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Mitigating abiotic stress in crop plants using nanoformulations for climate-resilient agriculture

S Anbuselvan[1], A Alagesan[2*], D Rajakumar[1], J Balamurugan[3] & B Karthikeyan[4]

[1]Department of Agronomy, V.O. Chidambaranar Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam 628 252, Tamil Nadu, India

[2]Department of Agronomy, Dr. M.S. Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai 614 902, Tamil Nadu, India

[3]ICAR-Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Pudukottai 622 303, Tamil Nadu, India

[4]Department of Agricultural Microbiology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Chettinad, Sivagangai 630 102, Tamil Nadu, India

***Correspondence:** A Alagesan, alagesan@tnau.ac.in

Abstract

Abiotic stresses, particularly drought, salinity, heat and heavy metal toxicity, increasingly constrain crop productivity and threaten sustainable food production. Although nanoformulations have demonstrated considerable potential for stress mitigation, the evidence remains fragmented across nanoparticle types, crops, stress conditions and application doses and a critical synthesis linking dose response, underlying mechanisms, multi-omics evidence, field performance and safety is still lacking. This review critically synthesises advances in nanoformulations for abiotic stress management, emphasising dose-dependent responses, physiological and biochemical mechanisms, molecular regulation, multi-omics insights, environmental implications and agricultural applications. Nanoformulations are broadly categorised into metal and metal-oxide, silicon-based, carbon-based, biopolymeric and composite or smart systems to provide a focused assessment of their functional roles. Their stress-alleviating effects are primarily associated with enhanced antioxidant defence, osmotic adjustment, photosynthetic protection, ion homeostasis, nutrient utilisation and stress responsive signalling. Quantitative evidence highlights their potential; for example, foliar application of zinc oxide nanoparticles (ZnO NPs) at 100 mg L^{-1} increased grain yield by 42.2 % and root dry weight by 63.1 % in salt-stressed wheat under experimental conditions. Multi-omics approaches integrating transcriptomics, proteomics, metabolomics and ionomics are increasingly revealing NP–plant interactions and stress-response networks. Their convergence with artificial intelligence (AI)-assisted precision agriculture could further support prediction of stress responses and optimisation of nanoformulation dose, timing and crop-specific application. However, dose standardisation, multi-season and multi-location field validation, NP transformation and persistence, soil microbiome effects, food-chain transfer and harmonised safety assessment remain major challenges. Future research should integrate smart nanoformulations, multi-omics and AI-driven precision deployment with rigorous field and environmental safety validation. Nanoformulations therefore represent a next-generation strategy for precise, sustainable and climate-resilient agriculture.

Keywords: abiotic stress; climate-resilient agriculture; multi-omics; nanoformulations; plant stress responses; sustainable agriculture

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1. Introduction

Global food security faces a crisis at an unprecedented level from the combined forces of population expansion and abiotic stress driven by climate change. In major food crops worldwide, total crop loss from the combined effects of drought, salinity, excessive heat and heavy metals may exceed 50 % (1). At present, salinised lands already span over 800 million ha on the global scale, a value predicted to further increase due to global warming (2). In fact, under such environmental stresses, damage to plants is caused by the overproduction of reactive oxygen species (ROS), which leads to damage of proteins and nucleic acids and disruptions in cellular metabolism. The cellular damage induced by ROS then leads to growth inhibition and productivity reduction. Conventional approaches for managing crop stress, including intensive irrigation and excessive use of mineral fertilisers and synthetic pesticides, can improve crop productivity but may also contribute to environmental concerns such as freshwater depletion, irrigation induced salinisation, greenhouse-gas emissions and deterioration of soil and water quality (3). Although conventional breeding and transgenic approaches have contributed to the development of stress-tolerant crop lines, improving abiotic stress tolerance remains challenging because these traits are typically polygenic and strongly influenced by genotype × environment interactions (4).

Nanotechnology is an emerging and highly promising field within agricultural science. Nanoparticles

(NPs) can be engineered to have physicochemical characteristics (e.g. A high surface-area-to-volume ratio, quantum confinement effect or an adjustable surface chemistry), which may facilitate specific interactions with plant biochemistry (5, 6). In comparison with bulk input applications, nanoformulations can be engineered for controlled release, stimulated responses, improved delivery and greater bioavailability. A large body of literature, initiated by findings on carbon nanotube-mediated plant growth promotion and germination enhancement (7), has shown that a wide range of nano formulations can improve the plant stress response in numerous experimental and some field conditions. The existence of contradictory results such as enhanced growth or phytotoxicity at varying levels of NP concentration, deviates between lab and field experiments and a lack of standardisation in dosages have all indicated that a critical synthesis is needed, rather than a descriptive accumulation (8). This review aims to address this research gap, providing a structured critical analysis on the different classes of nano formulations, the uptake mechanism of NPs by plants and the signalling mechanism mediating plant stress response both at the physiological and molecular levels, as well as an in depth evaluation on omics studies, field performance, commercial viability and regulatory consequences, economic considerations, environmental impact, all to clearly identify present gaps and to outline future directions.

Methodology and literature review

A systematic literature search was conducted to identify relevant studies on the role of nanoformulations in mitigating abiotic stresses in crop plants. The literature search covered publications from January 1997 to March 2025 and was performed using Web of Science, ScienceDirect, Scopus and Google Scholar. Search terms were developed around three major concepts: nanoformulations or NPs, abiotic stresses and crop plants. Boolean operators AND and OR were used to combine related terms. The principal search concepts included combinations of terms such as: “nanoformulations” OR “nanoparticles” OR “nanomaterials” OR “nano-fertilisers” AND “abiotic stress” OR drought OR salinity OR “heat stress” OR “cold stress” OR “heavy metal stress” OR “oxidative stress” AND crop OR plant OR agricultural crops. Additional searches were conducted using specific combinations addressing nanoparticle uptake and translocation, antioxidant defence, omics approaches, sustainable nano-fertilisers, environmental risks and regulatory aspects. Database-specific search strings were adapted to the syntax of each database. A total of 1657 records were initially identified. Duplicate records (n = 387) were removed through database filtering and manual verification, leaving 1270 records for title and abstract screening. Following screening, 978 records were excluded and 292 full-text articles were assessed for eligibility. After full-text evaluation, 192 articles were excluded based on predefined eligibility criteria, resulting in 100 studies being included in the final qualitative synthesis. The inclusion criteria comprised peer-reviewed research articles published in English between 2010 and 2025 that evaluated nanoformulations, including NPs, nanocomposites and nano-encapsulated products, for mitigating drought, salinity, heat, cold, heavy-metal or oxidative stress in agriculturally important crops. Studies reporting physiological, biochemical, molecular, growth, yield or stress-response parameters were considered eligible. Studies were excluded when they were unrelated to abiotic stress mitigation, lacked adequate information on the nanoformulation, focused primarily on non-crop or model plants without clear agricultural relevance, were conference abstracts, editorials or opinion papers, or represented duplicate or overlapping publications. The overall methodology used for literature identification, screening, eligibility assessment and final study selection is illustrated in Fig. 1. The search strategies used and the number of articles retrieved from each database are summarised in Table 1.

Table 1. Strategies for collecting appropriate review articles

Search strategy	Database Group 1 (Web of Science and Google Scholar)	Database Group 2 (ScienceDirect and Scopus)
Nanoformulations and abiotic stress management in crop plants	92	129
Nanoparticles and drought stress tolerance in plants	78	113
Nanoparticles and salinity stress mitigation in crops	84	119
Nanoformulations and heat stress tolerance in plants	49	75
Nanoparticles and heavy metal stress alleviation in crops	63	88
Nanoparticle uptake, translocation and fate in plants	56	81
Nanotechnology and plant antioxidant defence mechanisms	73	102
Omics approaches in nanoparticle–plant interactions	41	65
Nano-fertilisers and sustainable agriculture	99	135
Environmental risks and regulatory aspects of agricultural nanomaterials	45	70

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Quality assessment and evidence reliability

To assess the methodological rigor and reliability of the selected studies were evaluated using predefined criteria, including (i) experimental design and replication; (ii) crop species and growth conditions; (iii) NP/nanoformulation type and physicochemical characteristics; (iv) particle size and concentration/dose; (v) method and timing of application; (vi) abiotic stress treatment and severity; (vii) appropriateness of control treatments; (viii) physiological, biochemical or molecular response measurements; (ix) statistical analysis and significance testing; and (x) sufficient methodological information to permit reproducibility. Studies providing clear information across these criteria were considered to provide stronger evidence for the synthesis. Greater emphasis was placed on studies with well-defined controls, adequate replication, characterised nanoformulations and clearly described stress-treatment protocols. The quality assessment was used to inform the interpretation of the evidence rather than to exclude studies solely based on their quality.

Study screening and data extraction

Study screening and data extraction were independently performed by two reviewers. Titles and abstracts were initially screened against the predefined inclusion and exclusion criteria. Potentially relevant articles were subsequently subjected to full-text assessment. The same eligibility criteria were applied during both stages. Disagreements between the reviewers regarding study eligibility or extracted information were resolved through discussion and consensus; when consensus could not be reached, a third reviewer was consulted. For each eligible study, information was extracted on crop species, nanoformulation type, NP characteristics, concentration, application method, abiotic stress type, experimental conditions, physiological and biochemical responses, molecular or omics responses, mechanisms of stress mitigation and major outcomes. The extracted information was subsequently organised into comparative tables and synthesised thematically according to NP class, stress type and biological response.

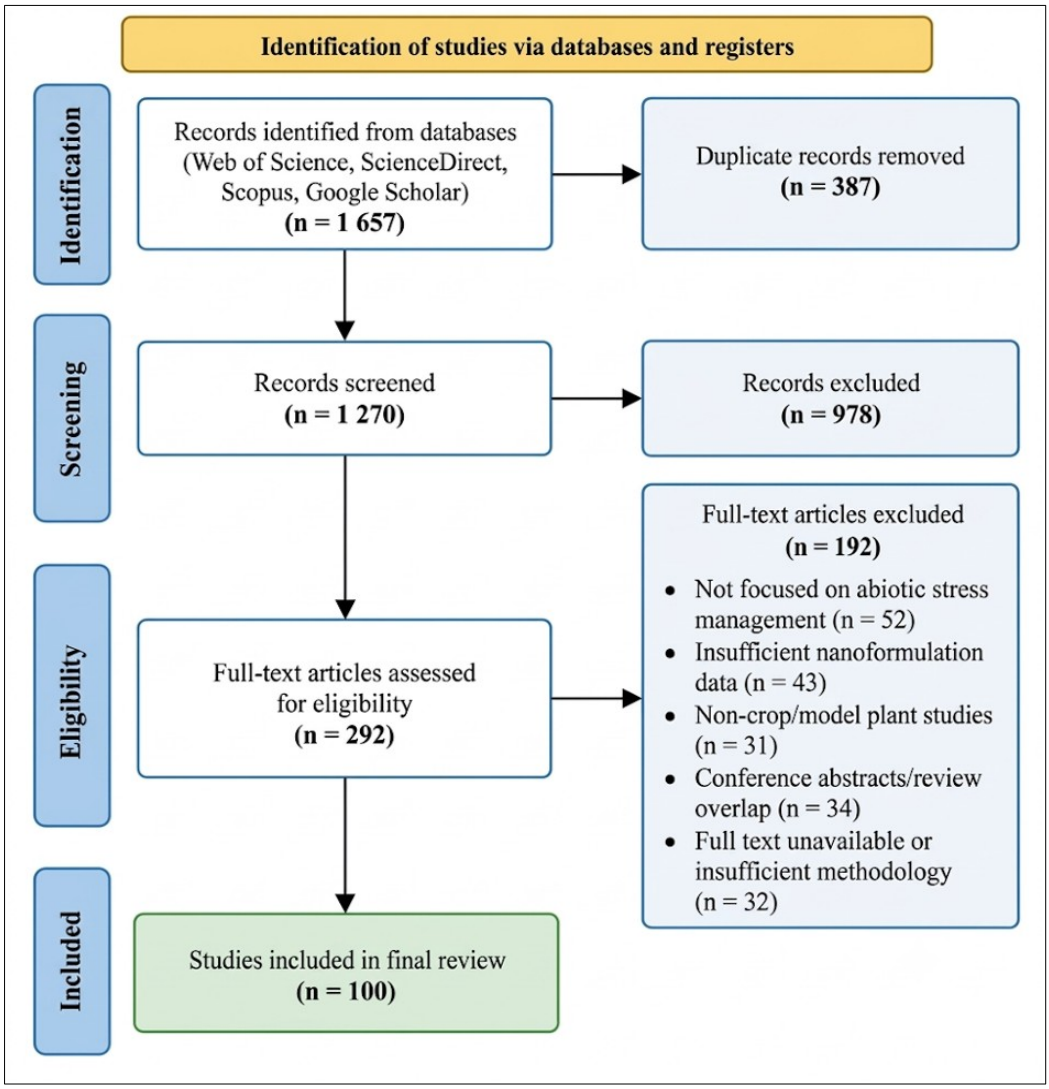


Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram outlining the study selection process.

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Categorisation of nanoformulations

The wide range of nanoformulations employed in stress-resilience studies reflects the diversity of nanomaterial chemistry and the complex nature of plant responses to abiotic stress. Classification based on chemical composition and structural characteristics provides a consistent framework for comparing nanoformulations with distinct physicochemical properties, which directly influence their uptake, transport, reactivity, stability and potential risks. In contrast, classification solely by function, mode of action, or application method may overlap across different nanomaterial types because a single nanoformulation can exhibit multiple functions and may be applied through different routes. Accordingly, nanoformulations can be broadly categorised into metal NPs, metal oxide NPs, silicon-based nanoparticles, carbon-based NPs, biopolymeric NPs and composite/smart nanoformulations. These categories may enhance abiotic stress tolerance through different mechanisms, including modulation of antioxidant defence, regulation of ion homeostasis, improvement of water and nutrient uptake, enhancement of photosynthetic performance, reduction of oxidative damage and regulation of stress-responsive signaling pathways. This composition and structure-based categorisation therefore facilitate a comparative assessment of their efficacy, mechanisms of stress mitigation and potential environmental and phytotoxicological risks. Several nanoformulation examples, coupled with their effects on abiotic stress responses across species are shown in Table 2.

Table 2. Role of nanoparticles in alleviating abiotic stress in crop plants through physiological, biochemical and molecular mechanisms of stress tolerance

Nanoparticle type	Abiotic stress	Plant species (Scientific name)	Experimental approaches	Dosage	Physiological / Biochemical response	Reference
Chitosan NPs	Drought	Wheat *(Triticum aestivum)*	Field experiment	90 ppm	Elevated chlorophyll content and relative water content; stomatal regulation improved	(29)
Chitosan-Se NPs and TiO_2 NPs	Salinity	Bitter gourd *(Momordica charantia)*	Pot experiment	100 mg L^{-1} TiO_2 & 20 mg L^{-1} Cs-Se NPs Cs-Se	Enhanced photosynthetic rate, plant growth parameters and marketable yield	(30)
Si NPs	Drought	Wheat *(Triticum aestivum)*	Pot experiment	100 mg kg^{-1}	Attenuation of oxidative stress markers; increased chlorophyll concentration and water use efficiency	(31)
Si NPs	Heavy metal	Wheat *(Triticum aestivum)*	Pot experiment	25 mg kg^{-1}	Boosted SOD and POD activity; decreased H_2O_2, electrolyte leakage, MDA and Cd accumulation	(32)
SiO_2 NPs	Drought	Potato *(Solanum tuberosum)*	Field experiment	50 mg L^{-1}	Improved vegetative growth parameters and significant reduction in drought-induced yield losses	(9)
ZnO NPs	Drought	Eggplant *(Solanum melongena)*	Field experiment	100 ppm	Augmented chlorophyll content, shoot biomass and relative water content under deficit irrigation	(33)
ZnO NPs	Drought	Tomato *(Solanum lycopersicum)*	Pot experiment	25 mg L^{-1}	Up-regulation of antioxidant enzyme activities and enhancement of overall drought tolerance index	(34)
ZnO NPs	Drought	Maize *(Zea mays)*	Pot experiment	100 mg L^{-1}	Stimulated melatonin biosynthesis and up-regulated antioxidant-related gene expression	(35)
ZnO NPs	Salinity	Eggplant *(Solanum melongena)*	Pot experiment	80 mg L^{-1}	Improved shoot growth, soluble sugar accumulation, total protein and free amino acid content	(36)
ZnO NPs	Temperature	Rice *(Oryza sativa)*	Hydroponic experiment	100 mg L^{-1}	Enhanced antioxidant gene expression and reduction in chilling-induced oxidative damage	(37)
Se NPs	Temperature	Tomato *(Lycopersicon esculentum)*	Field experiment	75 ppm	Improved seedling vigour, root length and overall morphological growth characteristics under heat	(38)
Fe NPs	Drought	Strawberry *(Fragaria x ananassa)*	Laboratory experiment	1 mg L^{-1}	Reduced reactive oxygen species accumulation and strengthened antioxidant enzymatic defence	(39)

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Fe_2O_3 NPs	Heavy Metal	Rice *(Oryza sativa)*	Pot experiment	100 mg L^{-1}	Increased plant height; improved fresh and dry biomass under cadmium toxicity	(6)
Ag NPs	Salinity	Pearl millet *(Pennisetum glaucum)*	Laboratory experiment	20 mg L^{-1}	Mitigated oxidative damage; enhanced superoxide dismutase and catalase activities under salt stress	(40)
Ag NPs	Temperature	Wheat *(Triticum aestivum)*	Pot experiment	75 mg L^{-1}	Up-regulation of heat-shock and antioxidant defence genes under high temperature stress	(11)

NPs: nanoparticles; Chitosan NPs: chitosan nanoparticles; Chitosan–Se NPs: chitosan–selenium nanoparticles; Si NPs: silicon nanoparticles; SiO_2 NPs: silicon dioxide nanoparticles; ZnO NPs: zinc oxide nanoparticles; Se NPs: selenium nanoparticles; Fe NPs: iron nanoparticles; Fe_2O_3 NPs: iron oxide nanoparticles; TiO_2 NPs: titanium dioxide nanoparticles; MgO NPs: magnesium oxide nanoparticles; Ag NPs: silver nanoparticles; SOD: superoxide dismutase; POD: peroxidase; H_2O_2: hydrogen peroxide; MDA: malondialdehyde; AMF: arbuscular mycorrhizal fungi; N: nitrogen; P: phosphorus; K: potassium; NaCl: sodium chloride.

Metal nanoparticles

Metal NPs (silver (Ag), gold (Au), copper (Cu), selenium (Se) and zinc (Zn)) are mainly produced by the techniques of chemical reduction, green synthesis and physical deposition. The roles of metal NPs in stress reduction are attributed to a variety of mechanisms, such as the direct antioxidant ability, ion-dependent enzyme activities and membrane stabilisation (9, 10). The Ag NPs (10–50 nm) can not only act as antimicrobial and antioxidant, but can be used to modulate reactive oxygen species (ROS) accumulation and enhance the survival in combination of heat stress (11). Biosynthesised Cu NPs (9–18 nm) alleviated the adverse effects of salinity stress in barley (*Hordeum vulgare* L.). The Cu NPs applied at 25 and 50 mg L^{-1} under 100 and 200 mM sodium chloride (NaCl) stress improved plant growth, photosynthetic pigments and gas-exchange parameters. Moreover, Cu NP application reduced salt-induced oxidative damage by decreasing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulation and enhancing antioxidant enzyme activities, with the effects depending on the Cu NP concentration and salinity level (12). The Se NPs have been reported to enhance antioxidant defence and improve plant tolerance to abiotic stress by promoting the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), thereby reducing oxidative damage under stress conditions (13). A significant limitation of metal nanoparticle application is ionic dissolution and the consequent active metal ions accumulation in the soil.

Metal oxide nanoparticles

Metal oxide nanoparticles (MONPs), including zinc oxide (ZnO), titanium dioxide (TiO_2), iron oxide (Fe_2O_3), cerium dioxide (CeO_2), manganese dioxide (MnO_2) and nickel oxide (NiO), represent one of the most extensively investigated nanoformulation classes in crop stress management. Their comparative advantage is their ability to simultaneously influence nutrient availability, antioxidant metabolism, photosynthetic activity and ion homeostasis. Nevertheless, their effectiveness is highly dependent on NP composition and stress type.

Among MONPs, ZnO NPs show comparatively strong evidence under salinity, because Zn released from the particles can support enzymes such as carbonic anhydrase and superoxide dismutase while influencing sodium (Na^+)/potassium (K^+) homeostasis through regulation of ion transport processes (14, 15). Applications of 50–100 mg L^{-1} ZnO have been associated with substantial yield improvement in salt-stressed wheat, although this concentration range should not be interpreted as universally optimal because responses vary with crop, soil conditions and exposure route. The TiO_2 NPs (25–50 nm), in contrast, appear particularly effective where photosynthetic impairment is a major component of drought or heat stress, with reported increases in photosynthetic efficiency and biomass accumulation (16). Cerium dioxide (CeO_2) NPs differ mechanistically from ZnO and TiO_2 because their major advantage is redox buffering: reversible Ce^{3+}/Ce^{4+} cycling enables SOD-like ROS-scavenging activity and potentially prolonged protection against oxidative damage (17). Iron oxide (Fe_2O_3) NPs have a more specialised role under heavy-metal stress, where surface adsorption and co-precipitation can reduce cadmium (Cd) bioavailability; however, their performance is strongly influenced by soil pH and therefore may be less transferable across field conditions (18).

Collectively, MONPs provide a broader mechanistic portfolio than individual metal NPs, but their optimal application is stress-specific: ZnO is particularly relevant to ionic and nutritional imbalance, TiO_2 to photosynthetic dysfunction, CeO_2 to oxidative stress and Fe_2O_3 to heavy-metal immobilisation. This specialisation represents a major strength but also limits the identification of a single universally superior MONP. Furthermore, dissolution, aggregation and transformation in soil can alter their bioavailability and toxicity. Thus, the apparent high efficacy of MONPs in controlled experiments should be interpreted cautiously until supported by field-scale studies incorporating soil chemistry and long-term environmental fate.

Silicon-based nanoparticles

Silicon (Si) is a non-essential but beneficial element to plants and nano-SiO_2 (5–50 nm) is one of the most field applicable nanoformulations given the advantage of low cost, high biosafety and multi-functional mechanism. During drought, nano-SiO_2 upregulates expression of aquaporin (tonoplast intrinsic protein (TIP) and plasma membrane intrinsic protein (PIP) family) to enhance

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efficient transport of water in cells while accumulating on leaf surface to reduce stomatal conductance and cuticular water loss, achieving a 2–35 % improvement in water use efficiency in rice and wheat (6**)**. Under saline condition, Si NPs compete for binding of transporter site with Na^+, reduced apoplastic bypass flow of ions and increased lignin deposition in Casparian strip to enhance selective exclusion of ions (19). Higher surface area and better tissue penetration in nanoscale distinguish it from the bulk SiO_2. Mesoporous silica nanoparticles (MSNPs) loaded with osmolytes, phytohormones or gene-editing construct have been designed as intelligent nanocarriers, a novel approach of nanoformulation design (20).

Carbon-based nanomaterials

A structurally heterogeneous class including carbon nanotubes (CNT) (single-walled (SWCNT); multi-walled (MWCNT)), graphene oxide (GO), fullerenes (C_{60} and C_{70}) and carbon nanohorns is the carbon-based nanomaterials class that presents special properties at nanoscale. The CNTs (diameter 1–100 nm, length can reach few micrometre) promote the aquaporin of the water channels in root and increase the nutrient uptake by interaction with root epidermis, promoting WUE by 18-25 % during drought (21). However, there is a very strong dependence between toxicity and concentrations and functional groups on CNTs; pristine MWCNTs are more phytotoxic than carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) (22). Graphene oxide (2–10 nm thickness) at 50–200 mg L^{-1} promote root elongation through an auxin-like signalling pathway whereas higher concentrations cause cell wall damage. Limited cost and the difficulty in scaling synthesis and environmental fates tracing are two major limitations (23).

Chitosan nanoparticles

Chitosan nanoparticles (CS-NPs), which are synthesised by deacetylating chitin are the most tested and validated nanoformulation in stress resilience agriculture. Their positive charge on the surface allows them to interact electrostatically with cell walls without being cytotoxic at the application rate of 0.1–0.5 % (24). The CS-NPs can promote the accumulation of osmolytes (proline, glycine betaine), stress-responsive gene expressions (*DREB2A, RD29A*) and modulate the abscisic acid (ABA) signalling pathway under drought and salinity (24, 25). The dual functionality as nano-fertiliser carriers and direct elicitors of systemic induced stress tolerance is a main benefit of this nanomaterial. Wheat treated with CS-NPs plus biochar under salinity stress can increase the growth parameters by 28–42 % and promote antioxidant defence system (24). The biodegradable property and the generally recognised as safe (GRAS) status of these materials provide a major advantage from a regulatory perspective, facilitating their acceptance and potential commercialisation.

Composite and smart nanoformulations

Composite nanoformulations incorporate two or more classes of nanomaterials or combine nanoparticles with organic matrices to enable cooperative functionalities. Examples of composites include ZnO-CS composites, silica-coated Fe_2O_3 NPs and nano emulsions of phytohormones (26). The leading edge of nanoformulation development lies in the synthesis of smart or stimulus responsive nanoformulations. For instance, ABA or salicylic acid (SA) loaded mesoporous silica, gated with pH, temperature or ROS sensitive materials, delivers the contents to the intended location and duration of stress, reduces off-target risks and consequently, the required dosage by 30–50 % relative to their free counterpart (27). Fertilisers with controlled release properties were nano-encapsulated with polyurea, ethyl cellulose or starch to improve nitrogen use efficiency (NUE) (15–30 %), synchronising nutrient release with plant requirements (28). The complexity of composite nanoformulations has added to production costs and regulatory constraints which, likely delay large scale commercial applications of composite nanoformulations. Several nanoformulation examples, coupled with their effects on abiotic stress responses across species are shown in Table 2.

Nanoparticle uptake, translocation and fate in plant systems

The pathways by which nanoparticles are taken up, translocated and ultimately fate-determined within the plant system are of fundamental importance for designing effective and safe nano-formulations.

Foliar uptake through stomatal and cuticular pathways

Foliar application is an effective delivery route of nanoparticles bypassing physicochemical interactions that immobilise particles in the soil. Nanoparticles less than 20nm will pass directly through stomatal pores of a width of 20-60nm; alternatively hydrophilic nanoparticles less than 40–50 nm can diffuse through the cuticle along hydrophilic channels (41). Hydrophobic (lipophilic) NPs such as carbon-based nanomaterials are transported through lipid pathways in the cuticle's waxy layer. Once inside the leaf tissues, NPs are transported through the apoplast where they come into contact with cell wall and after interaction with the cell wall either continue their pathway through apoplastic continuum or cross the plasma membrane (41, 42).

Root uptake through symplastic and apoplastic pathways

Root uptake depends critically on size, surface charge and rhizosphere physicochemical state of the nanoparticles. Nanoparticles of a diameter < 20 nm can enter plants through apoplastic movement, whereas particles of a diameter < 10 nm or with surface functionalisation may pass the Casparian strip to enter the symplast via endocytosis or mediated transport (43). Charged particles interact with charged cell wall components (pectin and hemicellulose) so the cell wall may act as an obstacle for particle transport and especially positively charged particles can be retained by a negative cell wall charge (44). After entry into the stele, particles are loaded into the xylem vessel and acropetal (towards the shoot) transport via bulk flow can take place (45, 43).

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Vascular transport and intracellular internalisation

Nanoparticles travelling within the xylem are propelled by transpirational pull and can reach the leaf mesophyll cells within hours after root exposure. Transport through the phloem can facilitate the bidirectional translocation of NPs, enabling movement toward sink tissues such as roots, developing leaves, fruits and reproductive organs (43). Cellular internalisation can take place through endocytosis (clathrin-mediated endocytosis or caveolae-mediated endocytosis) or for very small (< 5nm) particles direct crossing the plasma membrane. Once inside the cell, NPs travel within vesicles that originates from endosomes and transfer into vacuoles; some particles may also be transferred to the organelles such as chloroplasts and mitochondria and directly interfere with organellar biochemistry (46, 47). After internalisation, the NPs undergo biotransformation (dissolution, oxidation, sulfidation and biomineralisation) and are then likely to be accumulated in vacuoles or cell walls which can serve as an ionic depot or can be excluded for the detoxification (48, 49). These interconnected processes ultimately influence plant growth, stress tolerance and toxicity responses, as illustrated in Fig. 2.

Methods of application of nanoformulations

The mode and route of nanoformulation application directly influence the quantity reaching target tissues, plant exposure duration and environmental dissemination. In the recent years six routes of application have been thoroughly investigated. Seed treatment with suspensions of NPs (generally 5–100 mg L^{-1} for 8–24 hr) has been described as the simplest and highly scalable root delivery route, induces 'nano-priming memory', a preconditioned state where genes involved in response to various stresses and pool of antioxidant enzymes expression get enhanced before seed germination and thus enhances tolerance in germinating seeds to various stresses (50). Foliar treatment (spraying or misting) applied directly on the leaves of the plant leads to direct and quick uptake without possible adsorption and immobilisation of the NPs in the soil but precise optimisation of surfactant concentration and droplet size is required for effective leaf adhesion and stomatal penetration (41). Soil treatment offers both the plant and soil micro-environment exposure of NPs but are subject to aggregation, adsorption with soil colloids and precipitation and result in greatly reduced bioavailability of NPs in the plant (51). Root dip method where seedling is dipped just prior to transplantation into NP suspension is a highly efficient method for nursery propagated plants and for immediate availability to roots at the seedling stage. Encapsulation of NPs in biodegradable matrices like alginate, starch and CS not only facilitates controlled release but also synchronised release of nutrients or compounds in response to plant physiology and thereby preventing the phytotoxic surge (52). Combined application with biofertilisers like *Rhizobium*, *Azospirillum* and plant growth regulators like gibberellins, cytokinins resulted in better effect than their individual applications (53).

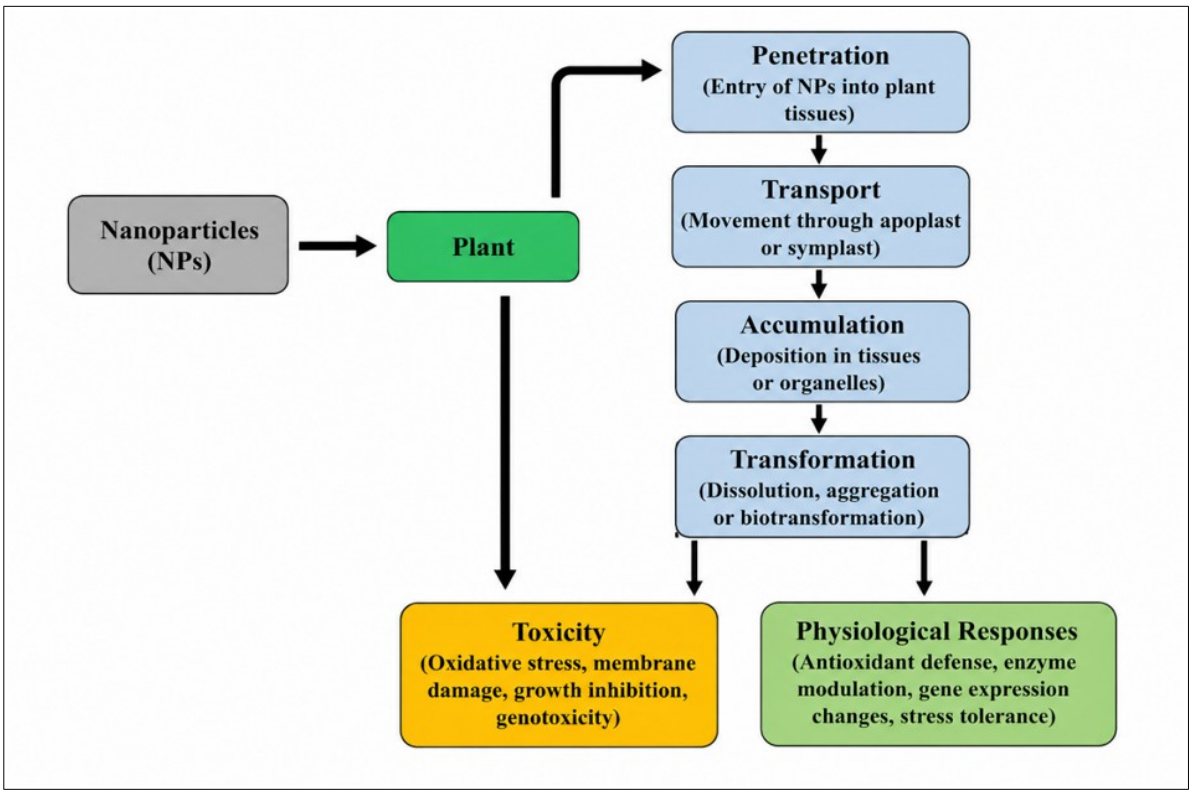


Fig. 2. Conceptual framework illustrating nanoparticle uptake, transport, accumulation, transformation and fate in plants, resulting in physiological adaptations or toxic effects.

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Abiotic stress amelioration mechanisms

Oxidative stress management

The ROS including superoxide radical (O_2^-), H_2O_2, hydroxyl radical (OH) and singlet oxygen ($^1O_2$) are the primary mediators of cellular damage under all forms of abiotic stress (54, 55). Under stress conditions, the balance between ROS production (at Photosystem II (PSII), mitochondrial electron transport chain, NADPH oxidases) and scavenging (enzymatic and non-enzymatic antioxidants) is disrupted, leading to oxidative damage (56). Nanoformulations alleviate this imbalance through multiple convergent mechanisms. The SOD is the first line of defence, converting O_2^- to H_2O_2; ZnO and Mn-based NPs activate Cu/Zn-SOD and Mn-SOD isoforms by supplying metal cofactors (14).The CAT decomposes H_2O_2 to water and O_2; TiO_2 NPs enhance CAT activity by reducing protein carbonylation under heat stress (5). Peroxidase (POD) and ascorbate peroxidase (APX) collectively maintain cellular H_2O_2 homeostasis; CeO_2 NPs, by virtue of their Ce^{3+}/Ce^{4+} redox cycling, directly mimic SOD and CAT activity without requiring transcriptional upregulation (17). Non-enzymatic antioxidants ascorbate, glutathione, tocopherols, flavonoids are elevated by chitosan and biogenic NP treatments, providing complementary protection (57, 58).

Osmotic adjustment

Under drought and salinity stress, plants accumulate compatible solutes to maintain osmotic potential and protect macromolecular structures. Proline, a multifunctional osmolyte is significantly elevated (2-5-fold) by NP treatments, including ZnO NPs (salinity), Si NPs (drought) and chitosan NPs (combined stress) (24, 59). Proline stabilises proteins, scavenges OH and serves as a N reserve during stress recovery. Glycine betaine, synthesised from choline via betaine aldehyde dehydrogenase (BADH) enzyme, is enhanced by Fe_2O_3 and TiO_2 NPs and provides membrane-level protection against osmotic perturbation. Soluble sugars (sucrose, trehalose) accumulate in NP-treated plants, contributing to osmoprotection and serving as signalling molecules that activate stress-responsive gene expression through SNF1-related protein kinase 1 (SnRK1) and target of rapamycin (TOR) kinase pathways (59).

Photosynthetic protection

Photosynthesis is the most vulnerable process under abiotic stress conditions such as heat, drought and salinity. Nanoparticle formulations offer multiple ways to protect photosynthetic capacity. Stabilisation of chlorophylls is achieved through ZnO NPs (Zn as a cofactor in chlorophyllase regulation) and Si NPs (which prevents membrane peroxidation in chloroplast) (6, 8). Nano TiO_2 treatment enhances Rubisco activity under heat stress by upregulating Rubisco activase, thereby sustaining photosynthetic carbon fixation and improving stress tolerance (60). Net photosynthesis rate, intercellular CO_2 concentration, stomatal conductance and biomass production were found to be increased in NP treated plants compared to untreated ones (6, 17).

Nutrient uptake and utilisation

Disruption of ion transporters and reduction in root hydraulic conductivity by stress conditions, affect nutrient uptake. Nanoformulations restore it by providing the respective micronutrient ions which serve as activators or cofactors for enzymes (e.g., ZnO, Fe_2O_3), enhancement of root surface area and root hair development, increased membrane potential and activity of ATPases (28, 61). Nano Fe_2O_3 helps in preventing metal toxicity when applied to soil and provides bioavailable iron which would be helpful under iron deficient and heavy-metal contaminated soils (62).

Hormonal regulation

Phytohormones coordinate plant responses to abiotic stress and nanoformulations may influence these responses through interconnected hormonal and redox signalling networks. Under drought and salinity, NP treatments have been associated with increased ABA levels, accompanied by stomatal closure, accumulation of late embryogenesis abundant (LEA) proteins and enhanced root hydrotropism (63). Si nanoparticles have been reported to enhance SA-associated responses under abiotic stress, including induction of pathogenesis-related (PR) proteins, suggesting a possible role in cross-protection against multiple stresses (64). Chitosan and metal NPs have also been associated with jasmonic acid (JA)-related oxidative responses and secondary-metabolite production, although the extent to which these effects result from direct JA pathway activation requires further validation. Similarly, NP-mediated changes in auxin transport and distribution have been associated with lateral root initiation and proliferation, potentially improving root-system architecture and water and nutrient acquisition under stress (23). Under drought, ZnO NP have been proposed to influence cytokinin-associated responses, with delayed leaf senescence and retention of photosynthetically active leaf area reported following treatment (14). Collectively, these findings suggest that nanoformulations influence multiple hormone-associated responses rather than a single hormonal pathway. However, the interactions among ABA, SA, JA and ethylene remain insufficiently resolved and the potential involvement of ROS and Ca^{2+} as intermediates in NP–hormone crosstalk requires further experimental validation.

Molecular regulation of abiotic stress responses

In molecular terms, nano-formulations induce a network of transcription factor pathways and signalling cascades which contribute to plant stress-adaptive gene expression. Nanoformulations have been reported to upregulate dehydration-responsive element-binding (DREB) transcription factors, including DREB1 (primarily associated with cold stress responses) and DREB2 (mainly involved in drought- and heat-stress responses), in plants exposed to abiotic stress (65). These transcription factors bind to dehydration-responsive element/C-repeat (DRE/CRT) cis-regulatory elements in the promoters of stress-responsive genes, such as *RD29A*, *KIN1* and *COR15A*, thereby enhancing their transcription and promoting cellular adaptation to

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osmotic stress. The WRKY transcription factors constitute a large plant-specific gene family involved in the regulation of stress responses. Members such as *WRKY18*, *WRKY40* and *WRKY70* have been reported to be upregulated in ZnO and TiO_2 NP treated plants under salinity and drought stress, respectively, contributing to improved ROS homeostasis and enhanced SA-mediated signalling (66). The NAC (NAM, ATAF and CUC) transcription factors (TFs) play important roles in regulating membrane stability, senescence, osmolyte accumulation and abiotic stress responses. Transcriptomic analyses have revealed nanoparticle-induced upregulation of NAC TFs in rice under heat stress, suggesting their involvement in NP mediated stress tolerance mechanisms (67). Myeloblastosis (MYB) TFs regulate anthocyanin biosynthesis, cell wall strengthening and stomatal response and CS-NP and Se-NP treatments upregulate expression of MYBs under cold and drought (68).

Mitogen-activated protein kinase (MAPK) cascades transduce signals from extracellular environment to transcriptional regulators. The ZnO NPs treatments increase expression of MPK3/MPK6 in Arabidopsis treated with salt stress and then promote the phosphorylation of WRKY33 and the expression of antioxidant genes (69). Silicon (Si) and CS NPs have been reported to modulate calcium (Ca) signalling networks involving Ca-dependent protein kinases (CDPKs) and calmodulin (CaM). By altering cytosolic Ca^{2+} oscillatory patterns and Ca signatures, these nanoparticles may improve the specificity and efficiency of downstream signalling events, ultimately contributing to enhanced abiotic stress tolerance (69). Nanoparticle application has been associated with the activation of ABA-dependent signalling cascades involving PYR/PYL receptors, PP2C phosphatases and SnRK2 kinases. Concurrently, enhanced expression of *NCED*, which encodes the rate-limiting enzyme in ABA biosynthesis, has been observed, suggesting a role for NPs in the regulation of ABA-mediated stress adaptation (67).

Beyond their damaging effects at high concentrations, ROS serve as essential signalling molecules in plants. Respiratory burst oxidase homolog D (RBOHD) is a plasma membrane-localised NADPH oxidase that generates ROS, particularly H_2O_2, which functions as a key secondary messenger in plant stress and defence signalling pathways this indicates that nanoparticle formulations can fine-tune ROS signalling dynamics, promoting adaptive signalling responses while preventing uncontrolled ROS accumulation and oxidative stress (10). The major mechanisms by which nanoformulations alleviate abiotic stress in plants are summarised in Fig. 3.

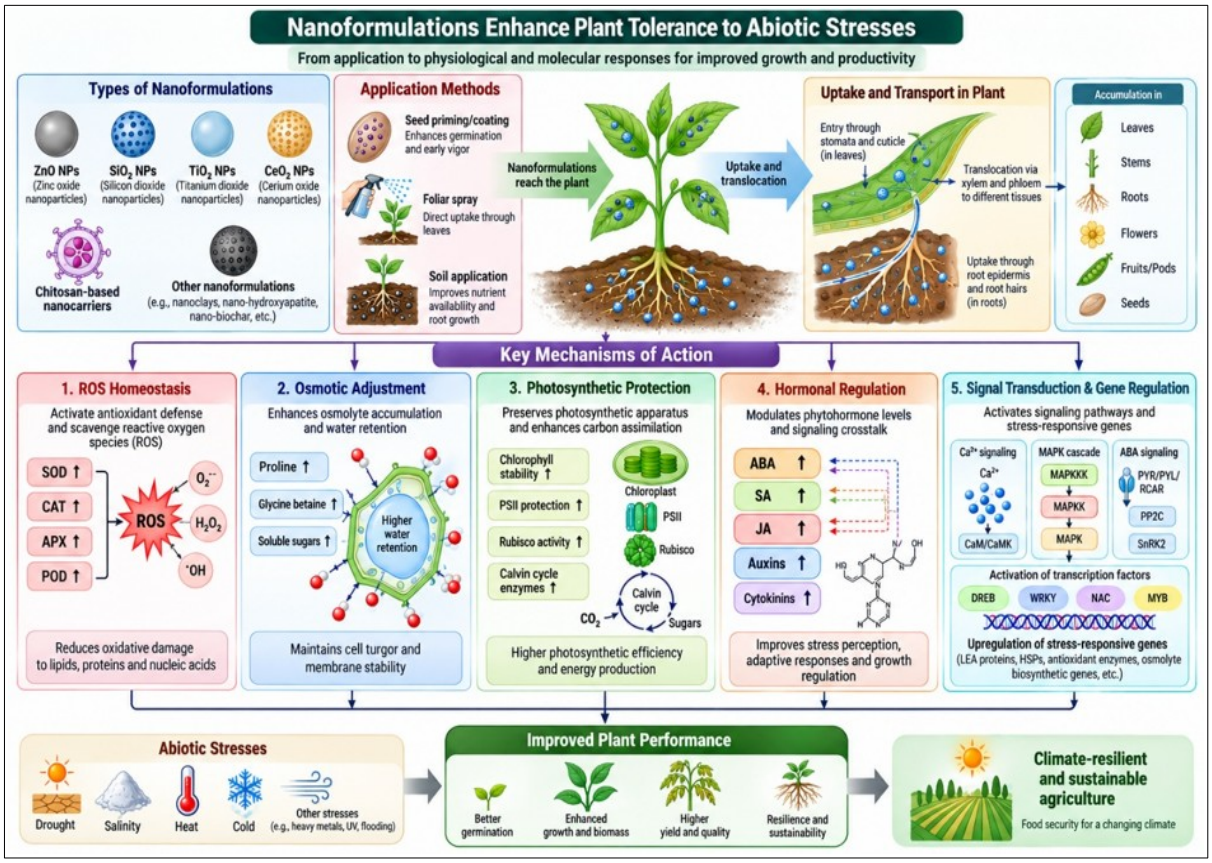


Fig. 3. Integrated regulatory network of physiological, biochemical and molecular responses underlying nanoformulation-mediated abiotic stress tolerance in crop plants.

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Omics approaches in nanoformulation research

Large-scale omics technologies have transformed our understanding of nanoformulation–plant interactions at the genomic, proteomic, metabolomic and ionomic levels, moving beyond descriptive observations toward mechanistic insights. These approaches have enabled a deeper understanding of NP–plant biology, uncovering novel interaction mechanisms such as protein corona formation, NP-induced epigenetic modifications and the reprogramming of metabolic networks. Collectively, these advances are paving the way for a new era of precision nano-agriculture.

Transcriptomics

The RNA sequencing (RNA-seq)-based transcriptomic analyses have provided an unbiased and comprehensive assessment of NP-induced changes in gene expression. In wheat exposed to salinity stress, ZnO NP treatment resulted in the differential expression of more than 1200 genes, with significant enrichment of pathways associated with ion transport (e.g., *SOS1* and *HKT1*), antioxidant defence (*APX* and *SOD*) and stress-responsive transcription factors, including members of the DREB and NAC families (70). Nanoparticle application of SiO_2 NPs on drought stressed rice induced expression of aquaporin gene families (OsPIP1;1, OsTIP1;1) while down-regulating genes associated with senescence indicating increased water use efficiency (6). Temporal transcriptomics also provided insight that NP-induced stress priming involved early activation of stress signalling genes (0–6 hr) followed by upregulation of genes for growth and metabolism (24–72 hr), demonstrating a novel temporal transition from stress signalling to growth-related processes signalling pattern. The CeO_2 NPs (CeO_2 NPs) applied to oxidatively stressed maize plants have been reported to induce the expression of specific clusters of glutathione S-transferase (GST) and peroxidase genes, responses that were not observed to the same extent following treatment with conventional antioxidants (17, 70). This suggests that CeO_2 NPs may activate unique molecular pathways beyond their direct antioxidant activity.

Although still an emerging technology in plant nanobiology, single-cell RNA sequencing (scRNA-seq) has provided valuable insights into tissue- and cell-type-specific responses to NP exposure. Such approaches have revealed differential transcriptional responses among cell populations and have indicated preferential NP accumulation in actively growing tissues, including meristems and vascular bundles (71).

Proteomics

The use of two-dimensional gel electrophoresis (2-DE) and mass spectrometry-based proteomic techniques, has shown that there are significant alterations in the abundance of specific proteins and the modifications of proteins after exposure to NPs. The proteins that are associated with stress tolerance, such as heat shock proteins (HSP70, HSP90 and small HSPs), LEA proteins and antioxidant enzymes have been frequently reported in plants that were treated with NPs (71). In soybean under drought stress, proteomic study after the treatment with CeO_2 NP (CeO_2 NP) showed the upregulation of important Calvin cycle enzymes, such as fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase), indicating improved carbon fixation efficiency and photosynthesis performance (17). Additionally, there are indications that different phosphorylation states of proteins, mediated by CDPKs and SNF1-related protein kinase 2 (SnRK2) family members, are an integral part of the signalling networks triggered by NPs, with distinct signatures (69).

Another emerging idea is the presence of a protein corona formed by the binding of plant endogenous proteins to the surface of NPs. The physicochemical characteristics of the NPs are important for determining the composition of this corona and can significantly affect the ability of these NPs to be taken up by cells, to traverse the cell and to elicit a biological response. For instance, ZnO NPs, exposed to root exudates are reported to have become covered by protein coronas composed of cell wall-modifying proteins and stress-associated chaperones, furnishing new insights in the molecular interactions that happen earlier than uptake by plant cells of NPs (70).

Metabolomics

Nuclear magnetic resonance (NMR) or mass spectrometry has demonstrated that in plants, treatments with NPs cause widespread metabolic reprogramming under abiotic stress conditions apart from osmolyte accumulation. For example, SiO_2 NPs boosted the amount of gamma-aminobutyric acid (GABA), putrescine and trehalose in drought-stressed rice, while decreasing the amount of abscisic acid glucosyl ester, thus improving ABA-mediated stress regulation (6). The CeO_2 NPs did encourage the accumulation of isoflavones and increase photosynthetic efficiency in drought stressed soybeans (17). Likewise, wheat subjected to both heat-stress and salinity showed higher production of polyamines and activity of the GABA shunt, which were correlated with stress tolerance (24). The production of chlorogenic and caffeic acid was also increased in salinity-stressed tomato owing to the enhanced phenylpropanoid metabolism by ZnO NPs (14). Together, these results demonstrate that NPs activate a new way of metabolic regulation, which is different from the response to conventional fertilisers or plant growth regulators.

Ionomics

Ionomics has contributed to understanding of the effects of NP treatments on uptake, redistribution and homeostasis of nutrients under stress conditions. The NPs typically cause more significant changes in plant ion profiles than conventional inputs. Under salinity stress, for instance, ZnO NPs decreased the level of Na^+ in the leaves, increased Ca and magnesium (Mg) and contributed to the stability of the membranes (14). In the same way, essential enzyme cofactors (manganese (Mn) and molybdenum (Mo)) were increased in drought stressed wheat with the use of TiO_2 NPs (5). The Fe_3O_4 NPs suppressed Cd uptake in heavy metal stressed plants, suggesting their role in limiting Cd uptake (18). Comparable ionomic responses

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have been reported across diverse crop species and stress conditions. Under salinity stress, Si NPs improved ionic homeostasis in rice by reducing Na^+ accumulation and increasing K^+ and Ca^{2+} levels, thereby improving the K^+/Na^+ balance (72, 73). In lentil, nano-silicon similarly enhanced the K^+/Na^+ ratio under NaCl stress, indicating a conserved role of Si-based nanomaterials in maintaining ionic balance across cereal and legume crops (74). Under drought stress, TiO_2 NPs enhanced the uptake of K, phosphorus (P) and N in wheat, suggesting improved acquisition of essential nutrients under water deficit (75). In contrast, under combined chromium (Cr) and drought stress, TiO_2 NPs in combination with *Pseudomonas* sp. reduced tissue Cr concentrations and improved tissue micronutrient concentrations in wheat, suggesting simultaneous restriction of toxic-ion accumulation and maintenance of essential nutrient homeostasis (76). Ionomic studies also indicate that the NP-mediated nutrient rebalancing is different between hydroponic and soil culture systems, thus underscoring the fact that the growth conditions must be taken into consideration when assessing the effects of NPs on plant nutrition.

Nanoproteomics and multi-omics integration

Nanoproteomics is a successful method to elucidate the interaction between plant proteins and NPs. It allows the identification of protein coronas formed on the surface of a NP and the identification of proteins that are adsorbed, activated or structurally altered after the exposure of a nanoparticle. More sophisticated techniques including single particle inductively coupled plasma mass spectrometry (ICP-MS), proteomics and cryo-electron microscopy (cryo-EM) have revealed that the composition of the tightly bound "hard" corona is quite different to that of the 'soft' corona. In root exudates, for instance, it was found that ZnO NPs form hard coronas consisting of expansins and β-1,3-glucanases that could act as mediators for the entry of NPs into plant tissues (70).

The interdisciplinary transcriptomics, proteomics and metabolomics are allowing a comprehensive understanding of the stress tolerance mechanism induced by NPs. In wheat, weighted gene co-expression network analysis (WGCNA) analysis of ZnO NP-induced transcriptomic data identified MAPK and ABA signalling pathways as key regulators of salinity tolerance (70). Similarly, multi-omics studies in CeO_2 NP-treated soybean under drought stress revealed a sequential response involving early ROS signalling, subsequent activation of DREB and NAC transcription factors and later accumulation of protective metabolites such as polyamines and trehalose (17). Machine learning approaches, including Random Forest and deep learning models, are increasingly being used to identify biomarker signatures associated with NP efficacy. Future integration of multi-omics, spatial transcriptomics and single-cell proteomics is expected to generate tissue-level maps of NP–plant interactions and accelerate the development of precision nano-agriculture.

Critical comparative analysis of nanoformulations

Careful critical comparison of existing nanoformulations demonstrates significant variations in performance, dosage-responses and consistent contradictions that complicate simplistic extrapolation. Translating laboratory studies into field-applicable recommendations is contingent on resolving these contradictions through mechanistic understanding rather than simple meta-analysis of experimental results.

Mechanistic basis for differential nanoformulation efficacy under salinity and drought

The effectiveness of different nanomaterials against abiotic stresses depends on the way they work. Nanoformulations of ZnO have a positive effect on the ability to cope with saline conditions due to the presence of many ways in which this occurs. The effect of Zn^{2+} release helps increase the expression of *SOS1* antiporters to promote Na^+ exclusion and maintain proper ion homeostasis, while the increase in the activity of the proton gradient created by H^+-ATPase in the plasma membrane improves the secondary Na^+ transport (8, 14). Besides, there is an interaction between nanomaterials and zinc, which acts as a cofactor for zinc-finger transcription factors like DREB proteins (26).

On the other hand, nanoformulations of SiO_2 prove to be effective when dealing with drought conditions due to aquaporins production, stomata regulation and silica accumulation in epidermal cells. These processes help reduce the loss of water not through stomata and provide better hydration (6, 13). These mechanisms of SiO_2 NPs influence can explain why their effectiveness decreases in saline conditions and ZnO NPs in drought conditions. Unlike ZnO and SiO_2 NPs, CeO_2 NPs have inherent redox activity, thereby enabling their use as mimics of antioxidant enzymes that effectively eliminate reactive oxygen species and prevent plants from various forms of abiotic stress. Nevertheless, their efficacy has been found to vary across species and high doses could interfere with vital processes like nitrogen fixation in legumes (77).

The protective potential of chitosan NPs lies in their ability to induce resistance by activating plant defence mechanisms as well as in the delivery of bioactive substances and osmoprotectants (25, 77). Consequently, they are highly suitable for dealing with multiple stresses simultaneously, yet the efficiency of these and other nanoformulations depends largely on soil properties, method of application, concentration and crop growth stages, amongst other parameters (53).

Contradictory findings and dose-dependent responses

Contradictory effects in NP studies are frequently observed when dosages, particle properties and experimental conditions differ. For instance, while CeO_2 NPs increased drought resistance in soybean plants at 10–50 mg L^{-1}, they inhibited nodulation and decreased Bradyrhizobium symbiosis at concentrations above 100 mg L^{-1}, due to interference with flavonoid signalling (17, 77). Hence, it becomes clear that there is an optimal dose range, unique to each crop and environmental stress type.

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Hormesis is frequently observed following nanoparticle application, in which low or moderate doses stimulate plant growth and stress tolerance, whereas concentrations exceeding a crop- and NP-specific threshold can induce phytotoxic effects. This beneficial-to-toxic transition has been reported across different crop species, although the effective threshold varies with nanoparticle type, particle size, concentration, exposure duration and plant genotype. For example, Ag NPs increased heat tolerance in wheat at concentrations of 5–20 mg L^{-1}, whereas higher concentrations were associated with DNA damage (11). Similarly, low concentrations of metal or metal-oxide NPs can enhance antioxidant defence and nutrient acquisition in crops such as rice, maize and legumes, while excessive doses may promote ROS accumulation, membrane injury, nutrient imbalance and growth inhibition. Furthermore, different growing systems also influence the effectiveness of NPs. In hydroponic conditions, plants are constantly exposed to NPs. However, in soils, the application rate might be lower because of NPs adsorption on soil particles (51) and a significant portion of NPs does not reach plant roots. Hence, several factors could result in contradictions in studies on NPs impact. Standardising these factors and considering NP surface area as a key exposure metric may help explain and reconcile many of the reported discrepancies (78).

Crop-specific responses and knowledge gaps

There is marked variation in the efficacy of nanoformulations from monocot plants compared to dicots, due to the differences in their root system, cell walls, stress signalling pathways and microbial associations (79). Monocot plants such as wheat and rice respond well to the application of ZnO and SiO_2 NPs, especially under conditions of salinity and drought stress, compared to several dicot plants, specifically legumes (6). The reason for this could be attributed to the differences in oxidative stress responses and the unique physiological conditions required for the symbiotic N fixation by the legumes. The responses of legumes may be variable due to the impact of the NPs on plant-microbe interactions, including rhizobial signalling and nodulation, which may affect plant growth indirectly (77). Although the field of agricultural nanotechnology is increasing in popularity, a vast majority of the studies are conducted using only a few model organisms, including *Arabidopsis*, wheat, rice and tomato. Key crops like millets, sorghum, cassava, yams and teff are underrepresented in the studies related to agriculture nanotechnology (80). Additionally, very few studies exist on NP activity under combination stress conditions like drought and heat stress and salinity and heavy metals. These knowledge gaps highlight the need for broader crop coverage and more realistic field-based evaluations of nanoformulations.

Laboratory, pot and field evaluation of nanoformulations

One of the major difficulties in nano-agriculture is the ability to convert good laboratory data into consistent field data. The more complex the environment and agronomic conditions the more complex the experiments become as the nanoformulations are translated from *in vitro* studies into pot experiments, greenhouse trials, field plots and eventually multi-location farmer-participatory trials. Thus, good laboratory results do not always mean good field results.

Several nanoformulations have been tested under pot and greenhouse conditions and proved to be effective. The positive effects of NPs in improving salinity tolerance were observed in *Cucurbita pepo* L., whereas Fe_3O_4 NPs alleviated Cd toxicity in radish (18, 19). While the advantages of yield improvement are typically less pronounced when compared to hydroponic systems because NPs can be absorbed by the soil particles, they can also interact with soil properties, microbial communities and irrigation practices, there is still a significant yield advantage observed in soil. For many systems it can be observed that the efficiency drops by about 30–40 % from hydroponic to soil systems (51). The summary of major nanoformulations in comparison with each other is presented in Table 3, showing the optimum application concentration, mechanism of action and efficacy of each nanoformulation under different abiotic stress conditions.

Long term field experiments are especially valuable for assessing environmental sustainability. For example, ZnO nanotechnology application to salt-affected wheat fields multiple times resulted in greater wheat yields and greater soil accumulation of Zn, which could affect soil health and soil microbial communities over time (14). Future research should therefore focus not only on agronomic benefits but also on the cumulative environmental effects of NP use. Overall, the successful adoption of nano-agriculture will depend on rigorous multi-season, multi-site field validation and the development of application guidelines based on realistic field conditions rather than laboratory experiments alone.

Table 3. Nanoformulation-based interventions for enhancing crop tolerance to drought, salinity, heat and heavy metal stresses through physiological and biochemical modulation

Nanoformulation	NP Type	Concentration	Crop	Target Stress	Improvement	Mechanism	Reference
ZnO NPs	Metal oxide NPs	100 mg L^{-1}	Wheat *(Triticum aestivum)*	Salinity (10 dS m^{-1})	Grain yield +42.2 %; chlorophyll a + 24.6 %; root dry weight +63.1 %	Ion homeostasis (K^+/Na^+ ratio); SOD/CAT/POD antioxidant activation	(14)
ZnO NPs	Metal oxide NPs	100 ppm	Eggplant	Drought	Fruit yield +22.6 %;	Stomatal	(33)

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			(Solanum melongena)	(60 % ETc)	water productivity + 66.1 %; RWC improved	regulation; aquaporin upregulation; ROS scavenging	
Chitosan–MgO Composite NPs	Biopolymer-metal oxide composite	100 mg L^{-1}	Rice *(Oryza sativa)*	Salinity (200 mM NaCl)	Root length +37.47 %; dry biomass + 32.86 %; CAT activity +67.21 %	Ion exclusion; chitosan-mediated membrane modulation; antioxidant enzyme induction	(81)
Se NPs	Elemental Se NPs (biosynthesized)	10 mg L^{-1}	Wheat *(Triticum aestivum)*	Drought + heat (60 % FC; 38 °C)	SOD activity +196 U mg^{-1} FW min^{-1}; antioxidant activity increased by 30–45 %	GPX/TrxR activation; HSP70/90 upregulation; ABA regulation	(82)
TiO_2 NPs	Metal oxide NPs	100 mg L^{-1}	Moldavian dragonhead *(Dracocephalum moldavica)*	Salinity	Chlorophyll, RWC and biomass improved by 25–35 %; MDA and H_2O_2 reduced; photosynthesis + 20–30 %	Photocatalytic ROS scavenging	(83)
ZnO NPs	Metal oxide NPs	150 ppm	Wheat *(Triticum aestivum)*	Drought (tillering stage)	Grain number + 12.5 %; grain weight + 12.4 %; total yield +25.5 %; Zn +58.6 %	Stomatal regulation; osmolyte accumulation; SOD/CAT/POD activation	(84)
SiO_2 NPs	Metal oxide NPs	50–100 mg L^{-1}	Maize *(Zea mays)*	Drought and salinity	RWC (+20–35 %) and shoot length improved; proline and antioxidant activity increased	Cell-wall silicification; aquaporin (PIP) upregulation; antitranspirant effects	(85)
Engineered carbon dots (CDs)	Carbon-based nanoparticles / carbon dots	50, 100 mg L^{-1}	Rice *(Oryza sativa)*	Cadmium(Cd) stress/toxicity	At 100 mg L^{-1}, grain yield +18 %; Cd accumulation reduced by 40 % in roots and 46 % in grains; grain nutritional quality maintained	Enhanced antioxidant defense (ABA, GABA, SOD, POD); metabolic reprogramming; ascorbate-glutathione, zeatin and triterpenoid saponin pathways; rhizosphere bacterial reshaping and Fe cycling, increasing Fe/Cd sequestration in root-surface Fe plaques	(86)
Selenium nanoparticles (Se-NPs)	Selenium-based nanoparticles	5, 10 mg L^{-1}	Rice *(Oryza sativa)*	Cd and Pb toxicity	At 10 mg L^{-1}, grain Cd and Pb reduced by 56 % and 32 %, respectively; Se, Zn, Fe and protein increased; phytic acid reduced	Restricted Cd/Pb uptake and translocation; Se biofortification and mineral nutrient uptake; reduced potential Cd/Pb bioavailability and bioaccessibility	(87)
Silica nanoparticles	Silica-based	500 mg L^{-1}	Maize	Water deficit and	Photosynthesis, stomatal conductance,	Enhanced CAT/SOD/POD	(88)

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(SiNPs)	nanoparticles	*(Zea mays)*	soil salinity	RWC, chlorophyll and carotenoids improved; proline, electrolyte leakage, MDA and Na^+ reduced; SiNP + PGPR further improved yield and N, P, K uptake	activity; improved K^+/Na^+ balance and water status; membrane stabilization; regulation of Na^+ uptake, transport and distribution

NP: nanoparticle; NPs: nanoparticles; ZnO: zinc oxide; MgO: magnesium oxide; Se: selenium; TiO_2: titanium dioxide; SiO_2: silicon dioxide; ABA: abscisic acid; ROS: reactive oxygen species; RWC: relative water content; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; GPX: glutathione peroxidase; TrxR: thioredoxin reductase; HSP70: heat shock protein 70; HSP90: heat shock protein 90; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; FC: field capacity; ETc: crop evapotranspiration; FW: fresh weight; K^+: potassium ion; Na^+: sodium ion; Cd: cadmium; Pb: lead; PIP: plasma membrane intrinsic protein; GABA: gamma-aminobutyric acid; ppm: parts per million; mg L^{-1}: milligrams per litre; dS m^{-1}: decisiemens per metre.

Regulatory challenges and risk assessment

The current regulatory framework for nano-agricultural products is scattered, contradictory and severely lags behind both scientific and commercial advances. These issues are the main obstacles to the appropriate use of these products at scale while, at the same time, allow some underexplored materials on the market before the completion of safety studies.

International and regional framework

Several tests adapted to nanomaterials (TG 201-203, 317, 318) has been published by the Organisation for Economic Co-operation and Development (OECD), addressing various aspects of solubility, agglomeration rates and environmental parameters relevant to the agriculture industry (54). These guidelines outline standard methods for the physicochemical analysis, aquatic toxicity, terrestrial organism toxicity and environmental fate assessment of nanomaterials. They are merely guidance documents; hence there is not even distribution in their application by various national regulatory bodies. The United States Environmental Protection Agency (US EPA) regulate the agricultural nanomaterials, via both Toxic Substances Control Act (TSCA) (for the existing and the new substances) and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (for the pesticidal use), but nano-pesticides must be presented with physicochemical analyses and environment fate information that differ significantly from the requirements for conventional pesticides (72). Nano pesticides require demonstrations that they do not pose risks related to nano-specific properties beyond what is already covered by the conventional risk assessment; nevertheless, the US EPA has not yet established any standards or residue tolerances to nano-specific materials in food crops, causing uncertainty to nano-input producers. In food and feed chains, European Food Safety Authority (EFSA) developed extensive guidance on nano-risk assessment although the actual regulations for nano-fertilisers and nano-biostimulators under European Union (EU) Fertilising Products Regulation (2019/1009) have not been developed yet. The EFSA's guidelines underline the need for characterisation of nanomaterials during each stage of the food chain, taking into account transformations and dissolution of NPs into plant matrices and soil. Similarly, Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO), through Joint Expert Committee on Food Additives (JECFA) and Codex Alimentarius, has outlined provisional guidance for the safety of nano-food, emphasising the importance of investigating fate and transformation throughout the food chain. Lack of an international classification of nanomaterials may lead to materials being considered nanomaterials in EU regulations while not in US or India, fragmenting the market and leading to inconsistency in safety standards.

Indian framework

India has progressively strengthened its regulatory framework for nano-enabled agricultural inputs. A major development was the inclusion of nano-fertilisers under the Fertiliser (Inorganic, Organic or Mixed) Control Order (FCO), 1985, through Notification S.O. 884(E) dated 24 February 2021, followed by the notification of specifications for Nano Urea (Liquid) under S.O. 885(E), establishing a formal regulatory framework for its manufacture and commercialisation (89). The framework was subsequently extended to nano diammonium phosphate (DAP), which was notified under the FCO based on bio-efficacy trials and toxicological testing, with specified manufacturers permitted to produce the product (90). Further regulatory notifications have expanded the range of approved nano-fertilisers to include products such as nano urea, nano urea plus and nano DAP, with these products required to comply with specifications prescribed under the FCO (91). In addition, the Department of Biotechnology (DBT) has developed guidelines for the evaluation of nano-based agri-inputs and food products, providing a framework for assessing the safety of nano-enabled products with respect to human, animal and environmental health. Despite these developments, further regulatory strengthening is required for nano-pesticides, residue assessment, environmental fate and post-market monitoring, particularly because internationally harmonised standards and maximum residue limits for many nanomaterials remain limited.

Main challenges of risk assessment

Several methodological obstacles pose unique challenges to nano-agricultural risk assessment. The transformation of NPs into secondary materials (sulfidation, phosphatisation, association with soil organic matter, dissolution) that exhibit significantly different properties from the original materials often complicates regulatory tests, which evaluate only the initial product (51). The definition of dose metric (particle

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number, surface area, mass concentration) that could best describe toxicity of nanomaterials is controversial since these different measures have been observed to produce different relative ranks in toxicity studies of the same set of NPs (78). Toxicity studies conducted on groups of animals exposed simultaneously to multiple NPs are rarely found in the regulatory context and the combined toxicity of NPs has not been addressed. A further area requiring attention is the potential for NP exposure to induce transgenerational effects in plants through epigenetic modifications such as DNA methylation, histone remodelling and small RNA-mediated regulation. Coordination of an international effort is required, focussing on the creation of nano-specific test methods, on harmonisation of characterisation protocols and procedures, accreditation of analytical facilities and harmonisation of regulatory guidelines, in order to address these long-term biological responses, which are still poorly understood and are not included in the existing regulatory assessment frameworks. Initiatives led by organisations such as the International Organisation for Standardisation Technical Committee on Nanotechnologies (ISO/TC 229) could play a pivotal role in establishing globally accepted standards for the safe and effective use of nanomaterials in agriculture (92).

Economic viability and adoption potential

A defining feature in the adoption of any agricultural innovation, the nano-formulations must yield a positive benefit-cost ratio under realistic farm conditions to be considered successful at scale. The economic viability of nano-agricultural products must take into consideration multiple dimensions, i.e., input cost reduction, yield effects, externalities, requirements for supply-chain investment and affordability at the smallholder level-dimensions which are seldom addressed in published literature. Early economic assessments suggest that nano-fertilisers have the potential to reduce fertiliser-related input costs while maintaining or improving crop yields. However, these outcomes vary considerably across studies and production systems, highlighting the need for further independent validation through large-scale, multi-location field trials before broad economic conclusions can be drawn (93, 94). Although nano-fertilisers generally have a higher unit price than conventional bulk fertilisers , their substantially lower application rates can improve overall economic efficiency by reducing transportation, storage and handling costs. For example, in contrast to conventional urea, which is often applied at rates exceeding 100 kg ha^{-1} in cereal-based cropping systems, nano urea is generally recommended at only 2–4 bottles (500 mL each) per hectare, substantially reducing the quantity of fertiliser material required for field application (94).

Furthermore, life-cycle assessments indicate that nitrogen fertiliser manufacture is associated with substantial greenhouse gas emissions, suggesting that reductions in conventional urea consumption could generate additional environmental and economic benefits under future carbon-pricing frameworks. In many smallholder farming systems of South Asia, fertiliser expenditure constitutes a substantial proportion of total variable production costs, often accounting for 20–50 % or more depending on crop type, input intensity and local market conditions. Consequently, reductions in fertiliser use through nano-fertiliser technologies could generate significant economic benefits. However, the relatively high unit cost of nano-products and limited access to agricultural credit may constrain adoption among resource-poor farmers (95).

Broader adoption challenges beyond economic concerns lie with farmer awareness, technical literacy of farmers and associated operators (application machinery must be properly calibrated for foliar delivery and nano-input concentration), supply chain infrastructure (e.g., need for cold chain storage for biological nano-products) and trust in claims based on independent validation rather than the statements of manufacturers. Many of these challenges are similar to, if more pronounced than, those experienced during the adoption of biofertilisers and precision agriculture products. Farmer participatory trials under IFFCO's nano-agriculture programme show promising adoption rates, particularly with repeated trials, with cost savings associated with the use of nano-fertilisers identified as the primary driver for success (93). Modelling the application of nano urea at the national scale in India indicates potential annual subsidy savings between 1.5 and 2.0 billion US dollars if one application is substituted per season for cereals in a one-to-one ratio with top-dressed conventional urea, thus presenting an incentive for government support of this innovation and potentially explaining rapid regulatory adoption (93).

The global market for nano-agriculture was valued at approximately 3.5 billion US dollars in 2022 and is projected to expand by 12–15 % annually from 2023 to 2030 driven by the need for resource efficient crop inputs under water-limited and degraded soil conditions it is based on industrial report (96). However, limited product portfolio (nano-fertilisers dominate) and geographic distribution (India, China, USA account for most sales) mean the vast majority of the global agricultural system is still largely excluded from the current nano-agriculture market; extensive and independent trials with economic accounting across the diverse global farming system are key to informed policy formulation.

Toxicity and environmental concerns

A complete risk assessment framework for nano-agricultural products needs to include analyses of phytotoxicity, ecotoxicity, disruption to soil microbial communities, bioaccumulation, transference through food webs and potential human health impacts areas that are typically characterised by gaps in our knowledge and that may require balanced application of the precautionary principle alongside potential agricultural benefits.

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Phytotoxicity and ecotoxicology

Phytotoxicity of NPs is influenced by a combination of dose, size, surface chemistry and the particular species of plant under consideration; NP toxicity usually demonstrates concentration-dependent effects. Concentrations of ZnO NPs at 3 to10 fold that are beneficial can inhibit root growth, induce chlorosis and damage membrane lipid integrity in various plant species. The lowest observed effect concentration (LOEC) for phytotoxicity in a species of lettuce, *Lactuca sativa* L., is as low as 100 mg L^{-1}, although for wheat and rice the LOEC is > 500 mg L^{-1} (97). The Ag NPs are the most phytotoxic NP among those studied to date. This effect seems largely due to Ag ion release, which inhibits respiration and disrupts cell membrane integrity via the formation of a complex with membrane sulfhydryl containing proteins. Epigenetic alterations to whole genome methylation profiling can even be induced by sub-toxic concentrations of Ag NPs. When applied to soil, earthworms' avoidance behaviour with ZnO NPs in amended soil can be observed at 500 mg kg^{-1} and there are observable changes to intestinal tract epithelium in earthworms at 200 mg kg^{-1} of ZnO NP-amended soil (98). It is shown that in soil, natural food webs are dramatically altered toward stress-tolerant organisms when the concentration of CeO_2 NPs is 100 mg kg^{-1} or greater. The ecotoxicity observed toward aquatic organisms is equally important to consider when waterbodies or drainage channels receive nano-input runoff from agricultural applications. The TiO_2 NPs reduce the growth of algae at a concentration as low as 1 mg L^{-1} due to UV induced photo-catalytic generation of ROS, whereas *Daphnia magna* is known to be sensitive to ZnO NPs with an EC50 of 1-3 mg L^{-1} and zebrafish embryos are killed by exposure at a LC50 of 5-10 mg L^{-1} (99). These toxicity thresholds should be compared to realistic field exposure concentrations.

Soil microbiome, food chain and human health

Interference of agricultural NPs to soil microbiome is a real and growing concern. Studies involving 16S rRNA amplicon sequencing showed that ZnO NPs amended to soil at 500 mg kg^{-1} lead to soil bacteria diversity reduction (Shannon index 20-35 %) and there is a higher sensitive decrease on phylum Acidobacteria and beneficial *Pseudomonas fluorescens* bacteria which are involved in production of siderophores and solubilisation of phosphate (51). Arbuscular mycorrhizal fungi (AMF) that are beneficial to P acquisition on most non-cereal crops, is more sensitive on Ag NPs and Cu NPs, in which AMF spore germination rate is reduced up to 40–60 % by Cu NPs in pot study when the content is 50 mg kg^{-1} soil (28). However, in agronomic condition, even for that very sensitive element, at the concentration and frequency recommended for crop input, the change in soil bacteria diversity is normally in the natural range of temporal variation for most nano formulations, especially Si NPs and CeO_2 NPs. A major distinction of the differences between acute single application effect and long-term chronic effects after years of repeated annual nano-inputs for crop relevant nano-inputs, is still seldom investigated. Most soil microbiome-related studies evaluate communities only at 30–90 days post nano-amendment. Beyond the initial application period, the environmental fate, transformation, persistence and residual accumulation of NPs in agricultural soils remain insufficiently understood. Their persistence may be influenced by soil properties, climatic conditions, microbial activity and transformation into secondary chemical species, highlighting the need for long-term field studies to assess their behaviour and potential ecological consequences. Transfer of NPs through the soil-plant-herbivore food chain was documented in mesocosm study for CeO_2 and ZnO NPs, most of the bioaccumulation factor values are smaller than 1, thus, biomagnification for NPs is limited. Furthermore, NPs may interact with commonly applied fertilisers, pesticides and other agrochemicals, potentially altering their mobility, bioavailability and toxicity. Such combined or synergistic effects remain poorly characterised and should be incorporated into future environmental risk assessments under realistic agricultural conditions. However, biotransformation products, such as zinc sulphide (ZnS) or $CePO_4$, compared with their parent materials (ZnO and CeO_2), show differences in bioavailability and potential toxicity (64). Potential transport of NPs and their transformation products through soil profiles also raises concerns regarding groundwater contamination, particularly in sandy soils, high-rainfall regions and irrigated agricultural systems. Leaching studies under realistic field conditions are therefore required to determine whether repeated nano-inputs could result in accumulation or contamination of groundwater resources. The key food safety question, is the potential of health risk caused by nano-input residues (in edible part of crops), is still underdeveloped. Current evidence suggests that NP residues in edible crop tissues are generally low and do not indicate immediate food safety concerns under recommended application conditions but, questions remain about their potential chronic health effects and interactions within the human digestive system. Therefore, more studies and some standardised risk assessment procedures are needed to draw final conclusions on the long-term safety of consumers.

Knowledge gaps and limitations

Based on the challenges discussed in the present study, this section synthesises the key limitations identified throughout the review and highlights major knowledge gaps and future research priorities, rather than repeating the earlier discussions. First and by far the most concerning limitation is the overreliance on single-season laboratory or pot-scale studies. Data on soil NP accumulation over multiple years under multi-location field trials, long-term soil health implications, crop rotation effects and cumulative environmental loading are almost entirely missing. Without these data, confident predictions on annual application rates and crop rotation compatibility are not possible (27). Secondly, standardising dose remains a core unsolved issue: concentrations developed in hydroponic systems seldom translate directly into soil and soil-optimal doses can vary three to five-fold depending on soil organic

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matter, clay mineralogy and pH level (51). Development of crop- and soil-specific dose-calibration protocols derived through multi-location field-based dose response studies must be conducted before any NP formulation can be responsibly advocated (51, 70). Thirdly, food safety data remain limited, with relatively few studies examining NP uptake, biotransformation and residue persistence in edible crop tissues, while most rely on single time point analyses and therefore cannot adequately assess residue dissipation kinetics required for establishing maximum residue limits (MRLs) (100). Fourthly, the absence of international harmonisation of NP regulations contributes to market fragmentation, which can hinder investment in robust testing and permit the development of inadequately evaluated products (100). Fifthly, functional responses of soil microbiomes to NPs remain poorly understood beyond changes in community diversity and the effects of long-term NP exposure on key ecosystem functions such as nutrient cycling and microbial symbioses remain largely unknown (70). Sixthly, omics studies of NP and crop interactions are limited to a narrow range of crop and NP combinations and integrated systems-biology approaches remain scarce, restricting mechanistic understanding of nanoparticle-mediated stress responses (71). Seventhly, NP transformation, dissolution, re-speciation, colloidal interactions and persistence pathways within soil, water and plant systems remain inadequately characterised for many commercially important nanoformulations, particularly under realistic field conditions (51). Finally, NP-induced transgenerational epigenetic modifications, including changes in DNA methylation, histone modification and small RNA regulation, remain largely unexplored and are absent from most short-term laboratory and pot-scale investigations (51, 71). Addressing these knowledge gaps will require multidisciplinary research consortia, standardised NP reference materials, open-access databases, long-term field research networks, nano-specific testing protocols and internationally harmonised regulatory frameworks (27).

Future prospects

The future of nano-agriculture is likely to be influenced by further developments in materials science, digital agriculture, synthetic biology and changing regulations. Research work is underway on the development of smart nanoformulations that can release their drug payload in a controlled and stimulus-responsive manner, such as the ones that are triggered by ROS, pH changes or temperature changes and these technologies would allow the targeted application of nutrients, phytohormones, osmoprotectants and RNA based molecules, which would lead to improved stress tolerance and reduce environmental exposure (27). The other promising area is the use of nanotechnology in combination with RNA-based crop protection and gene regulation technologies. One of the promising alternatives to the classical genetic modification is the delivery of RNA molecules that can modulate stress-responsive pathways, which can be made possible using nanocarriers. Meanwhile, rapid technological progress in the field of artificial intelligence (AI) and remote sensing and precision agriculture will aid in optimising the timing, dosage and spatial targeting of nano-input applications. A fusion of hyperspectral imaging, field sensors and wireless communication with predictive analytics has the potential to aid in making decisions for crop stress management and optimising resource use (50).

Biodegradable and renewable nanomaterials, such as cellulose nanofibres, cellulose nanocrystals, lignin NPs and naturally occurring clay nanotubes are also gaining interest because of the sustainability issues they pose and they could offer an alternative to certain metal-based nanomaterials without the need for using metal ores as feedstocks; in addition, agricultural and industrial biomass residues could be used to produce these materials (55). In the future, nano-agriculture is likely to be part of a larger climate-smart agriculture ecosystem that embraces effective water management, soil health enhancement, carbon sequestration and strategies for climate resilience (21). An emerging frontier is the integration of nanotechnology with gene-editing approaches for precise improvement of abiotic-stress tolerance. Nanoparticle-based delivery systems have demonstrated the potential to transport DNA and other genetic materials into plant cells, providing a foundation for the development of nano-enabled gene-editing strategies for targeted modification of stress-responsive genes (20). Future research should also consider NP interactions with the broader plant-associated microbiome, extending beyond soil bacterial communities to include diverse rhizosphere, endophytic and phyllosphere microorganisms. Such interactions may influence nutrient acquisition, plant growth promotion and stress responses and therefore should be incorporated into the evaluation of nanoformulations (8, 77). Furthermore, nanoformulations should be evaluated under more realistic climate-change scenarios involving combinations of drought, heat, salinity, elevated temperature and other interacting environmental stresses rather than under isolated stress conditions. Such integrated evaluations, together with multi-season and multi-location field studies, will be important for determining the consistency, environmental safety and practical effectiveness of nanoformulations under changing agricultural environments (70). Beyond the scientific advances, the successful adoption of these technologies will require the development of cost-effective delivery systems, effective regulation and access for smallholder farmers. Thus, further investment in interdisciplinary research, validation in the field and the development of supportive policy frameworks will be crucial to maximise the potential of nanotechnology in agriculture.

4. Conclusion

Nanoformulations show promising potential for improving crop responses to drought, salinity, heat and heavy metal stress, particularly under controlled experimental conditions. Reported improvements

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include 42.2 % higher grain yield and 63.1 % higher root dry weight with zinc oxide nano particles (ZnO NPs) in salt-stressed wheat, 37.5 % higher root length and 67.2 % higher catalase activity with Chitosan (CS)–magnesium oxide (CS–MgO) composite NPs in salt-stressed rice and 25.5 % higher yield in drought-stressed wheat. Selenium (Se) NPs also increased antioxidant activity by approximately 30–45 % under combined drought and heat stress in wheat. Among the evaluated materials, ZnO, titanium dioxide (TiO_2) and silicon (Si)-based and chitosan-based nanoformulations show substantial experimental promise, although their effectiveness depends on crop, dose, NP characteristics and application method. Importantly, these short-term responses do not yet establish long-term sustainability. Major gaps remain in dose optimisation, multi-season and multi-location field validation, NP transformation and persistence, soil microbiome effects, food-chain transfer and harmonised safety assessment. Future research should therefore prioritise field-based dose–response trials, long-term environmental-fate studies, residue and food-safety assessment, combined-stress experiments and standardised risk-assessment protocols. Overall, nanoformulations should be considered promising complementary tools for climate-resilient agriculture, while their wider adoption should depend on reproducible field performance, optimised application rates and demonstrated environmental and food safety.

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Authors' contributions

SA wrote the original manuscript draft. AA revised the manuscript. DR, JB and BK verified the contents. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly to improve language and clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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