (2016) Organic nanoparticles in foods: fabrication, characterization, and utilization. *Annu. Rev. Food Sci. Technol.* 7, 245–266
61. Chang, X. *et al.* (2020) Effects of carbon nanotubes on growth of wheat seedlings and Cd uptake. *Chemosphere* 240, 124931
62. Safdar, M. *et al.* (2022) Engineering plants with carbon nanotubes: a sustainable agriculture approach. *J. Nanobiotechnol.* 20, 275
63. Kargozar, S. *et al.* (2020) Quantum dots: a review from concept to clinic. *Biotechnol. J.* 15, 2000117
64. Li, Y. *et al.* (2021) Carbon dots as light converter for plant photosynthesis: augmenting light coverage and quantum yield effect. *J. Hazard. Mater.* 410, 124534
65. Her, S.C. and Liang, Y.M. (2022) Carbon-based nanomaterials thin film deposited on a flexible substrate for strain sensing application. *Sensors* 22, 5039
66. Nadendla, S.R. *et al.* (2018) HarpinPss encapsulation in chitosan nanoparticles for improved bioavailability and disease resistance in tomato. *Carbohydr. Polym.* 199, 11–19
67. Riseh, R.S. *et al.* (2022) Nano/microencapsulation of plant biocontrol agents by chitosan, alginate, and other important biopolymers as a novel strategy for alleviating plant biotic stresses. *Int. J. Biol. Macromol.* 222, 1589–1604
68. Zhang, X. *et al.* (2010) Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*). *Insect Mol. Boil.* 19, 683–693
69. Karny, A. *et al.* (2018) Therapeutic nanoparticles penetrate leaves and deliver nutrients to agricultural crops. *Sci. Rep.* 8, 7589
70. Zou, L. *et al.* (2015) A novel delivery system dextran sulfate coated amphiphilic chitosan derivatives-based nanoliposome: capacity to improve in vitro digestion stability of (–)-epigallocatechin gallate. *Food Res. Int.* 69, 114–120
71. Taherkhani, S. *et al.* (2014) Covalent binding of nanoliposomes to the surface of magnetotactic bacteria for the synthesis of self-propelled therapeutic agents. *ACS Nano* 8, 5049–5060
72. Gupta, A. *et al.* (2016) Nanoemulsions: formation, properties and applications. *Soft Matter* 12, 2826–2841
73. Salvia-Trujillo, L. *et al.* (2014) Impact of microfluidization or ultrasound processing on the antimicrobial activity against *Escherichia coli* of lemongrass oil-loaded nanoemulsions. *Food Control* 37, 292–297
74. Torney, F. *et al.* (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat. Nanotechnol.* 2, 295–300
75. Martin-Ortigosa, S. *et al.* (2014) Mesoporous silica nanoparticle-mediated intracellular Cre protein delivery for maize genome editing via loxP site excision. *Plant Physiol.* 164, 537–547
76. Corredor, E. *et al.* (2009) Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. *BMC Plant Boil.* 9, 45
77. Huang, X. *et al.* (2011) Magnetic virus-like nanoparticles in *N. benthamiana* plants: a new paradigm for environmental and agronomic biotechnological research. *ACS Nano* 5, 4037–4045
78. Zhao, L. *et al.* (2020) Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. *J. Agric. Food Chem.* 68, 1935–1947
79. Yan, X. *et al.* (2022) AgNPs-triggered seed metabolic and transcriptional reprogramming enhanced rice salt tolerance and blast resistance. *ACS Nano* 17, 492–504
80. Campos, E.V. *et al.* (2023) Encapsulated plant growth regulators and associative microorganisms: Nature-based solutions to mitigate the effects of climate change on plants. *Plant Sci.* 111688
81. Aazami, M.A. *et al.* (2023) Protective effects of chitosan based salicylic acid nanocomposite (CS-SA NCs) in grape (*Vitis vinifera* cv. 'Sultana') under salinity stress. *Sci. Rep.* 13, 883
82. Gohari, G. *et al.* (2023) Mitigation of salinity impact in spearmint plants through the application of engineered chitosan-melatonin nanoparticles. *Int. J. Biol. Macromol.* 224, 893–907
83. Panahirad, S. *et al.* (2023) Foliar application of chitosan-putrescine nanoparticles (CTS-Put NPs) alleviates cadmium toxicity in grapevine (*Vitis vinifera* L.) cv. Sultana: modulation of antioxidant and photosynthetic status. *BMC Plant Biol.* 23, 411
84. Sheikhalipour, M. *et al.* (2023) Seedling nanopriming with selenium-chitosan nanoparticles mitigates the adverse effects of salt stress by inducing multiple defence pathways in bitter melon plants. *Int. J. Biol. Macromol.* 124923
85. dos Santos Guaraldo, M. *et al.* (2023) Priming with sodium nitroprusside and hydrogen peroxide increases cotton seed tolerance to salinity and water deficit during seed germination and seedling development. *Environ. Exp. Bot.* 209, 105294
86. Giglou, M.T. *et al.* (2022) A new method in mitigation of drought stress by chitosan-coated iron oxide nanoparticles and growth stimulant in peppermint. *Ind. Crop. Prod.* 187, 115286
87. Khalili, N. *et al.* (2022) Chitosan-enriched salicylic acid nanoparticles enhanced anthocyanin content in grape (*Vitis vinifera* L. cv. Red Sultana) berries. *Polymers* 14, 3349
88. Mahmoudi, R. *et al.* (2022) Application of glycine betaine coated chitosan nanoparticles alleviate chilling injury and maintain quality of plum (*Prunus domestica* L.) fruit. *Int. J. Biol. Macromol.* 207, 965–977
89. Mahmoudi, R. *et al.* (2022) Postharvest chitosan-arginine nanoparticles application ameliorates chilling injury in plum fruit during cold storage by enhancing ROS scavenging system activity. *BMC Plant Biol.* 22, 555
90. Bahmani, R. *et al.* (2022) Evaluation of proline-coated chitosan nanoparticles on decay control and quality preservation of strawberry fruit (cv. Camarosa) during cold storage. *Horticulturae* 8, 648
91. Nasr, F. *et al.* (2021) Chitosan-phenylalanine nanoparticles (Cs-Phe Nps) extend the postharvest life of persimmon (*Diospyros kaki*) fruits under chilling stress. *Coatings* 11, 819
92. Zahedi, S.M. *et al.* (2023) Proline-functionalized graphene oxide nanoparticles (GO-pro NPs): A new engineered nanoparticle to

ameliorate salinity stress on grape (*Vitis vinifera* l. cv sultana). *Plant Stress* 7, 100128
93. Ganjavi, A.S. *et al.* (2021) Glycine betaine functionalized graphene oxide as a new engineering nanoparticle lessens salt stress impacts in sweet basil (*Ocimum basilicum* L.). *Plant Physiol. Biochem.* 162, 14–26
94. Gohari, G. *et al.* (2020) Putrescine-functionalized carbon quantum dot nanoparticles (Put-CQD) effectively prime grape (*Vitis vinifera* cv. Sultana) against salt stress. *BMC Plant Biol.* 21, 120
95. Panahirad, S. *et al.* (2023) Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticle: A promising stress-protecting agent against cadmium stress in grapevine (*Vitis vinifera* cv. Sultana). *Plant Physiol. Biochem.* 197, 107653
96. Zhu, L. *et al.* (2022) Carbon-based nanomaterials for sustainable agriculture: their application as light converters, nanosensors, and delivery tools. *Plants* 11, 511
97. Masoudniaragh, A. *et al.* (2021) Using halloysite nanotubes as carrier for proline to alleviate salt stress effects in sweet basil (*Ocimum basilicum* L.). *Sci. Hortic.* 285, 110202
98. Shelar, A. *et al.* (2023) Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: challenges, risk assessment, and future perspectives. *Nano-Micro Lett.* 15, 54
99. Gade, A. *et al.* (2023) Nanofertilizers: the next generation of agrochemicals for long-term impact on sustainability in farming systems. *Agrochemicals* 2, 257–278
100. Xin, X. *et al.* (2020) Nano-enabled agriculture: from nanoparticles to smart nanodelivery systems. *Environ. Chem.* 17, 413–425
101. Patra, J.K. *et al.* (2018) Nano based drug delivery systems: recent developments and future prospects. *J. Nanobiotechnol.* 16, 71
102. Vega-Vásquez, P. *et al.* (2020) Nanoscale drug delivery systems: from medicine to agriculture. *Front. Bioeng. Biotechnol.* 8, 79
103. Idumah, C.I. (2023) Design, development, and drug delivery applications of graphene polymeric nanocomposites and bionanocomposites. *Emergent Mater.* 6, 777–807
104. Xu, C. *et al.* (2019) Biodegradable nanoparticles of polyacrylic acid-stabilized amorphous $CaCO_3$ for tunable pH-responsive drug delivery and enhanced tumor inhibition. *Adv. Funct. Mater.* 29, 1808146
105. Li, C. *et al.* (2023) Recent progress in nanotechnology-based drug carriers for resveratrol delivery. *Drug Deliv.* 30, 2174206
106. Venugopalan, V.K. *et al.* (2022) Smart fertilizers – a way ahead for sustainable agriculture. *J. Plant Nutr.* 45, 2068–2076
107. Lowry, G.V. *et al.* (2019) Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nat. Nanotechnol.* 14, 517–522
108. Lv, J. *et al.* (2019) Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges. *Environ. Sci. Nano* 6, 41–59
109. Husted, S. *et al.* (2022) What is missing to advance foliar fertilization using nanotechnology? *Trends Plant Sci.* 28, 90–105
110. Schwab, F. *et al.* (2016) Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – critical review. *Nanotoxicology* 10, 257–278
111. Pacheco, I. and Buzea, C. (2018) Nanoparticle uptake by plants: beneficial or detrimental? In *Phytotoxicity of Nanoparticles* (Faisal, M. *et al.*, eds), pp. 1–61, Springer
112. Deng, C. *et al.* (2020) Bok choy (*Brassica rapa*) grown in copper oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation, biodistribution and nutritional disturbance. *J. Hazard. Mater.* 398, 122978
113. Zhao, L. *et al.* (2016) Metabolomics to detect response of lettuce (*Lactuca sativa*) to $Cu(OH)_2$ nanopesticides: oxidative stress response and detoxification mechanisms. *Environ. Sci. Technol.* 50, 9697–9707
114. Ghafariyan, M.H. *et al.* (2013) Effects of magnetite nanoparticles on soybean chlorophyll. *Environ. Sci. Technol.* 47, 10645–10652
115. Hong, J. *et al.* (2014) Evidence of translocation and physiological impacts of foliar applied CeO_2 nanoparticles on cucumber (*Cucumis sativus*) plants. *Environ. Sci. Technol.* 48, 4376–4385
116. Eichert, T. and Goldbach, H.E. (2008) Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces – further evidence for a stomatal pathway. *Physiol. Plant.* 132, 491–502
117. Gohari, G. *et al.* (2020) Modified multiwall carbon nanotubes display either phytotoxic or growth promoting and stress protecting activity in *Ocimum basilicum* L. in a concentration-dependent manner. *Chemosphere* 249, 126171
118. Ahmed, B. *et al.* (2021) Nanoparticles in the soil–plant system: a review. *Environ. Chem. Lett.* 19, 1545–1609
119. Peng, C. *et al.* (2020) Bioavailability and translocation of metal oxide nanoparticles in the soil-rice plant system. *Sci. Total Environ.* 713, 136662
120. Aslani, F. *et al.* (2014) Effects of engineered nanomaterials on plants growth: an overview. *Sci. World J.* 2014, 641759
121. Wang, W.N. *et al.* (2013) Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *J. Nanopart. Res.* 15, 1417
122. Khodakovskaya, M. *et al.* (2009) Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano* 3, 3221–3227
123. Yu, H. *et al.* (2022) Integrating machine learning interpretation methods for investigating nanoparticle uptake during seed priming and its biological effects. *Nanoscale* 14, 15305–15315
124. Raliya, R. *et al.* (2016) Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using ZnO nanoparticles synthesized by soil fungi. *J. Agric. Food Chem.* 64, 3111–3118
125. Wang, P. *et al.* (2016) Nanotechnology: a new opportunity in plant sciences. *Trends Plant Sci.* 21, 699–712
126. Dong, R. *et al.* (2019) Recent developments in luminescent nanoparticles for plant imaging and photosynthesis. *J. Rare Earths* 37, 903–915
127. Demirer, G.S. (2023) Detecting and quantifying nanoparticle-mediated biomolecule delivery in plants. *Nat. Rev. Methods Primers* 3, 16
128. Detection methods of nanoparticles in plant tissues. In *New Visions in Plant Science* (Yan, A. *et al.*, eds), 74101, InTechOpen
129. Proença, P.L. *et al.* (2022) Fluorescent labeling as a strategy to evaluate uptake and transport of polymeric nanoparticles in plants. *Adv. Colloid Interf. Sci.* 305, 102695
130. Sun, D. *et al.* (2014) Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. *Plant Cell Rep.* 33, 1389–1402
131. Wang, H. *et al.* (2019) Deep learning enables cross-modality super-resolution in fluorescence microscopy. *Nat. Methods* 16, 103–110
132. Wu, H. *et al.* (2017) Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. *ACS Nano* 11, 11283–11297
133. Wong, M.H. *et al.* (2016) Lipid exchange envelope penetration (LEEP) of nanoparticles for plant engineering: a universal localization mechanism. *Nano Lett.* 16, 1161–1172
134. Scheringer, M. (2008) Nanoecotoxicology: environmental risks of nanomaterials. *Nat. Nanotechnol.* 3, 322–323