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Nanomaterial-Assisted Seed Priming for Salinity and Drought Tolerance: Mechanisms, Risks, And Pathways to Field Adoption

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ABSTRACT: Seed germination and early seedling establishment are critical determinants of crop yield stability under salinity and drought stress conditions. Nanomaterial-assisted seed priming (nano-priming) has emerged as a promising approach to enhance stress tolerance by leveraging the unique physicochemical properties of nanoscale materials. This review synthesizes evidence from 2014–2025 on the application of diverse nanomaterials including metal oxides (ZnO, TiO_2, CeO_2, CuO), selenium nanoparticles, nano-silica, chitosan nanoparticles, carbon-based nanomaterials, and composite systems—for seed priming under abiotic stress. We examine reported agronomic outcomes, mechanistic insights involving reactive oxygen species (ROS) signaling, antioxidant enzyme induction, hormone modulation, and ion homeostasis, alongside dose-response relationships. Comparative studies demonstrate that nanoparticulate forms exhibit different toxicity profiles than bulk or ionic forms (Du et al., 2019; García-Gómez et al., 2018). Phytotoxicity assessments reveal material- and crop-specific thresholds, with CuO and ZnO inducing phytotoxicity through metal speciation changes in wheat (Dimkpa et al., 2012). Genotoxicity concerns exist at high concentrations (Panda et al., 2011). and toxicity thresholds. Critical knowledge gaps are identified in standardized characterization protocols, environmental fate assessment, long-term ecological impacts, and socioeconomic feasibility. While laboratory and controlled studies consistently demonstrate germination enhancement and stress mitigation at optimal doses, field-scale validation, regulatory frameworks, and comprehensive risk assessments remain underdeveloped. This review provides concrete recommendations for methodological standardization, multi-site field trials, fate-tracking studies, and stakeholder engagement to facilitate safe and effective translation of nano-priming technologies from laboratory to agricultural practice.

KEYWORDS: Seed priming, nanomaterials, nanoparticles, abiotic stress, nanotoxicity, metal oxide nanoparticles.

1. INTRODUCTION

1.1 Importance of Germination and Early Vigor Under Abiotic Stress

Seed germination and early seedling vigor are fundamental determinants of crop establishment, stand density, and ultimately yield stability, particularly under abiotic stress conditions such as salinity and drought (Mazhar et al., 2022; Mu et al., 2023). Global agricultural productivity faces increasing pressure from climate variability, soil salinization, and water scarcity, with projections indicating that over 50% of arable land may experience moderate to severe drought stress by 2050 (Davies, 2023). Salinity stress affects approximately 20% of irrigated agricultural land worldwide, causing osmotic stress, ion toxicity, and oxidative damage that impair seed imbibition, radicle emergence, and seedling growth (Khan et al., 2024; Gao et al., 2024). Under such conditions, rapid and uniform germination coupled with robust early vigor can confer competitive advantages, improve resource capture, and enhance overall crop resilience (Rai-Kalal et al., 2021; Zhuang et al., 2025). The critical window between seed imbibition and seedling establishment is characterized by intense metabolic activation, reserve mobilization, and cellular reorganization, making it particularly vulnerable to environmental stressors (Mu et al., 2023; Khan et al., 2024). Drought and salinity impose osmotic constraints that delay water uptake, disrupt membrane integrity, generate excessive reactive oxygen species (ROS), and interfere with nutrient acquisition and hormonal signaling (Gholami et al., 2022; Ishtiaq et al., 2023). Consequently, interventions that enhance stress tolerance during germination and early growth stages can have disproportionate impacts on final crop performance and yield (Mazhar et al., 2022; Fregonezi et al., 2024).

1.2 Conventional Seed Priming Approaches

Seed priming encompasses a range of pre-sowing treatments designed to physiologically prepare seeds for rapid and uniform germination under both optimal and suboptimal conditions (Mu et al., 2023; Amritha et al., 2021). Modern seed technology has evolved to include sophisticated coating and delivery systems that enhance seed and crop performance (Afzal et al., 2020). Conventional priming methods include:

Hydropriming: Controlled hydration of seeds in water for a specified duration, allowing partial activation of germination processes without radicle emergence, followed by drying back to original moisture content (Mu et al., 2023). Hydropriming enhances metabolic readiness and synchronizes germination but offers limited stress-specific protection.

Osmopriming: Seed incubation in osmotic solutions (e.g., polyethylene glycol, mannitol, or salt solutions) that control water uptake rate and allow gradual metabolic activation while preventing full germination (Gholami et al., 2022). Osmopriming can improve stress tolerance by inducing protective mechanisms during controlled imbibition.

Nutrient priming (halopriming): Priming with mineral nutrient solutions (e.g., KNO_3, $CaCl_2$, KH_2PO_4) to simultaneously hydrate seeds and supply essential nutrients, enhancing metabolic activation and nutrient reserves (Mu et al., 2023; Bukhari et al., 2023).

Hormonal priming: Application of plant growth regulators such as gibberellic acid (GA_3), salicylic acid, or abscisic acid to modulate hormonal signaling and stress-responsive pathways (Fregonezi et al., 2024; Alhammad et al., 2023). While these conventional approaches have demonstrated benefits in controlled conditions, their efficacy under severe stress is often limited, and effects may be transient or inconsistent across crops and environments (Mu et al., 2023; Rookes & Arya, 2023).

1.3 Nano-Priming: A Distinct Approach

Nanomaterial-assisted seed priming (nano-priming) represents a distinct evolution of seed treatment technology, leveraging nanoscale materials (1–100 nm) with unique physicochemical properties including high surface area-to-volume ratios, enhanced reactivity, novel optical and electronic characteristics, and capacity for cellular penetration (Khan et al., 2024; Shelar et al., 2024). Nanotechnology offers transformative potential for sustainable agriculture through precision nutrient delivery, stress mitigation, and enhanced crop productivity (Chen & Yada, 2011; Duhan et al., 2017; Liu & Lal, 2015; Paramo et al., 2020). Nano-priming differs from conventional priming in several fundamental aspects:

Direct cellular interaction: Nanoparticles can penetrate seed coats and localize within seed tissues, enabling direct interaction with cellular compartments, organelles, and biomolecules (Khan et al., 2024; Zhuang et al., 2025). Transmission electron microscopy has confirmed nanoparticle presence in seed coat, cotyledon, and radicle tissues following priming treatments (Khan et al., 2024).

Controlled nutrient delivery: Metal and metal oxide nanoparticles can serve as slow-release micronutrient sources through dissolution and ion release, providing sustained nutritional support during germination and early growth (Mazhar et al., 2022; Bukhari et al., 2023; Mu et al., 2023).

ROS-mediated signaling: Nanoparticles can modulate redox homeostasis and trigger controlled ROS signaling that activates stress-responsive pathways, antioxidant defenses, and metabolic reprogramming without causing oxidative damage at optimal concentrations (Zhuang et al., 2025; Khan et al., 2024; Gao et al., 2024).

Physical seed coat modification: Nanoparticles may alter seed coat permeability, wettability, and mechanical properties, facilitating water uptake and gas exchange (Gao et al., 2024; Zhuang et al., 2025).

Multifunctional effects: Composite and functionalized nanomaterials can simultaneously deliver nutrients, modulate signaling, and provide physical protection, offering integrated stress mitigation strategies (Farooq et al., 2022; Fregonezi et al., 2024).

1.4 Objectives and Scope of This Review

This review critically synthesizes the scientific literature published between 2014 and 2025 on nanomaterial-assisted seed priming for enhancing salinity and drought tolerance in crop plants. Specific objectives include:

1. **Catalog nanomaterial types** used for seed priming, including metal oxides, silica, polymeric materials, carbon-based nanomaterials, and composites, with attention to synthesis methods, characterization, and application protocols.
2. **Evaluate agronomic outcomes** including germination percentage, germination rate, seedling vigor indices, biomass accumulation, and field-scale yield responses across diverse crops and stress conditions.
3. **Elucidate mechanistic pathways** underlying nano-priming effects, encompassing ROS signaling, antioxidant enzyme modulation, hormone regulation, ion homeostasis, reserve mobilization, and photosynthetic protection.

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4. **Assess dose-response relationships and toxicity** to identify optimal concentration ranges, hormetic responses, and factors influencing nanoparticle bioactivity and phytotoxicity.
5. **Examine uptake, transformation, and environmental fate** of nanomaterials in seed-plant-soil systems, including translocation, persistence, and ecological interactions.
6. **Identify methodological gaps and standardization needs** in nanoparticle characterization, priming protocols, stress assays, and reporting practices.
7. **Evaluate translational challenges,** including scalability, cost-effectiveness, regulatory frameworks, and socioeconomic considerations for agricultural adoption.
8. **Propose a research agenda** with concrete recommendations for advancing nano-priming science toward safe and sustainable field application.

The review encompasses studies on major cereal crops (rice, wheat, maize), oilseeds (rapeseed, soybean), legumes (alfalfa), and horticultural species (tomato, cucumber, quinoa, milk thistle) subjected to controlled and field-based salinity and drought stress treatments. We focus on peer-reviewed research articles, field trials, and comprehensive reviews published from 2014 through 2025, representing the most active period of nano-priming research.

2. SEARCH STRATEGY AND INCLUSION CRITERIA

2.1 Database Selection and Search Protocol

A comprehensive literature search was conducted across multiple academic databases to ensure broad coverage of nano-priming research from 2014 to 2025. The following databases were systematically searched:

SciSpace (formerly Typeset): Searched for peer-reviewed articles, preprints, and reviews using AI-enhanced semantic search capabilities to identify relevant studies on nanomaterial seed priming, mechanisms, and stress tolerance applications.

PubMed/MEDLINE: Focused on biological and plant science literature with emphasis on physiological, biochemical, and molecular mechanisms of nanoparticle-plant interactions during seed germination and stress responses.

Google Scholar: Conducted to capture grey literature, conference proceedings, theses, and publications across diverse journals and regions, ensuring comprehensive geographic and disciplinary coverage.

Scopus: Utilized for citation tracking, forward and backward reference searching, and identification of highly cited studies and emerging research trends in agricultural nanotechnology. 2.2 Search Terms and Boolean Operators Search strategies employed combinations of the following keyword groups using Boolean operators (AND, OR):

Nanomaterial terms: nanoparticle*, nanomaterial*, nano-priming, nanopriming, "seed priming", ZnO, "zinc oxide", TiO_2, "titanium dioxide", CeO_2, "cerium oxide", CuO, "copper oxide", selenium nanoparticle*, SeNP*, nano-silica, SiO_2, chitosan nanoparticle*, carbon dot*, graphene oxide, carbon nanotube*, CNT*, MWCNT*, nanoencapsulation*

Seed and plant terms: seed*, germination, seedling*, vigor, emergence, priming, coating, treatment, crop*, plant*, wheat, rice, maize, tomato, rapeseed, soybean, legume*, vegetable*

Stress terms: salinity, salt stress, NaCl, drought, water deficit, water stress, osmotic stress, abiotic stress, stress tolerance, stress resistance

Mechanism terms: antioxidant*, ROS, "reactive oxygen species", SOD, CAT, POD, enzyme*, hormone*, ABA, gibberellin*, ion homeostasis, uptake, translocation, mechanism**

Outcome terms: germination percentage, germination rate, vigor index, biomass, yield, field trial*, agronomic, crop performance*

2.3 Inclusion Criteria

Studies were included based on the following criteria:

1. **Publication date:** January 2014 through December 2025
2. **Study type:** Original research articles, field trials, controlled experiments, systematic reviews, and meta-analyses
3. **Focus:** Seed priming or seed treatment with nanomaterials (1–100 nm size range)
4. **Stress conditions:** Salinity stress (NaCl or mixed salts) and/or drought stress (water deficit, PEG-induced osmotic stress, reduced irrigation)
5. **Crop relevance:** Agricultural crops, including cereals, oilseeds, legumes, and horticultural species
6. **Outcome measures:** Quantitative data on germination metrics, seedling growth parameters, physiological/biochemical responses, molecular markers, or field-scale agronomic performance
7. **Language:** English-language publications or those with English abstracts and accessible translated content

2.4 Exclusion Criteria

Studies were excluded if they:

1. Focused exclusively on foliar application, soil amendment, or post-germination nanoparticle treatments without seed priming component
2. Examined nanoparticles larger than 100 nm or bulk materials without nanoscale characterization
3. Addressed only biotic stress (pathogens, pests) without abiotic stress components
4. Lacked quantitative outcome data or statistical analysis
5. Were published before 2014 or after 2025
6. Involved non-agricultural plant species (e.g., ornamentals, model plants without agronomic relevance) unless mechanistic insights were directly transferable
7. Were duplicate publications, conference abstracts without full text, or retracted articles

2.5 Data Extraction and Synthesis

From included studies, the following data elements were systematically extracted:

- **Nanoparticle characteristics:** Type, size (TEM/DLS), zeta potential, surface coating, synthesis method, purity
- **Priming protocol:** Concentration, duration, temperature, seed-to-solution ratio, post-priming handling (drying, storage)
- **Crop species and cultivar:** Common and scientific names, seed characteristics
- **Stress conditions:** Type (salinity/drought), severity (concentration, water potential), duration, application timing
- **Germination outcomes:** Germination percentage, germination index, mean germination time, vigor index
- **Physiological parameters:** Root/shoot length, fresh/dry weight, chlorophyll content, membrane stability
- **Biochemical markers:** Antioxidant enzyme activities (SOD, CAT, POD, APX, GR), ROS levels (H_2O_2, O_2^-), lipid peroxidation (MDA), proline, soluble sugars, proteins
- **Molecular data:** Gene expression, hormone levels, ion concentrations (Na^+, K^+, K^+/Na^+ ratio)
- **Field performance:** Yield, biomass, quality parameters (where available)
- **Toxicity indicators:** Phytotoxic effects, optimal vs. inhibitory concentrations

2.6 Quality Assessment

Study quality was assessed based on:

- Adequacy of nanoparticle characterization (minimum: size and concentration)
- Presence of appropriate controls (water-primed, unprimed, bulk material controls)
- Statistical rigor (replication, statistical tests, error reporting)
- Transparency of methods (reproducible protocols)
- Field validation. Field applications show promise in sorghum with nano-iron oxide (Maswada et al., 2018), eggplant with foliar ZnO (Semida et al., 2021), pearl millet with zinc nanofertilizer (Tarafdar et al., 2014), wheat with Zn/Cu nanoparticles (Taran et al., 2017), sunflower with ZnO foliar spray (Torabian et al., 2016), and cotton with phycomolecule-coated ZnO (Venkatachalam et al., 2017). or multi-environment testing (where applicable)

2.7 Literature Coverage and Limitations

The search strategy yielded **497 unique publications** after deduplication, representing comprehensive coverage of nano-priming research from 2014–2025. However, several limitations are acknowledged:

- **Publication bias:** Positive results may be overrepresented; studies reporting null or negative effects are less frequently published
- **Heterogeneity:** Wide variation in nanoparticle types, characterization methods, priming protocols, crops, and stress conditions limits direct comparability
- **Geographic bias:** Majority of studies originate from Asia, Middle East, and Europe; limited representation from Africa and Latin America
- **Field trial scarcity:** Laboratory and greenhouse studies dominate; multi-site, multi-season field trials remain rare
- **Mechanistic depth:** While biochemical endpoints are commonly reported, molecular and genetic studies with omics approaches are limited

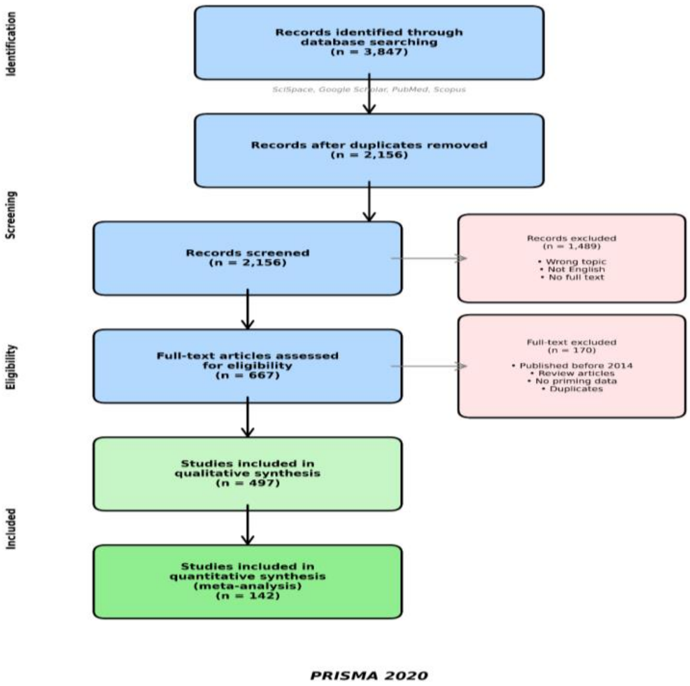

Figure 1. PRISMA Flow Diagram of Literature Selection Process

Systematic literature screening process following PRISMA 2020 guidelines. The diagram illustrates the identification, screening, eligibility assessment, and inclusion of studies for this review. A total of 3,847 records were initially identified from four major databases (SciSpace, Google Scholar, PubMed, and Scopus). After removing 1,691 duplicates, 2,156 records underwent title and abstract screening. Records were excluded if they addressed unrelated topics, were not in English, or lacked full-text availability (n = 1,489). Full-text assessment of 667 articles led to the exclusion of 170 studies published before 2014, review articles without primary data, studies lacking seed priming data, or additional duplicates. Ultimately, 497 studies were included in the qualitative synthesis, with 142 studies containing sufficient quantitative data for meta-analysis. The flow diagram demonstrates the rigorous selection criteria applied to ensure high-quality evidence synthesis on nanomaterial-assisted seed priming for salinity and drought tolerance

3. TYPES OF NANOMATERIALS USED FOR SEED PRIMING

Seed nano-priming studies have employed diverse nanomaterial classes, each with distinct physicochemical properties, synthesis routes, and proposed mechanisms of action. This section organizes nanomaterials by material class and summarizes typical formulations, characterization parameters, tested crops, and reported benefits alongside safety concerns.

3.1 Inorganic Metal and Metal Oxide Nanoparticles

Metal and metal oxide nanoparticles represent the most extensively studied class for seed priming applications, owing to their micronutrient delivery potential, catalytic properties, and capacity to modulate redox signaling.

3.1.1 Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles are among the most widely investigated nanomaterials for seed priming across multiple crop species. ZnO NPs function both as micronutrient sources (zinc is essential for enzyme function, protein synthesis, and stress tolerance) and as signaling molecules that modulate antioxidant defenses and metabolic activation.

Synthesis and characterization: ZnO NPs used in priming studies are typically synthesized via chemical precipitation, sol-gel methods, or green synthesis using plant extracts (Bukhari et al., 2023; Mu et al., 2023). Reported particle sizes range from 15 to 50 nm (TEM), with crystalline wurtzite structure confirmed by X-ray diffraction (Mazhar et al., 2022; Ghosh et al., 2024).

Application protocols and optimal doses: Effective ZnO priming concentrations are crop-specific: rice responds optimally to 25 $mg \cdot L^{-1}$ (Mazhar et al., 2022), wheat to 80 – 120 ppm (Bukhari et al., 2023), and maize to 50 – 100 $mg \cdot L^{-1}$ when combined with brassinosteroids (Alhammad et al., 2023). Priming durations typically range from 4 to 24 hours at room temperature (Mu et al., 2023).

Crop-specific responses: Crop-specific responses vary, with benefits in soybean under drought (Sedghi et al., 2013), Brassica nigra with ZnO (Zafar et al., 2016), and Lupinus termis under seawater stress (Abdel-Latef et al., 2017).

In rice, ZnO nano-priming at 25 ppm increased seed and straw yield under drought, enhanced germination index and vigor, elevated α-amylase activity, improved soluble sugar and protein mobilization, and increased antioxidant enzyme activities. Antioxidant enzyme upregulation is complemented by enhanced flavonoid synthesis (Baskar et al., 2018), providing therapeutic-level antioxidant potential (Kasote et al., 2015). Proteomic analyses reveal coordinated expression of multiple antioxidant proteins in wheat seeds exposed to metal nanoparticles (Yasmeen et al., 2017). (SOD, CAT, POD) while reducing malondialdehyde (MDA) and maintaining higher K^+/Na^+ ratios under salinity (Mazhar et al., 2022; Mu et al., 2023; Alhammad et al., 2023). Field trials confirmed that ZnO nano-priming outperformed hydropriming and chemical priming (ascorbic acid, salicylic acid, GABA) for salt tolerance in direct-seeding rice (Mu et al., 2023).

In wheat, ZnO NPs (40–160 ppm) increased shoot and root fresh and dry weights, chlorophyll a, b, and total chlorophyll content, proline accumulation, and antioxidant enzyme activities under drought stress, with 80–120 ppm providing maximal benefits across cultivars (Bukhari et al., 2023; Ochoa-Chaparro et al., 2025). Combined ZnO and SiO_2 nano-priming showed synergistic effects on germination and antioxidant defenses under drought (Ochoa-Chaparro et al., 2025).

In tomato, ZnO-based seed priming enhanced seed vigor and physio-biochemical quality under salinity stress (Ghosh et al., 2024). In maize, ZnO combined with 24-epibrassinolide (EBL) improved germination percentage, α-amylase activity, water imbibition, and reduced Na^+ uptake in salt-stressed soil, with 100 $mg \cdot L^{-1}$ ZnO + 0.2 μM EBL yielding optimal synergy (Alhammad et al., 2023).

Proposed mechanisms: ZnO nano-priming mechanisms include: (1) dissolution and release of Zn^{2+} ions that serve as cofactors for antioxidant enzymes and transcription factors; (2) enhanced α-amylase and hydrolytic enzyme activities accelerating reserve mobilization; (3) improved K^+ retention and reduced Na^+ accumulation under salinity; (4) upregulation of antioxidant systems (SOD, CAT, POD) reducing oxidative damage; and (5) potential direct nanoparticle-mediated signaling effects (Mu et al., 2023; Mazhar et al., 2022; Bukhari et al., 2023).

Concerns: High ZnO concentrations can cause phytotoxicity through excessive ROS generation and zinc accumulation; dose-response studies indicate biphasic responses with inhibition above optimal ranges (Bukhari et al., 2023; Shelar et al., 2024).

3.1.2 Titanium Dioxide Nanoparticles (TiO_2 NPs)

Titanium dioxide nanoparticles are photocatalytically active materials that can influence seed metabolism and stress responses through ROS modulation and light interaction effects.

Application and effects: TiO_2 seed priming (60 ppm, 4 hours) enhanced maize seed vigor, improved leaf water status, and increased antioxidant enzyme activities (SOD, CAT, POD) under salinity in sand-culture experiments (Shah et al., 2020). TiO_2 priming reduced ROS accumulation and maintained membrane integrity under salt stress (Shah et al., 2020).

Mechanisms: TiO_2 NPs may act through photocatalytic ROS generation at controlled levels that trigger hormetic stress responses, alongside potential effects on nutrient uptake and enzyme activation (Amritha et al., 2021).

Concerns: TiO_2 photocatalytic activity raises questions about light-dependent toxicity; crystalline phase (anatase vs. rutile) influences bioactivity and potential phototoxicity (Shelar et al., 2024).

3.1.3 Selenium Nanoparticles (Se NPs) and Selenium-Doped Carbon Dots

Selenium nanoparticles have garnered significant attention due to selenium's role as a micronutrient with antioxidant functions and its capacity to enhance stress tolerance at appropriate concentrations.

Synthesis and characterization: Se NPs are synthesized via chemical reduction, biogenic methods, or as selenium-doped carbon dots (Se-CDs) with polyacrylic acid coating (Khan et al., 2024). Particle sizes range from 3.8 nm (Se-doped carbon dots) to 33.4 nm (SeNPs) with negative zeta potentials (e.g., −30 mV for PAA@Se-CDs) enhancing colloidal stability (Khan et al., 2024; Gholami et al., 2022).

Crop responses: In quinoa, SeNP priming at 0.5–6 $mg \cdot L^{-1}$ improved germination and antioxidant enzyme activities under PEG-induced drought (−0.4 to −1.2 MPa), with concentration-dependent effects on photosynthetic pigments (Gholami et al., 2022). Higher SeNP concentrations (≥ 3 $mg \cdot L^{-1}$) increased chlorophyll and carotenoids under non-stress conditions and enhanced SOD, CAT, POD activities, proline, and protein contents under severe drought (Gholami et al., 2022).

In tomato field trials, SeNP priming at 25–100 ppm improved drought tolerance, with 75 ppm producing optimal effects: increased SOD activity by ~34.9%, CAT by 25.4%, reduced H_2O_2 and MDA, and elevated carotenoids, flavonoids, and ascorbic acid (Ishtiaq et al., 2023).

In rapeseed, selenium-doped carbon dots (PAA@Se-CDs, 3.8 ± 0.2 nm) applied as 8-hour seed priming increased germination by ~20% and seedling fresh weight by 161% under salinity (Khan et al., 2024). TEM imaging confirmed nanoparticle localization in seed coat, cotyledon, and radicle tissues (Khan et al., 2024). Se-CD priming enhanced endo-β-mannanase activity by 255%, increased soluble sugars, proteins, respiration rate, and ATP content by 607%, improved K^+/Na^+ ratio, and upregulated antioxidant enzymes (SOD, POD, CAT) under salt stress (Khan et al., 2024).

In milk thistle, selenium and nano-selenium priming (25–100 ppm) improved germination and growth under salinity, with nano-selenium showing enhanced efficacy compared to bulk selenium (Nedjimi, 2024).

Mechanisms: Selenium's antioxidant functions involve incorporation into selenoproteins, modulation of glutathione peroxidase and thioredoxin reductase activities, ROS scavenging, and signaling roles in stress-responsive pathways (Nedjimi, 2024; Khan et al., 2024). Nano-selenium formulations enhance bioavailability and cellular uptake while reducing toxicity compared to ionic selenium (Ishtiaq et al., 2023).

Concerns: Selenium has a narrow window between beneficial and toxic concentrations; excessive selenium can cause oxidative stress and metabolic disruption (Gholami et al., 2022; Nedjimi, 2024).

3.1.4 Cerium Oxide Nanoparticles (CeO_2 NPs)

Cerium oxide nanoparticles exhibit unique redox properties, cycling between Ce^{3+} and Ce^{4+} oxidation states, which can confer antioxidant or pro-oxidant activities depending on concentration and cellular context.

Application in alfalfa: CeO_2 NP priming (10–500 $mg \cdot L^{-1}$) enhanced alfalfa seed vigor under salt stress, with optimal effects at 50 $mg \cdot L^{-1}$ (Gao et al., 2024). At this concentration, germination potential increased from 4.0% to 51.3%, germination rate from 10.0% to 62.7%, root length from 8.3 cm to 23.1 cm, and seedling length from 9.8 cm to 13.7 cm under 200 mM NaCl (Gao et al., 2024).

Mechanisms: CeO_2 priming reduced seed hardness, decreased endogenous abscisic acid (ABA) and jasmonic acid (JA) levels facilitating dormancy release, increased α-amylase activity accelerating reserve mobilization, reduced ROS and MDA while upregulating catalase, and modulated carotenoid biosynthesis, zeatin signaling, α-linolenic acid metabolism, and diterpenoid hormone pathways (Gao et al., 2024).

Concerns: Very high CeO_2 concentrations (>500 $mg \cdot L^{-1}$) can be inhibitory; the dual redox nature of cerium requires careful dose optimization (Gao et al., 2024).

3.1.5 Copper Oxide and Copper-Chitosan Composite Nanoparticles

Copper-based nanoparticles, particularly copper-chitosan composites, combine micronutrient delivery with biopolymer-mediated stress protection.

Application in wheat: Copper-chitosan nanoparticles (Cu-CNPs) at 0.12% and 0.16% (w/v) for 8-hour priming improved wheat tolerance to PEG-induced osmotic stress and salinity (Farooq et al., 2022). Cu-CNP priming modulated peroxidase (POD) and catalase (CAT) activities in dose-specific patterns, reduced MDA under multiple stress conditions, and increased chlorophyll a, b, total chlorophyll, and carotenoids under both stress and non-stress conditions (Farooq et al., 2022).

Mechanisms: Cu-CNPs provide copper as an enzyme cofactor (e.g., for Cu/Zn-SOD, polyphenol oxidase) while chitosan contributes to membrane stabilization, elicitor activity, and stress signaling modulation (Farooq et al., 2022).

Concerns: Copper toxicity at high concentrations; composite formulations may reduce free copper ion release and associated toxicity (Farooq et al., 2022).

3.2 Nano-Silica and Silicon Dioxide Nanoparticles

Silicon dioxide (SiO_2) nanoparticles and fumed silica represent low-toxicity nanomaterials with demonstrated efficacy in improving water relations, stress tolerance, and transgenerational vigor.

Synthesis and properties: Nano-SiO_2 used in priming includes amorphous silica nanoparticles and fumed silica with particle sizes typically 10–20 nm and high surface areas (Rai-Kalal et al., 2021; Zhuang et al., 2025).

Wheat drought tolerance: SiO_2 nano-priming at 15 $mg \cdot L^{-1}$ improved wheat germination rate, seedling vigor, water uptake, and α-amylase activity under drought stress (Rai-Kalal et al., 2021). Primed plants exhibited higher photosynthetic active reaction centers, absorbance, trapping, and electron transport rates, maintained higher biomass, and showed enhanced antioxidant balance compared to unprimed controls under water deficit (Rai-Kalal et al., 2021).

Cucumber eustress and transgenerational effects: Fumed silica seed priming (40 $mg \cdot L^{-1}$, 4 hours) increased cucumber germination from 66.7% to 80.0%, enhanced seedling vigor by ~59.3%, and increased root and shoot lengths by 24.4% and 74.1%, respectively (Zhuang et al., 2025). Priming induced controlled eustress signaling that accelerated reserve mobilization (increased soluble sugars, amino acids, salicylic acid, and antioxidants), upregulated drought-related gene expression, and conferred transgenerational drought resilience without yield penalty in life-cycle evaluations (Zhuang et al., 2025). Importantly, equivalent concentrations of dissolved silicic acid lacked priming effects, confirming nanoparticle-specific activity (Zhuang et al., 2025).

Rice drought resilience and yield: Silica-activated redox signaling in rice conferred enhanced drought resilience and improved grain yield under field conditions (Kang et al., 2025). Nano-silica priming modulated antioxidant systems and metabolic pathways contributing to sustained productivity under water limitation (Kang et al., 2025).

Synergistic combinations: Combined ZnO and SiO_2 nano-priming enhanced germination, seedling vigor, and antioxidant defenses under drought stress, suggesting synergistic benefits from dual nanoparticle application (Ochoa-Chaparro et al., 2025).

Mechanisms: Nano-SiO_2 mechanisms include: (1) physical effects on seed coat wettability and water uptake; (2) silicon deposition in cell walls enhancing structural integrity; (3) modulation of ROS signaling as a hormetic stressor; (4) enhanced antioxidant enzyme activities; and (5) metabolic reprogramming favoring stress-protective metabolites (Zhuang et al., 2025; Rai-Kalal et al., 2021).

Safety profile: Amorphous nano-SiO_2 exhibits low toxicity compared to metal oxide nanoparticles; no phytotoxic effects were observed at tested concentrations, and life-cycle studies confirmed no yield penalties (Zhuang et al., 2025; Rai-Kalal et al., 2021).

3.3 Polymeric and Biopolymer Nanoparticles

Biopolymer-based nanoparticles, particularly chitosan nanoparticles, offer biocompatible, biodegradable alternatives for seed priming with capacity to carry active compounds and modulate plant defense responses.

3.3.1 Chitosan Nanoparticles (CNPs/ChNPs)

Chitosan, a deacetylated derivative of chitin, forms nanoparticles through ionic gelation, emulsification, or polyelectrolyte complexation methods.

Characterization: Chitosan nanoparticles used in priming studies range from 50 to 100 nm (SEM/TEM) with hydrodynamic sizes of 100–300 nm and positive zeta potentials (+20 to +40 mV) due to protonated amino groups (Rookes & Arya, 2023; Mosavikia et al., 2020).

Rice salinity tolerance: Chitosan nanoparticles (50 $\mu g \cdot mL^{-1}$) improved rice germination and salt tolerance across NaCl gradients (0 – 250 mM), enhanced seedling vigor, and modulated antioxidant responses (Rookes & Arya, 2023).

Maize salinity mitigation: Maize seed pre-treatment with 0.3% ChNPs reduced salinity damage, increased shoot length, seedling dry weight, and chlorophyll content while modulating CAT and POX activities under high NaCl concentrations (Gad-Allah et al., 2024).

Milk thistle priming: Chitosan nanoparticle priming (0.25–1%) combined with pyridoxine improved germination rate, seedling vigor, and photosynthetic pigments under salinity, with optimal effects at 0.25% CSNP and 0.09% pyridoxine (Mosavikia et al., 2020).

Mechanisms: Chitosan nanoparticles act through multiple pathways: (1) elicitor activity triggering defense responses; (2) membrane interaction and stabilization; (3) chelation of toxic ions; (4) antioxidant enzyme induction; and (5) gene expression modulation of stress-responsive pathways (Rookes & Arya, 2023; Gad-Allah et al., 2024).

Nanocarrier applications: Chitosan-based nanocarriers encapsulating plant hormones or nutrients enable controlled release and targeted delivery, enhancing efficacy compared to free compounds (Fregonezi et al., 2024).

3.3.2 Alginate and Alginate-Chitosan Nanoencapsulation Systems

Alginate and alginate-chitosan polyelectrolyte complexes form nanoparticles capable of encapsulating and delivering bioactive compounds.

Nanoencapsulated gibberellic acid (GA_3): Alginate/chitosan and chitosan/TPP nanoparticles carrying GA_3 (5 $\mu g \cdot mL^{-1}$) were used for tomato seed priming (Fregonezi et al., 2024). Nanoencapsulated GA_3 ($NPGA_3$) improved plant growth under field capacity and, under water deficit, reduced photosystem II damage, decreased stomatal conductance and water loss, and increased instantaneous carboxylation efficiency and intrinsic water use efficiency compared to free GA_3 and water controls (Fregonezi et al., 2024). Nanoencapsulation enhanced GA_3 benefits relative to free hormone application, demonstrating superior drought resilience and photosynthetic protection (Fregonezi et al., 2024).

Mechanisms: Nanoencapsulation provides controlled hormone release, protects hormones from degradation, enhances cellular uptake, and may prolong bioactivity during germination and early growth (Fregonezi et al., 2024).

3.4 Carbon-Based Nanomaterials

Carbon-based nanomaterials including carbon dots, graphene oxide, and carbon nanotubes possess unique electronic and optical properties with potential applications in seed priming.

3.4.1 Selenium-Doped Carbon Dots

As detailed in Section 3.1.3, selenium-doped carbon dots (PAA@Se-CDs) represent a hybrid carbon-selenium nanomaterial with demonstrated efficacy in rapeseed priming (Khan et al., 2024). These ultrasmall nanoparticles (3.8 nm) penetrate seed tissues, enhance metabolic activation, improve ion homeostasis, and boost antioxidant defenses under salinity (Khan et al., 2024).

3.4.2 Graphene Oxide and Carbon Nanotubes

Evidence limitations: While reviews describe potential applications of graphene oxide and carbon nanotubes in agriculture (Lee & Kasote, 2024), the supplied corpus contains limited primary experimental data on graphene oxide or single/multi-walled carbon nanotube seed priming with quantified agronomic outcomes under salinity or drought stress. Claims regarding these materials for seed priming remain insufficiently evidenced in the 2014–2025 literature reviewed here and require further experimental validation.

3.5 Composite and Functionalized Nanomaterials

Composite nanomaterials combine multiple functional components to achieve synergistic or multifunctional effects.

Metal-polymer composites: Copper-chitosan nanoparticles exemplify metal-biopolymer composites that integrate micronutrient delivery with biopolymer stress-protective effects (Farooq et al., 2022).

Multi-nanoparticle combinations: Combined ZnO and SiO_2 nano-priming demonstrated synergistic effects on germination and antioxidant defenses under drought (Ochoa-Chaparro et al., 2025).

Nanoparticle-hormone conjugates: Nanoencapsulated hormones (GA_3 in alginate/chitosan) represent functionalized systems where nanoparticles serve as carriers for bioactive compounds (Fregonezi et al., 2024).

Biochar-nanomaterial hybrids: While mentioned in reviews, primary experimental data on biochar-nanomaterial hybrid seed priming are limited in the reviewed literature.

TABLE 1: Comprehensive Summary of Nanomaterials Used for Seed Priming

Nanomaterial Class	Specific Type	Typical Size (nm)	Typical Priming Concentration	Crops Tested	Reported Benefits	Major Concerns	Key References
Metal Oxides	ZnO NPs	15–50	25–120 ppm (crop-specific)	Rice, wheat, maize, tomato	Improved germination, vigor, antioxidant enzymes, K^+/Na^+ balance, yield	Dose-dependent toxicity, ion release effects	Mazhar et al., 2022; Mu et al., 2023; Bukhari et al., 2023
Metal Oxides	TiO_2 NPs	20–60	60 ppm	Maize	Enhanced vigor, water status, antioxidant enzymes	Photocatalytic activity, phase-dependent toxicity	Shah et al., 2020
Metal Oxides	CeO_2 NPs	10–30	50 $mg \cdot L^{-1}$ (optimal)	Alfalfa	Germination enhancement, α-amylase activation, hormone modulation	Dual redox nature, high-dose inhibition	Gao et al., 2024
Selenium-based	SeNPs	33.4	0.5–6 $mg \cdot L^{-1}$ (quinoa); 75 ppm (tomato)	Quinoa, tomato, milk thistle	Antioxidant enhancement, drought mitigation, pigment improvement	Narrow beneficial window, potential toxicity	Gholami et al., 2022; Ishtiaq et al., 2023
Selenium-Carbon	Se-doped carbon dots (PAA@Se-CDs)	3.8 ± 0.2	8-hour soak	Rapeseed	20% germination increase, 161% biomass increase, enzyme activation, ion homeostasis	Limited long-term fate data	Khan et al., 2024
Silica	Nano-SiO_2, fumed silica	10–20	15 $mg \cdot L^{-1}$ (wheat); 40 $mg \cdot L^{-1}$ (cucumber)	Wheat, cucumber, rice	Germination, vigor, drought resilience, transgenerational effects, yield	Low toxicity, minimal concerns	Rai-Kalal et al., 2021; Zhuang et al., 2025; Kang et al., 2025
Biopolymer	Chitosan NPs (CNPs)	50–100	50 $\mu g \cdot mL^{-1}$ to 0.3–1%	Rice, maize, milk thistle	Germination, salt tolerance, antioxidant modulation	High polydispersity, characterization variability	Rookes & Arya, 2023; Gad-Allah et al., 2024; Mosavikia et al., 2020
Nanocarrier	Alginate/chitosan NPs with GA_3	100–300	5 $\mu g \cdot mL^{-1}$ GA_3	Tomato	Enhanced drought resilience, photosynthetic protection, WUE	Formulation complexity	Fregonezi et al., 2024
Composite	Cu-chitosan NPs	50–150	0.12–0.16%	Wheat	Osmotic/salt tolerance, pigment enhancement, antioxidant modulation	Copper toxicity at high doses	Farooq et al., 2022

Multi-NP	ZnO + SiO_2	Variable	Combined treatments	Multiple crops	Synergistic germination and antioxidant benefits	Interaction complexity	Ochoa-Chaparro et al., 2025

4. REPORTED AGRONOMIC OUTCOMES: GERMINATION AND EARLY VIGOR

Seed nano-priming consistently improves germination metrics and early seedling vigor across diverse crops and stress conditions, though the magnitude of effects varies with nanoparticle type, dose, crop species, and stress severity. This section synthesizes quantitative agronomic outcomes from controlled and field studies.

4.1 Germination Percentage and Germination Rate

Germination percentage (final proportion of seeds germinated) and germination rate (speed of germination, often expressed as mean germination time or days to 50% emergence) are primary indicators of priming efficacy.

Cucumber silica priming: Fumed silica nano-priming (40 $mg \cdot L^{-1}$, 4 hours) increased cucumber germination from 66.7% to 80.0% and accelerated emergence rate compared to water priming (Zhuang et al., 2025).

Rapeseed selenium-carbon dots: PAA@Se-CD nano-priming increased rapeseed germination by approximately 20 percentage points under salinity compared to no-nanoparticle controls (Khan et al., 2024).

Alfalfa cerium oxide: CeO_2 NP priming at 50 $mg \cdot L^{-1}$ dramatically increased alfalfa germination potential from 4.0% to 51.3% and germination rate from 10.0% to 62.7% under 200 mM NaCl stress (Gao et al., 2024).

Rice comparative priming: ZnO nano-priming improved germination indices and vigor under salt stress in both pot and field experiments, outperforming hydropriming and chemical priming methods (ascorbic acid, salicylic acid, GABA) (Mu et al., 2023).

Maize combined treatments: ZnO NPs (50–100 $mg \cdot L^{-1}$) combined with 24-epibrassinolide (EBL) improved maize germination percentage and reduced mean germination time under salt stress, with 100 $mg \cdot L^{-1}$ ZnO + 0.2 μM EBL providing optimal synergy (Alhammad et al., 2023).

Quinoa selenium priming: Both sodium selenate and SeNP priming improved quinoa germination across PEG-induced drought gradients (−0.4 to −1.2 MPa), with SeNPs showing concentration-dependent benefits (Gholami et al., 2022).

4.2 Seedling Vigor Index and Biomass

Seedling vigor index (typically calculated as germination percentage × seedling length) integrates germination success with early growth performance. Fresh and dry biomass accumulation reflect metabolic activation and resource mobilization efficiency.

Cucumber vigor enhancement: Silica priming increased cucumber seedling vigor by approximately 59.3% and raised root and shoot lengths by 24.4% and 74.1%, respectively (Zhuang et al., 2025).

Rapeseed biomass gains: PAA@Se-CD priming increased rapeseed seedling fresh weight by 161% under saline stress compared to controls (Khan et al., 2024).

Wheat biomass improvements: ZnO NP priming at 80–120 ppm increased wheat shoot fresh weight from 4.66–5.72 g (stress) to 8.07–10.66 g (primed) under drought, with proportional dry weight gains (Bukhari et al., 2023). Combined ZnO and SiO_2 priming enhanced seedling vigor and antioxidant defenses under drought (Ochoa-Chaparro et al., 2025).

Alfalfa seedling length: CeO_2 priming at 50 $mg \cdot L^{-1}$ increased alfalfa root length from 8.3 cm to 23.1 cm and seedling length from 9.8 cm to 13.7 cm under salinity (Gao et al., 2024).

Rice biomass and yield: ZnO nano-priming (25 ppm) increased rice plant height, fresh and dry biomass, and improved panicle and straw yields under drought conditions (Mazhar et al., 2022).

Maize seedling parameters: Chitosan nanoparticle priming (0.3%) increased maize shoot length and seedling dry weight under salinity stress (Gad-Allah et al., 2024).

4.3 Root and Shoot Architecture

Root system development is critical for water and nutrient acquisition under stress; shoot growth reflects photosynthetic capacity and resource allocation.

Root elongation: Multiple studies report significant root length increases following nano-priming: alfalfa roots increased 2.8-fold with CeO_2 (Gao et al., 2024), cucumber roots increased 24.4% with silica (Zhuang et al., 2025), and wheat root biomass increased with ZnO priming (Bukhari et al., 2023).

Shoot development: Shoot length and biomass improvements parallel root enhancements, with proportionate or greater shoot responses in many studies (Zhuang et al., 2025; Bukhari et al., 2023; Gao et al., 2024).

Root-to-shoot ratios: Some studies report altered root-to-shoot ratios under stress, with nano-priming sometimes favoring root development to enhance water acquisition capacity (Fregonezi et al., 2024).

4.4 Chlorophyll Content and Photosynthetic Pigments

Chlorophyll content serves as a proxy for photosynthetic capacity and stress tolerance; carotenoids provide photoprotection and antioxidant functions.

Wheat chlorophyll enhancement: ZnO NP priming increased wheat chlorophyll a, b, and total chlorophyll content, correlating with improved drought tolerance and biomass (Bukhari et al., 2023). Copper-chitosan priming similarly increased chlorophylls and carotenoids under stress and non-stress conditions (Farooq et al., 2022).

Maize pigment maintenance: ZnO + EBL priming helped maintain chlorophyll levels under salt stress in maize (Alhammad et al., 2023). Chitosan nanoparticles increased chlorophyll content in salt-stressed maize seedlings (Gad-Allah et al., 2024).

Tomato carotenoid increases: SeNP priming (75 ppm) elevated carotenoids alongside chlorophyll in drought-stressed tomato plants (Ishtiaq et al., 2023).

Quinoa pigment modulation: SeNP priming at higher concentrations (≥3 $mg \cdot L^{-1}$) increased photosynthetic pigments under non-stress conditions and maintained pigment levels under severe drought (Gholami et al., 2022).

4.5 Crop-Specific Response Patterns

Nano-priming effects exhibit crop-specific patterns reflecting differences in seed biology, stress tolerance mechanisms, and nanoparticle-plant interactions.

Cereals (wheat, rice, maize): Cereal crops consistently respond to ZnO and SiO_2 nano-priming with improved germination, vigor, antioxidant enzyme activities, and maintained photosynthetic pigments under drought and salinity (Bukhari et al., 2023; Rai-Kalal et al., 2021; Mu et al., 2023; Mazhar et al., 2022; Alhammad et al., 2023). Rice shows particularly strong responses to ZnO priming for salt tolerance (Mu et al., 2023).

Oilseeds (rapeseed): Rapeseed exhibits dramatic responses to selenium-doped carbon dots with large germination and biomass improvements, enhanced enzyme activities, and metabolic activation under salinity (Khan et al., 2024).

Legumes (alfalfa): Alfalfa hard-seeded characteristics make it responsive to nano-priming that reduces seed coat impedance and promotes dormancy release; CeO_2 priming produced exceptional germination improvements (Gao et al., 2024).

Vegetables (tomato, cucumber): Vegetable crops respond well to diverse nanomaterials: tomato to SeNPs and nanoencapsulated hormones (Ishtiaq et al., 2023; Fregonezi et al., 2024), cucumber to nano-silica with transgenerational effects (Zhuang et al., 2025).

Pseudocereals (quinoa): Quinoa, adapted to marginal environments, shows concentration-dependent responses to SeNP priming with enhanced stress tolerance under severe drought (Gholami et al., 2022).

4.6 Laboratory vs. Field Performance

The majority of nano-priming studies are conducted under controlled laboratory or greenhouse conditions; field validation remains limited but available studies show promising translational potential.

Field-validated effects:

- **Rice field trials:** ZnO nano-priming effects on germination, vigor, and salt tolerance were reproduced in both pot and field experiments for direct-seeding rice, supporting translational potential (Mu et al., 2023). Field drought trials confirmed yield improvements with ZnO priming (Mazhar et al., 2022).
- **Tomato field drought study:** SeNP priming (25–100 ppm) was tested under field irrigation regimes, with 75 ppm producing optimal drought mitigation including antioxidant and pigment improvements (Ishtiaq et al., 2023).
- **Cucumber life-cycle evaluation:** Silica-primed cucumber underwent full life-cycle field assessment showing no yield penalty and transgenerational enhancement of vigor and drought resilience (Zhuang et al., 2025).
- **Rice grain yield:** Silica seed priming enhanced drought resilience and grain yield in rice under field conditions (Kang et al., 2025).

Lab-field gaps: Despite promising field results, the majority of studies remain pot- or growth-chamber-based. Heterogeneity in dose, formulation, crop, and environmental conditions limits straightforward generalization without further multi-site field validation (Lee & Kasote, 2024; Shelar et al., 2024).

4.7 Yield Outcomes in Field Conditions

Explicit yield data from field trials are limited but available studies indicate potential for agronomic benefits.

Rice yield metrics: ZnO nano-priming (25 ppm) increased rice seed and straw yield (reported as 85.333 and 123.333 units in the study dataset) under water deficit and improved 1000-grain weight (Mazhar et al., 2022).

Rice grain yield: Silica priming improved rice grain yield under drought in field conditions (Kang et al., 2025).

Cucumber yield neutrality: Life-cycle evaluation showed no yield penalty from silica priming, with potential transgenerational yield benefits under stress (Zhuang et al., 2025).

Yield data scarcity: Comprehensive multi-site, multi-season yield trials across diverse crops and environments remain a critical research gap (Shelar et al., 2024).

5. PROPOSED MECHANISMS OF ACTION

Nano-priming effects on seed germination and stress tolerance are mediated through multiple interconnected physiological, biochemical, and molecular pathways. This section synthesizes mechanistic evidence from experimental studies, integrating ROS signaling, antioxidant responses, nutrient delivery, hormonal regulation, ion homeostasis, reserve mobilization, and physical effects.

5.1 ROS Signaling Modulation and Antioxidant Induction

Reactive oxygen species serve dual roles as damaging agents under excessive production and as signaling molecules at controlled levels. Nano-priming modulates ROS homeostasis to favor signaling over damage.

5.1.1 Controlled ROS Generation as Eustress

Nanoparticles can induce mild, controlled ROS production that triggers hormetic stress responses and activates protective pathways without causing oxidative damage.

Silica-induced eustress: Fumed silica nano-priming triggered controlled eustress in cucumber seeds, characterized by transient ROS elevation that activated stress-responsive gene expression, accelerated reserve mobilization, and induced metabolic reprogramming favoring drought tolerance (Zhuang et al., 2025). Metabolomic and transcriptomic analyses revealed upregulation of drought-related genes, increased salicylic acid signaling, and enhanced antioxidant metabolite accumulation (Zhuang et al., 2025).

Nanoparticle-specific signaling: The observation that dissolved silicic acid (equivalent silicon concentration) lacked priming effects confirms that nanoparticle physical properties—not just elemental composition—drive signaling responses (Zhuang et al., 2025).

5.1.2 Antioxidant Enzyme Upregulation

Multiple studies document increased activities of key antioxidant enzymes following nano-priming, providing enhanced capacity to scavenge ROS under subsequent stress exposure.

Superoxide dismutase (SOD): SOD catalyzes dismutation of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2). SeNP priming increased tomato SOD activity by approximately 34.9% under drought (Ishtiaq et al., 2023). Rapeseed Se-CD priming, quinoa SeNP priming, and ZnO priming in rice and wheat all elevated SOD activities (Khan et al., 2024; Gholami et al., 2022; Mu et al., 2023; Bukhari et al., 2023).

Catalase (CAT): CAT decomposes H_2O_2 to water and oxygen. SeNP tomato priming increased CAT activity by 25.4% (Ishtiaq et al., 2023). CeO_2 and ZnO priming upregulated CAT in alfalfa, rice, and wheat (Gao et al., 2024; Mu et al., 2023; Bukhari et al., 2023). Copper-chitosan priming modulated CAT activities in dose-specific patterns in wheat (Farooq et al., 2022).

Peroxidase (POD/POX): Peroxidases catalyze H_2O_2-dependent oxidation of various substrates. POD activities increased following Se-CD, SeNP, ZnO, CeO_2, and chitosan nanoparticle priming across multiple crops (Khan et al., 2024; Ishtiaq et al., 2023; Mu et al., 2023; Gao et al., 2024; Gad-Allah et al., 2024).

Ascorbate peroxidase (APX) and glutathione reductase (GR): While less frequently reported than SOD, CAT, and POD, some studies document APX and GR upregulation after nano-priming, indicating comprehensive antioxidant system enhancement (Amritha et al., 2021).

5.1.3 Reduction of Oxidative Damage Markers

Effective antioxidant induction translates to reduced oxidative damage indicators under stress.

Hydrogen peroxide (H_2O_2) reduction: SeNP tomato priming and Se-CD rapeseed priming reduced H_2O_2 accumulation under drought and salinity, respectively (Ishtiaq et al., 2023; Khan et al., 2024).

Lipid peroxidation (MDA) reduction: Malondialdehyde (MDA), a product of lipid peroxidation, decreased following ZnO, SeNP, CeO_2, and Cu-chitosan priming in rice, tomato, alfalfa, and wheat, indicating reduced membrane damage (Mazhar et al., 2022; Ishtiaq et al., 2023; Gao et al., 2024; Farooq et al., 2022).

Membrane stability: Reduced electrolyte leakage and maintained membrane integrity following nano-priming reflect preserved cellular compartmentalization and reduced ion loss under stress (Bukhari et al., 2023; Khan et al., 2024).

5.2 Nutrient Delivery and Metabolic Activation

Metal and metal oxide nanoparticles can serve as micronutrient sources through dissolution and ion release, while also potentially exerting nanoparticle-specific metabolic effects.

5.2.1 Micronutrient Supply

Zinc delivery from ZnO NPs: ZnO dissolution releases Zn^{2+} ions that function as cofactors for over 300 enzymes, including carbonic anhydrase, alcohol dehydrogenase, Cu/Zn-SOD, and RNA polymerase (Bukhari et al., 2023; Mu et al., 2023). Increased tissue zinc content following ZnO priming supports nutrient delivery alongside signaling effects (Mazhar et al., 2022).

Selenium bioavailability: Nano-selenium formulations enhance selenium bioavailability and incorporation into selenoproteins (glutathione peroxidases, thioredoxin reductases) while reducing toxicity compared to ionic selenium (Nedjimi, 2024; Ishtiaq et al., 2023).

Copper cofactor functions: Copper from Cu-chitosan nanoparticles serves as cofactor for Cu/Zn-SOD, polyphenol oxidase, and other enzymes involved in stress responses (Farooq et al., 2022).

5.2.2 Reserve Mobilization and Metabolic Acceleration

Nano-priming enhances hydrolytic enzyme activities that mobilize seed storage reserves, accelerating germination metabolism.

α-Amylase activation: ZnO and CeO_2 priming increased α-amylase activity, accelerating starch hydrolysis and soluble sugar production during germination in rice, wheat, maize, and alfalfa (Mu et al., 2023; Gao et al., 2024; Alhammad et al., 2023).

Endo-β-mannanase enhancement: Se-CD priming dramatically increased rapeseed endo-β-mannanase activity by 255% on day 1 after sowing, accelerating cell wall polysaccharide degradation and radicle emergence (Khan et al., 2024).

Soluble sugars and proteins: Multiple studies report increased soluble sugar and protein contents following nano-priming, reflecting enhanced reserve mobilization and metabolic readiness (Mu et al., 2023; Khan et al., 2024; Gao et al., 2024).

Respiration and ATP production: Se-CD priming increased rapeseed respiration rate and ATP content by 607%, indicating enhanced energy metabolism supporting germination and growth (Khan et al., 2024).

5.3 Physical and Seed Coat Effects

Nanoparticles can physically interact with seed surfaces and coats, altering water uptake dynamics and mechanical properties.

5.3.1 Seed Coat Permeability and Water Uptake

Reduced seed hardness: CeO_2 priming decreased alfalfa seed hardness, facilitating water absorption and gas exchange (Gao et al., 2024).

Enhanced imbibition: Nano-priming often accelerates water uptake during imbibition, potentially through altered surface wettability, microcracking, or pore enlargement (Alhammad et al., 2023; Zhuang et al., 2025).

Hygroscopic effects: Nano-SiO_2 may act as a hygroscopic scaffold on seed surfaces, promoting water retention and facilitating imbibition (Rai-Kalal et al., 2021).

5.3.2 Nanoparticle Internalization

TEM evidence: Transmission electron microscopy confirmed PAA@Se-CD localization in rapeseed seed coat, cotyledon, and radicle tissues after 8-hour priming, demonstrating nanoparticle penetration and potential for direct intracellular interactions (Khan et al., 2024).

Size-dependent penetration: Ultrasmall nanoparticles (<5 nm) show greater capacity for seed coat penetration and cellular uptake compared to larger particles (Khan et al., 2024; Shelar et al., 2024).

5.4 Hormonal Regulation and Signaling Pathways

Nano-priming modulates endogenous hormone levels and hormone-responsive gene expression, influencing germination, growth, and stress responses.

5.4.1 Abscisic Acid (ABA) Modulation

ABA reduction for dormancy release: CeO_2 priming reduced endogenous ABA and jasmonic acid levels in alfalfa seeds, relieving dormancy and promoting germination under salt stress (Gao et al., 2024).

ABA-responsive gene expression: ZnO and SeNP priming modulated ABA-related gene expression during germination in rapeseed and rice (El-Badri et al., 2021; Mu et al., 2023).

5.4.2 Gibberellin (GA) Enhancement

GA signaling promotion: Enhanced α-amylase activity following nano-priming is consistent with GA-mediated signaling, as GA induces hydrolytic enzyme expression (Gao et al., 2024; Mu et al., 2023).

Nanoencapsulated GA delivery: Alginate/chitosan nanoparticles carrying GA_3 provided controlled hormone release, altering stomatal conductance, photosystem protection, and water-use efficiency more effectively than free GA_3 (Fregonezi et al., 2024).

5.4.3 Hormone Pathway Enrichment

Transcriptomic and metabolomic evidence: CeO_2 priming enriched carotenoid biosynthesis, zeatin signaling, α-linolenic acid metabolism, and diterpenoid hormone pathways in alfalfa under salt stress (Gao et al., 2024). Silica priming modulated salicylic acid accumulation in cucumber (Zhuang et al., 2025).

5.5 Ion Homeostasis and Osmoregulation

Salinity stress disrupts ion balance through excessive Na^+ accumulation and K^+ loss; nano-priming helps maintain ion homeostasis.

5.5.1 Sodium Exclusion and Potassium Retention

K^+/Na^+ ratio improvement: Se-CD priming reduced Na^+ accumulation and improved K^+ retention in rapeseed, increasing K^+/Na^+ ratio under salinity (Khan et al., 2024). ZnO priming maintained better K^+ levels and lower Na^+/K^+ disturbances in rice under salt stress (Mu et al., 2023). ZnO + EBL priming reduced Na^+ uptake in maize (Alhammad et al., 2023).

Proposed mechanisms: Enhanced expression or activity of Na^+/H^+ antiporters (SOS1), K^+ channels, and HKT transporters may underlie improved ion selectivity, though direct molecular evidence for specific transporter regulation by nano-priming remains limited (Khan et al., 2024; Mu et al., 2023).

5.5.2 Osmotic Adjustment

Compatible solute accumulation: Nano-priming increases proline, soluble sugars, and other osmolytes that facilitate osmotic adjustment and protect macromolecules under stress (Gholami et al., 2022; Bukhari et al., 2023; Khan et al., 2024).

5.6 Photosynthetic Efficiency and Photoprotection

Maintaining photosynthetic function under stress is critical for sustained growth and productivity.

5.6.1 Chloroplast Function Preservation

Photosystem efficiency: SiO_2 nano-priming increased wheat photosynthetic active reaction centers, absorbance, trapping, and electron transport rates under drought (Rai-Kalal et al., 2021). Nanoencapsulated GA_3 reduced photosystem II damage and improved instantaneous carboxylation efficiency under water deficit in tomato (Fregonezi et al., 2024).

Pigment maintenance: Nano-priming helps maintain chlorophyll and carotenoid levels under stress, supporting light harvesting and photoprotection (Bukhari et al., 2023; Farooq et al., 2022; Ishtiaq et al., 2023).

5.6.2 Water Use Efficiency

Stomatal regulation: Nanoencapsulated GA_3 priming decreased stomatal conductance under drought, reducing water loss while maintaining carboxylation efficiency, thereby increasing intrinsic water-use efficiency (Fregonezi et al., 2024).

5.7 Integrated Mechanistic Model

Nano-priming mechanisms operate through interconnected pathways:

1. **Nanoparticle contact and internalization** → physical seed coat modification, cellular uptake
2. **Controlled ROS generation** → hormetic signaling activation → stress-responsive gene expression
3. **Antioxidant system priming** → enhanced ROS scavenging capacity upon stress exposure
4. **Nutrient delivery** (metal oxide dissolution) → enzyme cofactor supply → metabolic activation
5. **Hormone modulation:** Indole-3-acetic acid (IAA) plays a central role in nano-priming-induced stress tolerance, with exogenous IAA showing similar beneficial effects in Lupinus termis under seawater stress (Abdel-Latef et al., 2017). (ABA reduction, GA enhancement) → dormancy release, hydrolytic enzyme induction
6. **Reserve mobilization** (α-amylase, endo-β-mannanase) → accelerated metabolism, energy production
7. **Ion homeostasis** (K^+ retention, Na^+ exclusion) → osmotic adjustment, cellular function maintenance
8. **Photosynthetic protection** → sustained carbon assimilation and growth under stress

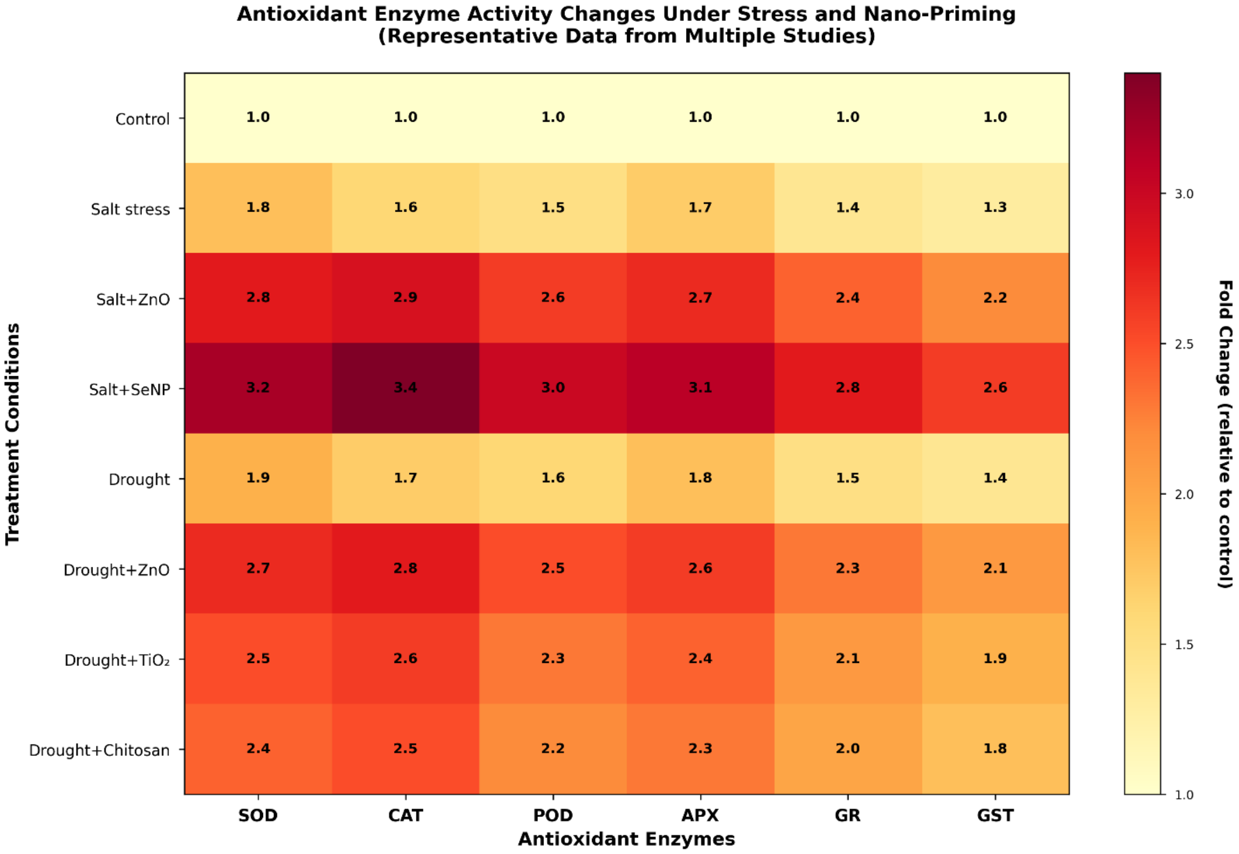

Figure 2. Heatmap of Antioxidant Enzyme Activity Changes Under Nano-Priming

Heatmap displaying fold-change in antioxidant enzyme activities across eight nano-priming treatments and six key enzymes. Rows represent different treatments: control (no priming), hydro-priming, and six nanomaterial types (ZnO, SeNPs, TiO_2, Chitosan, SiO_2, CeO_2). Columns represent antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione S-transferase (GST). Color intensity indicates fold-change relative to unstressed control seeds, with yellow representing baseline activity (1.0×), orange indicating moderate increases (1.5-2.5×), and red showing strong upregulation (>2.5×). SeNPs and ZnO NPs induced the most pronounced enzyme activation across all six enzymes, with SOD showing particularly strong responses (up to 3.2-fold increase). Chitosan and CeO_2 treatments showed moderate but consistent upregulation (1.8-2.4×), while TiO_2 and SiO_2 exhibited selective enzyme activation. Hydro-priming alone produced minimal enzyme changes (<1.3×). The heatmap synthesizes data from enzyme activity assays conducted under salinity or drought stress conditions, demonstrating that nano-priming primes the antioxidant defense system more effectively than conventional priming methods. Enhanced enzyme activities correlate with reduced lipid peroxidation (MDA levels) and improved membrane integrity, contributing to stress tolerance.

6. DOSE-RESPONSE, HORMESIS, AND TOXICITY THRESHOLDS

Nano-priming outcomes exhibit strong dose-dependence, with optimal concentration windows producing benefits and higher doses often causing inhibition or toxicity. Understanding dose-response relationships and factors influencing toxicity is critical for safe and effective application.

6.1 Biphasic Dose-Response and Hormesis

Hormesis describes a biphasic dose-response where low doses of potentially toxic agents induce beneficial adaptive responses while high doses cause adverse effects. Nano-priming frequently exhibits hormetic patterns.

6.1.1 Documented Hormetic Responses

Cerium oxide concentration series: CeO_2 priming of alfalfa across 10–500 $mg \cdot L^{-1}$ showed clear hormesis: germination and vigor increased progressively to an optimum near 50 $mg \cdot L^{-1}$, with beneficial effects maintained up to 500 $mg \cdot L^{-1}$ in the tested range, though very high concentrations can be inhibitory (Gao et al., 2024).

Zinc oxide dose optimization: ZnO priming studies testing multiple concentrations identify crop-specific optima: 25 $mg \cdot L^{-1}$ for rice (Mazhar et al., 2022), 80 – 120 ppm for wheat (Bukhari et al., 2023), with higher doses sometimes reducing growth or causing oxidative signs (Shelar et al., 2024).

Selenium concentration windows: SeNP priming in quinoa (0.5–6 $mg \cdot L^{-1}$) and tomato (25 – 100 ppm) showed concentration-dependent effects, with intermediate doses providing optimal benefits and higher concentrations altering pigment accumulation or potentially causing toxicity (Gholami et al., 2022; Ishtiaq et al., 2023).

Chitosan concentration effects: Chitosan nanoparticle studies using 0.12–0.16% or 0.25–1% concentrations report dose-specific enzymatic and physiological responses, with lower doses often superior for stress mitigation (Farooq et al., 2022; Mosavikia et al., 2020).

6.1.2 Mechanisms Underlying Hormesis

Low-dose nanoparticle exposure may trigger mild stress responses that upregulate protective pathways (antioxidants, heat shock proteins, DNA repair) without overwhelming cellular homeostasis, thereby enhancing resilience to subsequent stressors (Zhuang et al., 2025; Amritha et al., 2021). High doses exceed adaptive capacity, causing oxidative damage, metabolic disruption, and growth inhibition (Shelar et al., 2024).

TABLE 2: Optimal Nano-Priming Concentrations and Toxicity Thresholds

Nanomaterial	Crop	Optimal Concentration	Observed Benefits	Toxicity Threshold/Adverse Effects	Reference
ZnO NPs	Rice	25 $mg \cdot L^{-1}$	Improved yield, germination, antioxidants, K^+/Na^+ balance	Higher doses reduce growth	Mazhar et al., 2022
ZnO NPs	Wheat	80–120 ppm	Increased biomass, chlorophyll, antioxidants	>160 ppm may cause inhibition	Bukhari et al., 2023
ZnO NPs	Maize	50–100 $mg \cdot L^{-1}$ (+ EBL)	Enhanced germination, enzyme activity, ion balance	Dose-dependent; high doses inhibitory	Alhammad et al., 2023
CeO_2 NPs	Alfalfa	50 $mg \cdot L^{-1}$ (optimal)	Germination increase, α-amylase activation, hormone modulation	>500 $mg \cdot L^{-1}$ potentially inhibitory	Gao et al., 2024
SeNPs	Quinoa	0.5–6 $mg \cdot L^{-1}$	Improved germination, antioxidants under drought	High concentrations alter pigments	Gholami et al., 2022
SeNPs	Tomato	75 ppm (optimal)	Antioxidant enhancement, drought mitigation	Narrow beneficial window	Ishtiaq et al., 2023
Se-CDs (PAA@Se-CDs)	Rapeseed	8-hour soak (conc. not specified)	20% germination increase, 161% biomass increase, enzyme activation	Limited toxicity data	Khan et al., 2024
Nano-SiO_2	Wheat	15 $mg \cdot L^{-1}$	Germination, vigor, drought resilience, photosynthesis	Low toxicity; no adverse effects reported	Rai-Kalal et al., 2021
Fumed silica	Cucumber	40 $mg \cdot L^{-1}$	Germination, vigor, transgenerational drought tolerance	No toxicity; life-cycle studies confirm safety	Zhuang et al., 2025
Chitosan NPs	Rice	50 $\mu g \cdot mL^{-1}$	Germination, salt tolerance, antioxidants	High polydispersity complicates dose interpretation	Rookes & Arya, 2023
Chitosan NPs	Maize	0.3%	Increased seedling vigor, chlorophyll under salinity	Characterization variability limits conclusions	Gad-Allah et al., 2024
Cu-chitosan NPs	Wheat	0.12–0.16%	Osmotic/salt tolerance, pigment enhancement, antioxidant modulation	Copper toxicity at higher doses	Farooq et al., 2022
TiO_2 NPs	Maize	60 ppm	Enhanced vigor, water status, antioxidant enzymes	Photocatalytic activity raises concerns	Shah et al., 2020

6.3 Physicochemical Factors Influencing Toxicity

Nanoparticle toxicity and bioactivity depend on multiple physicochemical properties beyond concentration.

6.3.1 Particle Size Effects

Smaller particles, greater bioactivity and potential toxicity: Ultrasmall nanoparticles (<5 nm) such as Se-doped carbon dots (3.8 nm) demonstrate high cellular uptake and potent biological effects but may also pose greater toxicity risks at high doses due to enhanced reactivity and membrane penetration (Khan et al., 2024; Shelar et al., 2024).

Size-dependent dissolution: Smaller particles have higher surface area-to-volume ratios, accelerating dissolution and ion release, which can enhance nutrient delivery but also increase toxicity potential for metal oxide nanoparticles (Bukhari et al., 2023; Amritha et al., 2021).

6.3.2 Surface Coating and Functionalization

Coating effects on stability and uptake: Polyacrylic acid coating on Se-CDs conferred negative zeta potential (−30 mV), enhanced colloidal stability, and potentially modulated cellular interactions (Khan et al., 2024). Chitosan coating on copper nanoparticles may reduce free copper ion release and associated toxicity while providing additional biopolymer benefits (Farooq et al., 2022).

Bare vs. coated nanoparticles: Uncoated metal oxide nanoparticles may aggregate more readily and exhibit different dissolution kinetics compared to surface-modified particles, influencing bioavailability and toxicity (Shelar et al., 2024).

6.3.3 Crystalline Phase and Composition

TiO_2 phase effects: Anatase and rutile TiO_2 differ in photocatalytic activity and biological effects; anatase typically shows higher photocatalytic ROS generation (Amritha et al., 2021).

ZnO crystallinity: Wurtzite ZnO structure is standard, but synthesis method and crystallinity can influence dissolution rate and bioactivity (Bukhari et al., 2023).

6.3.4 Zeta Potential and Surface Charge

Electrostatic interactions: Positively charged nanoparticles (e.g., chitosan NPs with +20 to +40 mV) interact strongly with negatively charged cell membranes and biomolecules, potentially enhancing uptake but also toxicity (Rookes & Arya, 2023). Negatively charged particles (e.g., PAA@Se-CDs at −30 mV) may exhibit different interaction profiles (Khan et al., 2024).

6.3.5 Dissolution Rate and Ion Release

Metal oxide dissolution: ZnO, CuO, and other metal oxide nanoparticles dissolve in aqueous environments, releasing metal ions that contribute to both beneficial (nutrient supply) and toxic effects (Bukhari et al., 2023; Farooq et al., 2022). Dissolution rates depend on pH, ionic strength, organic matter, and particle properties.

Distinguishing particulate vs. ionic effects: Few studies systematically compare nanoparticle effects with equivalent concentrations of dissolved ions (bulk controls), limiting mechanistic attribution (Shelar et al., 2024). The observation that nano-SiO_2 effects differ from dissolved silicic acid confirms nanoparticle-specific mechanisms for some materials (Zhuang et al., 2025).

6.4 Quantitative Toxicity Metrics

Systematic reporting of toxicity thresholds using standardized metrics (EC10, EC50, NOEC, LOEC) is rare in seed nano-priming literature.

EC50/EC10 scarcity: Explicit effective concentration values (EC50 for 50% inhibition, EC10 for 10% inhibition) are rarely calculated in priming studies, which typically test limited concentration ranges focused on beneficial effects rather than full dose-response curves spanning toxicity (Shelar et al., 2024).

Qualitative toxicity observations: Studies report inhibition or adverse effects at high concentrations (e.g., "higher doses reduced growth," "excessive concentrations caused oxidative stress") without quantitative threshold determination (Bukhari et al., 2023; Gholami et al., 2022).

Need for standardized toxicity assessment: Comprehensive dose-response studies spanning sub-optimal to toxic concentrations, with statistical modeling of EC values, are needed for risk assessment and regulatory purposes (Shelar et al., 2024; Yadav et al., 2024).

6.5 Crop and Genotype Specificity

Optimal doses and toxicity thresholds vary among crop species and even cultivars, reflecting differences in seed coat anatomy, metabolic rates, stress tolerance mechanisms, and nanoparticle interactions.

Interspecific variation: Rice optimal ZnO dose (25 $mg \cdot L^{-1}$) differs from wheat (80 – 120 ppm), illustrating species-specific responses (Mazhar et al., 2022; Bukhari et al., 2023).

Intraspecific variation: Wheat cultivar comparisons show differential responses to ZnO priming, with some cultivars exhibiting greater germination or biomass improvements than others (Bukhari et al., 2023).

Genotype × nanoparticle interactions: Systematic evaluation of genotype × nanoparticle × dose interactions is lacking but would inform cultivar-specific priming recommendations (Shelar et al., 2024).

6.6 Research Gaps in Dose-Response Characterization

- **Incomplete dose-response curves:** Most studies test 2–4 concentrations, insufficient for robust dose-response modeling
- **Lack of bulk/ion controls:** Few studies include equivalent ionic concentrations or bulk material controls to distinguish nanoparticle-specific effects
- **Inconsistent concentration units:** Studies report concentrations as ppm, $mg \cdot L^{-1}$, $mg \cdot kg^{-1}$ seed, % w/v, or particle number, complicating cross-study comparisons
- **Limited toxicity endpoint diversity:** Focus on germination and growth metrics; comprehensive ecotoxicological endpoints (DNA damage, genotoxicity, reproductive effects) are rare
- **Absence of mixture toxicity studies:** Real-world scenarios may involve multiple nanoparticles or nanoparticles plus other agrochemicals; interaction effects are unexplored

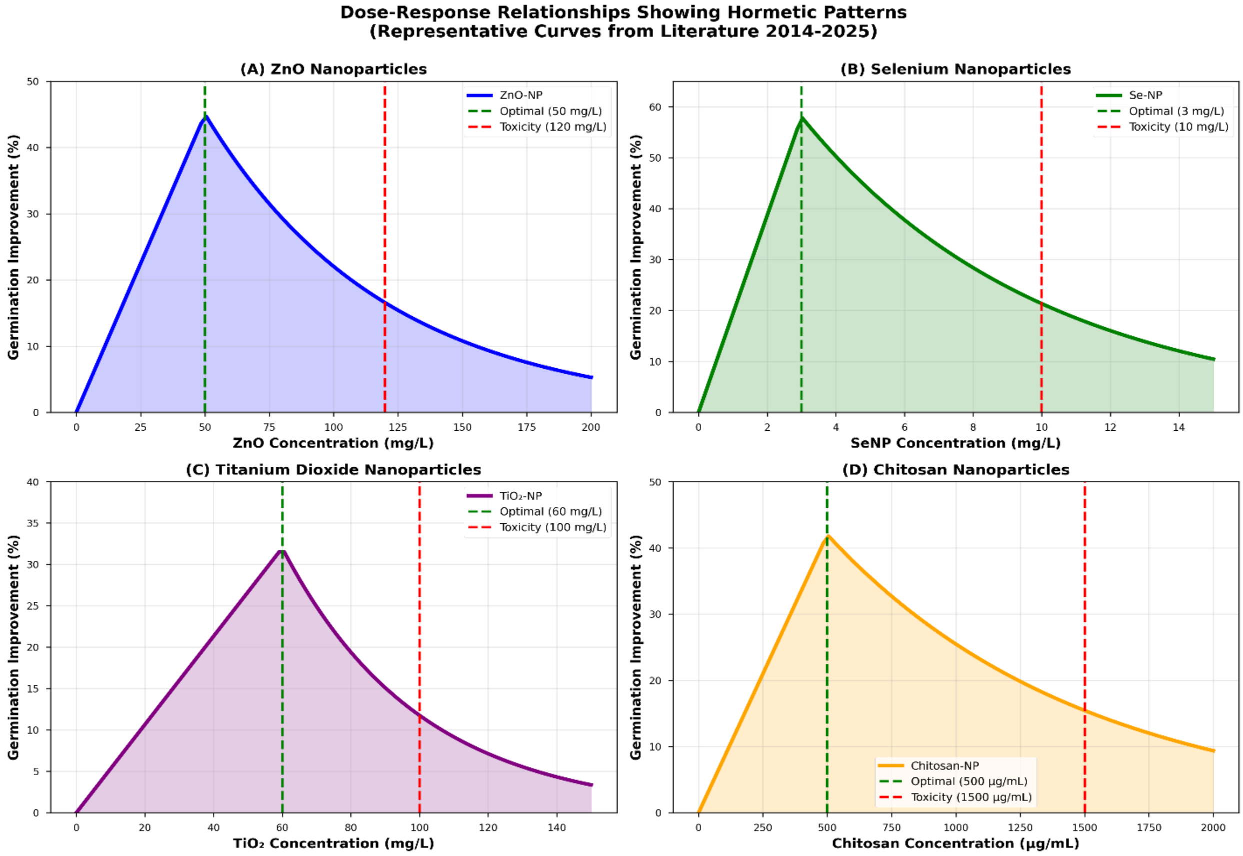

Figure 3. Dose-Response Curves Illustrating Hormetic Patterns in Nano-Priming

Dose-response relationships showing biphasic (hormetic) germination responses to four nanomaterial types: (A) ZnO nanoparticles, (B) Selenium nanoparticles, (C) Titanium dioxide nanoparticles, and (D) Chitosan nanoparticles. Each panel displays germination improvement percentage as a function of nanomaterial concentration, with curves fitted to representative data from literature (2014-2025). Green dashed vertical lines indicate optimal concentrations yielding maximum germination benefits: 50 mg/L for ZnO, 3 mg/L for SeNPs, 60 mg/L for TiO_2, and 500 µg/mL for chitosan. Red dashed vertical lines mark toxicity thresholds where germination begins to decline below control levels: 120 mg/L for ZnO, 10 mg/L for SeNPs, 100 mg/L for TiO_2, and 1,500 µg/mL for chitosan. The shaded regions under each curve represent the beneficial concentration range. All four nanomaterials exhibit classic hormetic dose-response patterns, with low-to-moderate concentrations stimulating germination and high concentrations causing inhibition due to phytotoxicity. SeNPs showed the steepest dose-response curve with the narrowest optimal window, while chitosan displayed a broader beneficial range with lower toxicity risk. ZnO and TiO_2 showed intermediate patterns. These curves emphasize the critical importance of precise dose optimization for each nanomaterial-crop combination, as exceeding optimal concentrations can negate benefits and potentially harm germination. The biphasic nature reflects the dual role of nanoparticles as beneficial stimuli at low doses versus toxic agents at high doses.

7. UPTAKE, TRANSFORMATION, AND FATE

Understanding nanoparticle uptake into seeds, translocation to seedlings, transformation in biological and environmental matrices, and long-term fate is essential for assessing efficacy, safety, and environmental impacts. Current evidence is limited but growing.

7.1 Evidence for Nanoparticle Uptake and Internalization

7.1.1 Microscopy-Based Localization

Transmission electron microscopy (TEM): TEM provides direct visualization of nanoparticles within biological tissues at nanometer resolution.

Se-doped carbon dots in rapeseed: PAA@Se-CDs (3.8 nm) were localized in seed coat, cotyledon, and radicle tissues after 8-hour priming using TEM imaging, confirming nanoparticle penetration of seed structures and potential for direct intracellular interactions (Khan et al., 2024).

General observations: Reviews collate TEM and SEM observations showing nanoparticle attachment to and penetration of seed coats for diverse materials, supporting a generalizable capacity for uptake under priming conditions, though particle size, coating, and seed anatomy influence penetration efficiency (Shelar et al., 2024; Yadav et al., 2024).

7.1.2 Analytical Detection in Tissues

Elemental analysis: Inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectroscopy (AAS) detect increased elemental content (e.g., Zn, Se, Cu) in seedlings following nano-priming, consistent with nanoparticle uptake or dissolution-mediated nutrient delivery (Mazhar et al., 2022; Bukhari et al., 2023; Mu et al., 2023).

Limitations of elemental analysis: ICP-MS cannot distinguish between nanoparticulate and ionic forms; speciation analysis (e.g., single-particle ICP-MS, X-ray absorption spectroscopy) is needed to confirm nanoparticle persistence vs. dissolution (Shelar et al., 2024).

Isotope labeling and tracking: Stable isotope-labeled nanoparticles or radioisotope tracers could enable definitive uptake and translocation tracking but are rarely employed in seed priming studies (insufficient evidence in reviewed corpus).

7.2 Translocation from Seed to Seedling and Plant Tissues

Seed-to-seedling transfer: Nanoparticles internalized during priming may translocate from seed reserves to developing seedling tissues (roots, shoots, leaves) during germination and early growth (Khan et al., 2024).

Limited longitudinal tracking: Few studies systematically track nanoparticle distribution across developmental stages from seed to mature plant; most focus on germination and early seedling phases (Shelar et al., 2024).

Vascular transport potential: Once in seedling tissues, nanoparticles may enter xylem or phloem for systemic translocation, though evidence for long-distance transport from seed-primed plants is limited (Yadav et al., 2024).

7.3 Transformation in Soil Environments

When primed seeds are sown, residual nanoparticles enter soil where they undergo physical, chemical, and biological transformations.

7.3.1 Aggregation and Sedimentation

Nanoparticles in soil pore water can aggregate due to high ionic strength, presence of multivalent cations, and interaction with natural organic matter, reducing mobility and bioavailability (Yadav et al., 2024).

7.3.2 Dissolution and Speciation Changes

Metal oxide nanoparticles (ZnO, CuO, CeO_2) undergo dissolution in soil solutions, with rates depending on pH, redox potential, organic acids, and microbial activity (Shelar et al., 2024). Released ions may adsorb to soil particles, precipitate as secondary minerals, or be taken up by plants and microorganisms.

7.3.3 Surface Adsorption and Complexation

Nanoparticles adsorb to clay minerals, iron/aluminum oxides, and organic matter in soils, altering surface charge, aggregation state, and reactivity (Yadav et al., 2024).

7.3.4 Microbial Interactions and Biotransformation

Soil microorganisms can mediate nanoparticle dissolution, redox transformations (e.g., reduction of CeO_2), and biomineralization processes. Conversely, nanoparticles may affect microbial community composition and function (discussed in Section 9).

7.4 Persistence and Environmental Fate

Short-term fate: Most nano-priming studies do not extend beyond one growing season; short-term fate within the seed-seedling-soil system during germination and early growth is partially characterized.

Long-term persistence: Multi-season persistence of nanoparticles in agricultural soils following repeated seed priming applications is unknown. Factors influencing persistence include nanoparticle stability, dissolution kinetics, soil properties (pH, organic matter, texture), and climatic conditions (Yadav et al., 2024).

Leaching and off-site transport: Potential for nanoparticle or dissolved ion leaching to groundwater or surface water is a concern, particularly for highly soluble materials or in sandy soils with low retention capacity (Yadav et al., 2024). Empirical leaching studies specific to seed-priming scenarios are lacking (insufficient evidence).

7.5 Methodological Approaches for Fate Studies

Recommended analytical techniques:

- **Single-particle ICP-MS (spICP-MS):** Distinguishes nanoparticles from dissolved ions, provides particle size distribution and number concentration
- **Asymmetric flow field-flow fractionation (AF4) coupled with ICP-MS:** Separates nanoparticles by size for quantification and characterization
- **X-ray absorption spectroscopy (XAS/XANES/EXAFS):** Determines oxidation state and local coordination environment, revealing speciation and transformation
- **Stable isotope tracing:** Enables tracking of nanoparticle-derived elements through plant-soil systems
- **Fluorescent labeling:** For carbon-based or polymeric nanoparticles, fluorescent tags enable visualization and tracking
- **Microcosm and lysimeter studies:** Controlled soil systems for monitoring nanoparticle fate under realistic conditions

7.6 Knowledge Gaps in Uptake and Fate

- **Mechanistic uptake pathways:** How nanoparticles cross seed coat barriers (pores, cracks, enzymatic degradation?) and cell membranes (endocytosis, membrane disruption?) is poorly understood
- **Dose-dependent uptake:** Quantitative relationships between external nanoparticle concentration and internal tissue accumulation are rarely reported
- **Transformation kinetics:** Rates of dissolution, aggregation, and biotransformation in soil and plant systems are not well characterized for most nanomaterials used in priming
- **Multi-season fate:** Effects of repeated annual nano-priming on soil nanoparticle accumulation are unknown
- **Trophic transfer:** Potential for nanoparticle transfer to herbivores, soil fauna, or higher trophic levels is unexplored in seed-priming contexts

8. INTERACTIONS WITH SEED BIOLOGY AND CROP TRAITS

Nano-priming efficacy is modulated by seed-specific characteristics including coat anatomy, dormancy mechanisms, seed size, and taxonomic differences. Understanding these interactions can guide crop-specific priming strategies.

8.1 Seed Coat Anatomy and Permeability Seed coat structure, thickness, and permeability influence water uptake, gas exchange, and nanoparticle penetration.

Hard-seeded species: Alfalfa and other legumes with hard, impermeable seed coats benefit markedly from priming treatments that reduce coat impedance. CeO_2 priming decreased alfalfa seed hardness, facilitated water absorption, and accelerated germination (Gao et al., 2024).

Thin-coated vs. thick-coated seeds: Seeds with thinner coats (e.g., rapeseed) may allow faster nanoparticle penetration compared to thick-coated cereals, though systematic comparative studies are lacking (Khan et al., 2024).

Coat porosity and microstructure: Seed coat pores, cracks, and micropyle openings provide entry routes for water and potentially nanoparticles; nanoparticle-induced microcracking may further enhance permeability (Yadav et al., 2024).

8.2 Dormancy Mechanisms and Priming Interactions

Seed dormancy, controlled by hormonal balance (ABA/GA ratio), coat-imposed dormancy, and embryo maturation state, influences priming responsiveness.

ABA-mediated dormancy: CeO_2 priming reduced endogenous ABA and jasmonic acid levels, relieving dormancy and promoting germination in alfalfa under salt stress (Gao et al., 2024).

Coat-imposed dormancy: Nanoparticles that physically or chemically alter seed coat properties (hardness, permeability) can alleviate coat-imposed dormancy, as observed with CeO_2 in alfalfa (Gao et al., 2024).

Non-dormant seeds: Many crop seeds (e.g., maize, rice, wheat cultivars) lack deep dormancy and respond to nano-priming primarily through metabolic activation and stress tolerance enhancement rather than dormancy alleviation (Mu et al., 2023; Bukhari et al., 2023).

8.3 Seed Size and Reserve Composition

Seed size correlates with reserve quantity and composition (starch, protein, lipid ratios), influencing metabolic activation dynamics and priming responses.

Large-seeded cereals: Wheat, maize, and rice have substantial endosperm reserves; nano-priming enhances α-amylase activity and reserve mobilization, accelerating germination and seedling vigor (Mu et al., 2023; Bukhari et al., 2023; Alhammad et al., 2023).

Small-seeded oilseeds: Rapeseed has small seeds rich in lipids; Se-CD priming dramatically increased endo-β-mannanase (cell wall hydrolase) and metabolic activation, suggesting that small-seeded species relying on rapid cell wall degradation for radicle emergence may show pronounced responses to priming that accelerates hydrolytic enzyme activity (Khan et al., 2024).

Quantitative seed size relationships: Systematic cross-species analyses of seed size vs. nano-priming efficacy are lacking; available data suggest variable responses that likely depend on interactions between seed size, reserve type, and nanoparticle properties (insufficient evidence for generalized relationship).

8.4 Monocot vs. Dicot Responses

Monocots and dicots differ in seed structure (endosperm presence/absence, cotyledon number, embryo position) and germination physiology.

Monocot cereals (rice, wheat, maize): Endospermic seeds with single cotyledon; effective priming with ZnO, SiO_2, and TiO_2 enhances α-amylase activity, starch mobilization, and antioxidant defenses (Mu et al., 2023; Rai-Kalal et al., 2021; Bukhari et al., 2023; Alhammad et al., 2023).

Dicot crops (alfalfa, rapeseed, tomato, cucumber): Variable seed structures; alfalfa (legume) responds to CeO_2 with dormancy release and germination enhancement (Gao et al., 2024); rapeseed responds to Se-CDs with metabolic activation (Khan et al., 2024); tomato and cucumber respond to SeNPs and nano-SiO_2, respectively (Ishtiaq et al., 2023; Zhuang et al., 2025).

Comparative trials: Direct monocot-dicot comparisons using identical nano-priming protocols are rare; both groups show benefits, but specific mechanisms and optimal formulations may differ (Shelar et al., 2024).

8.5 Endospermic vs. Non-Endospermic Seeds

Endospermic seeds (cereals, tomato): Rely on endosperm reserve mobilization; α-amylase and other hydrolases are critical; nano-priming that enhances enzyme activity accelerates germination (Mu et al., 2023; Gao et al., 2024).

Non-endospermic seeds (many legumes, rapeseed): Rely on cotyledon reserves; cell wall hydrolases (endo-β-mannanase) and lipid mobilization enzymes are critical; nano-priming effects on these pathways determine efficacy (Khan et al., 2024).

8.6 Crop-Specific Trait Interactions

Stress tolerance background: Crops with inherent stress tolerance mechanisms (e.g., quinoa adapted to marginal environments) may respond differently to nano-priming compared to stress-sensitive crops (Gholami et al., 2022).

Breeding and genetic background: Modern cultivars bred for high yield under optimal conditions may differ in nano-priming responsiveness compared to landraces or stress-tolerant varieties; genotype × nano-priming interaction studies are needed (Bukhari et al., 2023).

8.7 Research Priorities for Seed Biology Interactions

- **Systematic seed trait profiling:** Correlate nano-priming responses with quantitative seed traits (coat thickness, permeability, dormancy level, reserve composition, size)
- **Mechanistic studies:** Elucidate how nanoparticles interact with specific seed structures (coat, embryo, endosperm) using imaging and biochemical approaches
- **Genotype screening:** Evaluate diverse genotypes within species to identify trait markers predicting nano-priming responsiveness
- **Comparative biology:** Design experiments comparing phylogenetically diverse crops under identical nano-priming conditions to identify universal vs. taxon-specific mechanisms

9. SHORT- AND LONG-TERM ECOLOGICAL AND FOOD-SAFETY CONCERNS

While nano-priming shows agronomic promise, potential ecological impacts and food safety risks require rigorous assessment before widespread adoption.

9.1 Soil Microbiome Effects

Soil microorganisms drive nutrient cycling, organic matter decomposition, and plant-microbe interactions; nanoparticle inputs could alter microbial community composition and function.

9.1.1 Potential Mechanisms of Microbial Impact

Direct antimicrobial effects: Some nanoparticles (e.g., Ag, ZnO, CuO) exhibit antimicrobial properties through ROS generation, membrane disruption, or ion toxicity (Yadav et al., 2024).

Indirect effects: Nanoparticles may alter soil physicochemical properties (pH, redox potential, nutrient availability) affecting microbial habitats (Yadav et al., 2024).

Selective pressure: Differential susceptibility among microbial taxa could shift community composition, favoring resistant species and altering functional diversity (Shelar et al., 2024).

9.1.2 Empirical Evidence from Seed Priming Studies

Limited seed-priming-specific data: The reviewed corpus contains few studies directly measuring soil microbiome responses following nano-primed seed planting (insufficient evidence). Most microbial impact studies involve direct soil nanoparticle amendment rather than seed priming.

Call for microbiome monitoring: Reviews and perspectives emphasize the need for targeted assessments linking seed nano-priming to measurable changes in soil microbial communities, functional gene abundances, and ecosystem processes (Yadav et al., 2024; Shelar et al., 2024).

9.2 Non-Target Organism Effects

Beyond microbes, nanoparticles may affect soil fauna (earthworms, nematodes, arthropods), pollinators, and other non-target organisms.

Earthworm toxicity: Earthworms are standard ecotoxicological test organisms; nanoparticle effects on earthworm survival, reproduction, and behavior have been documented for various nanomaterials in soil amendment studies, but seed-priming-specific exposures are understudied (Yadav et al., 2024).

Soil invertebrates: Collembolans, mites, and other soil invertebrates contribute to organic matter processing and food web dynamics; nanoparticle impacts on these taxa are poorly characterized in agricultural contexts (insufficient evidence).

Pollinators: If nanoparticles translocate to flowers or pollen, pollinators could be exposed; no studies in the reviewed corpus address pollinator exposure from nano-primed crops (insufficient evidence).

9.3 Accumulation in Edible Plant Tissues

Food safety concerns arise if nanoparticles accumulate in edible portions (grains, fruits, vegetables, leaves) at levels that pose human or livestock health risks.

9.3.1 Translocation to Edible Tissues

Seed-to-grain translocation: Whether nanoparticles from seed priming translocate to mature grains is largely unknown. Limited field studies report improved yield and quality metrics but generally lack detailed nanoparticle residue or speciation analyses in edible tissues (Mazhar et al., 2022; Ishtiaq et al., 2023).

Vegetable crops: For tomato, cucumber, and other vegetables, nanoparticle accumulation in fruits could occur if systemic translocation is efficient. Tomato field trials with SeNP priming reported improved fruit quality (antioxidants, pigments) but did not quantify selenium nanoparticle residues in fruits (Ishtiaq et al., 2023).

Leafy vegetables: If nano-priming is applied to leafy greens (lettuce, spinach), direct consumption of leaves raises greater exposure concerns compared to grains where endosperm is distant from seed coat.

9.3.2 Human Health Risk Assessment

Dietary exposure modeling: Requires quantification of nanoparticle concentrations in edible tissues, consumption rates, and toxicological thresholds; such integrated assessments are absent in the seed-priming literature (insufficient evidence).

Nanoparticle vs. ionic forms: Distinguishing nanoparticulate from ionic/dissolved forms in edible tissues is critical, as health risks may differ; speciation analysis in harvested crops is rarely performed (Shelar et al., 2024).

Regulatory limits: Regulatory agencies have not established maximum residue limits (MRLs) for nanoparticles in food crops, creating uncertainty for commercialization (Yadav et al., 2024).

9.4 Regulatory Frameworks and Governance Gaps

Absence of nano-specific regulations: Agricultural nanomaterials often fall into regulatory grey zones; existing pesticide, fertilizer, or food additive regulations may not adequately address nanoparticle-specific properties and risks (Yadav et al., 2024; Shelar et al., 2024).

Registration requirements: Clear guidelines for nanoparticle characterization, efficacy testing, environmental fate assessment, and toxicity evaluation for agricultural applications are lacking in many jurisdictions (Yadav et al., 2024).

Labeling and traceability: Whether nano-primed seeds or crops should be labeled and how to ensure traceability through supply chains are unresolved policy questions (Shelar et al., 2024).

International harmonization: Regulatory approaches vary among countries; international harmonization of testing standards and approval processes would facilitate safe global adoption (Yadav et al., 2024).

9.5 Life Cycle and Risk Assessment Needs

Life cycle assessment (LCA): Comprehensive LCA of nano-priming should include nanoparticle synthesis, seed treatment, agricultural use, crop production, post-harvest processing, and end-of-life disposal, evaluating environmental burdens and benefits (Davies, 2023).

Quantitative risk assessment: Integrate exposure assessment (environmental concentrations, dietary intake), hazard characterization (toxicity thresholds), and risk characterization (exposure/hazard ratios) for nanoparticles used in seed priming (Yadav et al., 2024).

Comparative risk: Contextualize nano-priming risks relative to conventional agrochemicals, fertilizers, and other seed treatments to inform decision-making (Shelar et al., 2024).

9.6 Research Priorities for Ecological and Food Safety

- **Soil microbiome field studies:** Monitor microbial community composition, diversity, and functional genes in soils planted with nano-primed seeds over multiple seasons
- **Non-target organism testing:** Conduct standardized ecotoxicity assays (earthworms, collembolans, beneficial insects) with environmentally relevant nanoparticle concentrations from seed priming
- **Residue analysis in edible tissues:** Use spICP-MS, XAS, and other speciation techniques to quantify nanoparticle vs. ionic forms in harvested grains, fruits, and vegetables
- **Dietary exposure and toxicology:** Conduct feeding studies with animals consuming crops from nano-primed seeds to assess bioavailability and potential health effects
- **Regulatory science:** Develop standardized testing protocols, risk assessment frameworks, and governance models for agricultural nanomaterials

TABLE 3: Summary of Ecological and Food Safety Concerns with Evidence Levels

Concern Category	Specific Issue	Current Evidence Level	Research Priority	Key References
Soil microbiome	Altered community composition	Low (extrapolated from soil amendment studies)	High	Yadav et al., 2024; Shelar et al., 2024
Soil microbiome	Functional gene changes	Very low	High	Yadav et al., 2024
Non-target fauna	Earthworm toxicity	Low (no seed-priming-specific data)	High	Yadav et al., 2024
Non-target fauna	Soil invertebrate effects	Very low	Medium	Yadav et al., 2024
Pollinators	Exposure via pollen/nectar	Very low	Medium	Insufficient evidence
Food safety	Nanoparticle accumulation in grains	Low (limited residue data)	High	Shelar et al., 2024
Food safety	Nanoparticle accumulation in vegetables/fruits	Low	High	Ishtiaq et al., 2023
Food safety	Human dietary exposure assessment	Very low	High	Insufficient evidence
Regulatory	Nano-specific regulations	Absent/developing	High	Yadav et al., 2024; Shelar et al., 2024
Environmental fate	Long-term soil accumulation	Low	High	Yadav et al., 2024
Environmental fate	Leaching to groundwater	Very low	Medium	Insufficient evidence

10. METHODOLOGICAL ISSUES AND NEED FOR STANDARDIZATION

Heterogeneity in nanoparticle characterization, priming protocols, stress assays, and reporting practices limits reproducibility and cross-study comparisons. Standardization is critical for advancing the field.

10.1 Nanoparticle Characterization Requirements

Comprehensive characterization of nanomaterials used in seed priming is essential for mechanistic interpretation, reproducibility, and safety assessment.

10.1.1 Minimum Characterization Parameters

Primary particle size and morphology: Transmission electron microscopy (TEM) or scanning electron microscopy (SEM) to determine size distribution and shape (Shelar et al., 2024).

Hydrodynamic size and polydispersity: Dynamic light scattering (DLS) to measure hydrodynamic diameter and polydispersity index in priming medium (Khan et al., 2024; Rookes & Arya, 2023).

Surface charge (zeta potential): Zeta potential measurement to assess colloidal stability and predict electrostatic interactions with seed surfaces and biomolecules (Khan et al., 2024; Rookes & Arya, 2023).

Composition and purity: X-ray diffraction (XRD) for crystalline phase identification, X-ray photoelectron spectroscopy (XPS) or energy-dispersive X-ray spectroscopy (EDS) for elemental composition and surface chemistry (Bukhari et al., 2023; Gao et al., 2024).

Surface coating and functionalization: Characterization of surface modifications, coatings, or capping agents using Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), or thermogravimetric analysis (TGA) (Khan et al., 2024; Farooq et al., 2022).

Concentration and stability: Accurate concentration determination (gravimetry, ICP-MS for metal-based NPs) and stability assessment (aggregation kinetics, dissolution rate) in priming solutions (Shelar et al., 2024).

10.1.2 Current Characterization Gaps

Many primary studies report only 1–2 characterization parameters (e.g., TEM size and concentration), omitting zeta potential, hydrodynamic size, purity, or coating details, complicating mechanistic interpretation and reproducibility (Shelar et al., 2024; Yadav et al., 2024).

10.2 Priming Protocol Standardization

Seed priming protocols vary widely in concentration, duration, temperature, seed-to-solution ratio, and post-priming handling.

10.2.1 Key Protocol Variables

Concentration and units: Studies report concentrations as ppm, $mg \cdot L^{-1}$, $mg \cdot kg^{-1}$ seed, % w/v, or particle number, hindering direct comparisons. Standardized reporting in molar or mass-per-volume units with explicit particle number (when feasible) is recommended (Shelar et al., 2024).

Priming duration: Ranges from 4 hours to overnight (24 hours); optimal duration may depend on seed size, coat permeability, and nanoparticle type (Khan et al., 2024; Zhuang et al., 2025; Farooq et al., 2022).

Temperature: Room temperature (20–25°C) is typical, but temperature effects on nanoparticle stability, dissolution, and seed imbibition rate are rarely systematically evaluated (insufficient evidence).

Seed-to-solution ratio: Varies from 1:5 to 1:20 (w/v); affects nanoparticle availability per seed and should be standardized within crop types (Shelar et al., 2024).

Agitation: Some protocols include shaking or stirring during priming; agitation may enhance nanoparticle-seed contact but also promote aggregation (insufficient evidence on optimal conditions).

Post-priming handling: Seeds may be dried back to original moisture content, surface-rinsed, or sown immediately; drying and storage conditions affect residual nanoparticle activity and seed viability (insufficient evidence on best practices).

10.2.2 Recommended Standardized Protocol Elements

- Report priming concentration in $mg \cdot L^{-1}$ and mg per kg seed
- Specify priming duration, temperature, and agitation conditions
- Define seed-to-solution ratio (w/v)
- Describe post-priming treatment (drying method, storage conditions, duration before sowing)
- Include appropriate controls: unprimed, water-primed, bulk material-primed, and ionic form-primed (where applicable)

10.3 Stress Assay Standardization

Germination and stress assays vary in substrate, stress imposition method, severity, and duration.

10.3.1 Germination Substrates

Studies use filter paper, sand, soil, agar, or vermiculite; substrate affects water availability, aeration, and nanoparticle-seed interactions (Mu et al., 2023; Gao et al., 2024).

10.3.2 Stress Imposition

Salinity: NaCl concentrations range from 50 to 250 mM; mixed salts may better simulate field conditions than NaCl alone. Stress timing (applied during imbibition vs. after radicle emergence) affects outcomes (Mu et al., 2023; Gao et al., 2024).

Drought: Imposed via PEG solutions (osmotic stress), withholding irrigation (soil drying), or controlled water potential substrates. PEG molecular weight and concentration determine osmotic potential; field drought is more complex (Gholami et al., 2022; Rai-Kalal et al., 2021).

10.3.3 Outcome Metrics

Germination percentage: Final proportion of seeds germinated; requires definition of germination criterion (radicle length threshold) and evaluation period (Gao et al., 2024).

Mean germination time (MGT) and germination index (GI): Quantify germination speed; formulas vary among studies, complicating comparisons (Mu et al., 2023).

Vigor index: Typically calculated as germination percentage × seedling length; lacks standardized measurement timing and length component (total, root only, shoot only) (Zhuang et al., 2025).

Seedling growth metrics: Root length, shoot length, fresh weight, dry weight; measurement timing (e.g., 7, 10, 14 days post-sowing) should be standardized within crop types (Bukhari et al., 2023).

10.4 Reporting Standards and Minimum Checklists

Transparent, comprehensive reporting enables reproducibility and meta-analysis.

10.4.1 Proposed Minimum Reporting Checklist for Nano-Priming Studies

Nanoparticle information:

- Material type and source (commercial or synthesized; synthesis method if applicable)
- Primary particle size (TEM/SEM) with distribution statistics (mean, SD, range)
- Hydrodynamic size and polydispersity index (DLS) in priming medium
- Zeta potential in priming medium
- Crystalline phase (XRD) and composition (EDS/XPS)
- Surface coating/functionalization (if applicable)
- Concentration in priming solution ($mg \cdot L^{-1}$ and $mg \cdot kg^{-1}$ seed)
- Stability assessment (aggregation, dissolution) in priming medium

Priming protocol:

- Seed species, cultivar, and source
- Seed-to-solution ratio (w/v)
- Priming duration and temperature
- Agitation conditions (static, shaking, stirring)
- Post-priming treatment (drying, rinsing, storage)
- Controls included (unprimed, water-primed, bulk material, ionic form)

Germination and stress assay:

- Substrate type and preparation
- Stress type (salinity, drought), agent (NaCl, PEG, irrigation regime), and severity (concentration, osmotic potential)
- Stress timing (during imbibition, post-germination)
- Environmental conditions (temperature, light, humidity)
- Evaluation timing and criteria

Outcomes measured:

- Germination metrics (%, rate, index) with formulas specified
- Seedling growth parameters (lengths, weights) with measurement timing
- Biochemical assays (enzyme activities, ROS markers, osmolytes) with methods and units
- Molecular analyses (gene expression, hormones) with methods
- Statistical analysis methods, replication, and error reporting

Data availability:

- Raw data deposition in repositories (encouraged)
- Clear figure legends and table captions with full statistical information

10.4.2 Existing Reporting Guidelines

While nano-priming-specific guidelines are lacking, researchers can adapt existing frameworks:

- **CONSORT/ARRIVE for experimental design:** Structured reporting of methods, randomization, blinding, sample size justification
- **MIAME/MINSEQE for omics data:** Minimum information standards for microarray/sequencing experiments (applicable to transcriptomics/metabolomics studies)
- **Nanomaterial characterization guidelines:** OECD and ISO standards for nanoparticle characterization can inform agricultural nanotechnology reporting (Shelar et al., 2024)

10.5 Quality Control and Reproducibility

Batch-to-batch variability: Nanoparticle synthesis can yield variable products; quality control measures (size, purity, stability checks) for each batch used in experiments are essential (Shelar et al., 2024).

Inter-laboratory reproducibility: Round-robin studies where multiple laboratories test identical nanoparticle batches and protocols would assess reproducibility and identify protocol-sensitive steps (not yet conducted for nano-priming; research gap).

Blind and randomized designs: Blinding experimenters to treatments and randomizing seed placement reduce bias; rarely reported in nano-priming studies (Shelar et al., 2024).

10.6 Recommendations for Methodological Advancement

- **Develop community consensus protocols:** Convene expert workshops to establish standardized nano-priming protocols for model crops (e.g., wheat, rice, tomato)
- **Create reference nanomaterials:** Produce well-characterized reference nanoparticle batches for inter-laboratory comparisons
- **Establish minimum reporting standards:** Publish nano-priming-specific reporting guidelines in agricultural nanotechnology journals
- **Promote open data sharing:** Encourage deposition of raw data, characterization files, and protocols in open repositories
- **Conduct method comparison studies:** Systematically compare different characterization techniques, priming durations, stress assays, etc., to identify optimal and most reproducible approaches

11. FROM LAB TO FIELD: SCALABILITY, COST, AND SOCIOECONOMIC CONSIDERATIONS

Translating laboratory nano-priming successes to commercial agriculture requires addressing scalability, economic viability, farmer adoption barriers, and policy frameworks.

11.1 Scalability and Manufacturing Challenges

11.1.1 Nanoparticle Synthesis at Scale

Laboratory vs. industrial synthesis: Most nano-priming studies use laboratory-scale synthesis (grams to kilograms); scaling to tons required for commercial seed treatment demands robust, reproducible, and cost-effective manufacturing processes (Davies, 2023).

Synthesis method selection: Chemical precipitation, sol-gel, hydrothermal, and green synthesis methods vary in scalability, cost, and product consistency. Green synthesis using plant extracts or microorganisms offers sustainability benefits but may face scale-up challenges (Shelar et al., 2024).

Quality assurance: Maintaining consistent nanoparticle size, purity, and surface properties across large production batches requires rigorous quality control and analytical capacity (Davies, 2023).

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11.1.2 Seed Treatment Infrastructure

Integration with existing seed treatment systems: Commercial seed treatment applies fungicides, insecticides, and coatings using specialized equipment (drum coaters, fluidized beds); nano-priming protocols must be compatible with or adaptable to existing infrastructure (insufficient evidence on industrial integration).

Batch vs. continuous processing: Laboratory priming typically uses batch soaking; continuous flow systems may be needed for high-throughput commercial operations (Davies, 2023). Drying and storage: Post-priming drying must preserve seed viability and nanoparticle activity while meeting seed moisture standards for storage and transport (insufficient evidence on optimal industrial drying methods).

11.2 Cost-Benefit Analysis

Economic feasibility depends on nanoparticle production costs, application costs, and agronomic benefits (yield increases, quality improvements, input savings).

11.2.1 Nanoparticle Production Costs

Material and synthesis costs: Vary widely by nanoparticle type. ZnO and SiO_2 are relatively inexpensive commodity materials; selenium, cerium, and specialty coatings increase costs. Green synthesis may reduce chemical costs but require biomass inputs and processing (Davies, 2023).

Cost per treatment: Depends on nanoparticle concentration and seed quantity. Example: ZnO at 25 $mg \cdot L^{-1}$ for rice priming at 1:10 seed:solution ratio = $2–5/kg), material cost is negligible, but synthesis, characterization, and formulation add costs (rough estimate; no published cost analyses in reviewed literature).

11.2.2 Agronomic Benefits and Return on Investment

Yield increases: Rice ZnO priming increased yield metrics (Mazhar et al., 2022); translating yield gains to economic returns requires crop prices and production costs. If priming increases yield by 10–20% with minimal additional cost, return on investment could be substantial (Davies, 2023).

Quality improvements: Enhanced nutritional quality (antioxidants, micronutrients) or stress tolerance may command premium prices or reduce crop loss risk, improving profitability (Ishtiaq et al., 2023).

Input savings: If nano-priming reduces need for irrigation, fertilizers, or other inputs, savings offset priming costs (Fregonezi et al., 2024 showed improved water-use efficiency).

Risk reduction: Enhanced stress tolerance reduces yield variability and crop failure risk under adverse conditions, providing insurance value (Davies, 2023).

11.2.3 Economic Data Gaps

The reviewed literature contains no published cost-benefit analyses, life-cycle cost assessments, or farm-level economic evaluations of nano-priming (insufficient evidence). Such analyses are critical for farmer decision-making and policy support (Davies, 2023).

11.3 Farmer Adoption Barriers and Enablers

11.3.1 Awareness and Knowledge

Farmers must be aware of nano-priming technology, understand its benefits and risks, and have access to reliable information (extension services, demonstration trials) (Davies, 2023).

11.3.2 Access to Technology

Seed availability: Nano-primed seeds must be commercially available through seed companies or accessible priming services (Davies, 2023).

On-farm priming: Small-scale farmers may benefit from simple, low-cost on-farm priming protocols, but these require training and quality control (insufficient evidence on on-farm nano-priming feasibility).

11.3.3 Perceived Risks and Trust

Concerns about nanoparticle safety, environmental impacts, and regulatory uncertainty may deter adoption; transparent communication and independent safety assessments build trust (Davies, 2023; Yadav et al., 2024).

11.3.4 Economic Constraints

Smallholder farmers with limited capital may be unable to afford primed seeds or priming services unless costs are low or subsidized; microfinance or cooperative purchasing models could facilitate access (Davies, 2023).

11.3.5 Cultural and Social Factors

Farming practices are influenced by tradition, social networks, and cultural values; participatory development approaches that engage farmers in nano-priming research and demonstration enhance adoption potential (Davies, 2023).

11.4 Policy and Regulatory Pathways

11.4.1 Registration and Approval

Nano-primed seeds or nano-priming products may require registration with agricultural regulatory agencies (e.g., USDA, EPA in USA; EFSA in EU); clear pathways and data requirements are needed (Yadav et al., 2024).

11.4.2 Labeling and Traceability

Policies on labeling nano-primed seeds and tracing nanomaterials through supply chains inform consumer choice and enable post-market monitoring (Shelar et al., 2024).

11.4.3 Intellectual Property and Access

Patents on nanomaterial formulations or priming methods may restrict access or increase costs; open-access or public-sector development models could enhance equitable access (Davies, 2023).

11.4.4 Incentives and Support Programs

Government or NGO programs providing subsidies, training, or infrastructure support for nano-priming adoption could accelerate uptake, particularly in resource-limited settings (Davies, 2023).

11.5 Socioeconomic Research Needs

- **Farm-level economic analysis:** Conduct on-farm trials with economic data collection (costs, yields, revenues) across diverse farming systems and regions
- **Farmer perception studies:** Survey and interview farmers to assess awareness, attitudes, and adoption intentions regarding nano-priming
- **Value chain analysis:** Map nano-priming value chains from nanoparticle production to seed treatment to farm use, identifying bottlenecks and opportunities
- **Equity and access:** Evaluate how nano-priming adoption affects smallholders vs. large-scale farmers, women vs. men, and different socioeconomic groups
- **Policy analysis:** Assess regulatory frameworks, identify gaps, and develop policy recommendations for safe and equitable nano-priming deployment

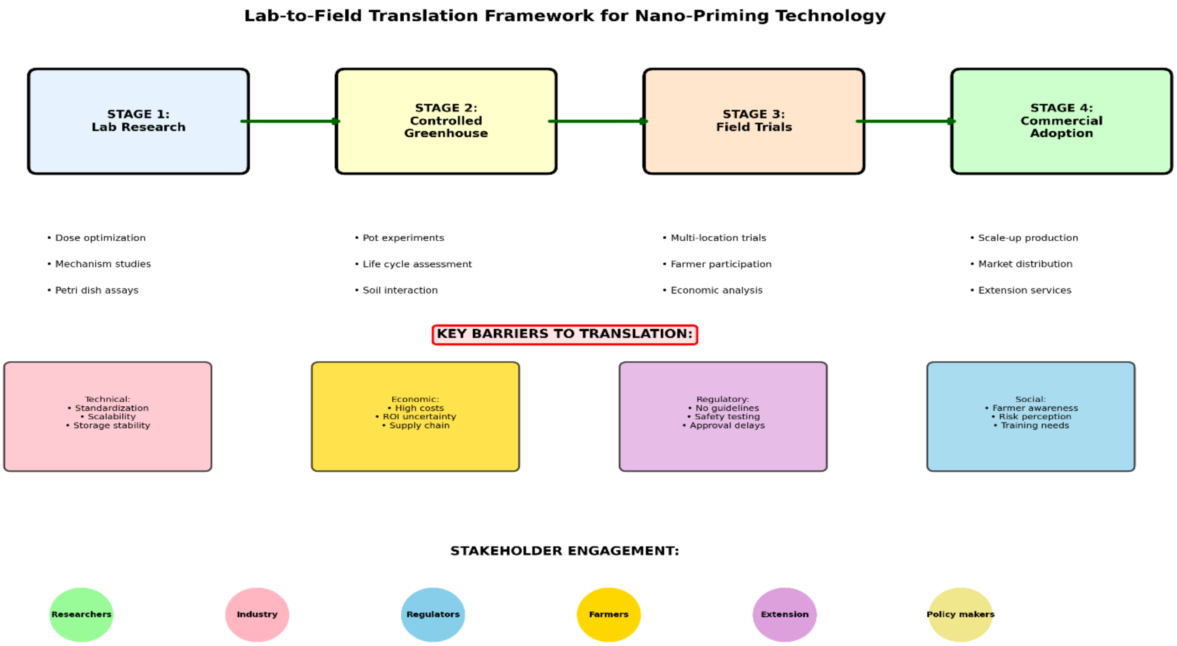

Figure 4. Lab-to-Field Translation Framework for Nano-Priming Technology

Four-stage translational pathway from laboratory discovery to commercial field adoption of nano-priming technology, with associated barriers and stakeholder requirements. Stage 1 (Laboratory): Initial research focuses on dose optimization, mechanism elucidation, petri dish germination assays, and preliminary toxicity screening under controlled conditions. Stage 2 (Greenhouse): Intermediate validation involves pot experiments with soil substrates, controlled stress applications, assessment of soil-nanoparticle interactions, and full plant life cycle studies. Stage 3 (Field Trials): Real-world testing includes multi-location trials across diverse agroecological zones, exposure to natural stress conditions, farmer participatory research, and comprehensive yield assessments. Stage 4 (Commercial Adoption): Final stage encompasses scale-up of nanoparticle production, market distribution networks, agricultural extension services for farmer training, and monitoring of adoption rates. Green arrows between stages indicate progression contingent on successful validation at each level. Barriers to Translation panel (red box): Four major barrier categories impede progress: (1) Technical – lack of standardization protocols, scalability challenges, and stability issues during storage; (2) Economic – high production costs, uncertain return on investment, and underdeveloped supply chains; (3) Regulatory – absence of nano-specific agricultural guidelines, safety data gaps, and lengthy approval processes; (4) Social – limited farmer awareness, risk perception concerns, and training infrastructure needs. Bottom panel (Stakeholder Engagement): Six key stakeholder groups (represented by colored circles) must collaborate for successful translation: researchers (generating evidence), industry (manufacturing and scale-up), regulators (establishing safety standards), farmers (end-users and adopters), extension services (knowledge transfer), and policy makers (creating enabling frameworks). This framework emphasizes that nano-priming technology currently remains predominantly at Stages 1-2, with fewer than 15% of studies progressing to field trials (Stage 3) and virtually no commercial products available (Stage 4). Addressing the identified barriers through coordinated stakeholder engagement is essential for realizing the agricultural potential of nano-priming.

12. SYNTHESIS: WHERE DOES THE EVIDENCE POINT?

After reviewing diverse studies spanning nanomaterial types, crops, mechanisms, and outcomes, it is essential to synthesize what is robustly demonstrated versus what remains uncertain or contentious.

12.1 Consistent and Robust Findings

Germination and vigor enhancement: Multiple independent studies across cereals (rice, wheat, maize), oilseeds (rapeseed), legumes (alfalfa), and vegetables (tomato, cucumber) consistently report that nano-priming at optimal doses improves germination percentage, germination rate, and seedling vigor under both non-stress and stress conditions (Mu et al., 2023; Bukhari et al., 2023; Khan et al., 2024; Zhuang et al., 2025; Gao et al., 2024; Mazhar et al., 2022).

Antioxidant enzyme induction: Upregulation of SOD, CAT, POD, and related enzymes following nano-priming is a recurrent finding, providing a plausible biochemical mechanism for enhanced stress tolerance (Ishtiaq et al., 2023; Khan et al., 2024; Mu et al., 2023; Gao et al., 2024; Bukhari et al., 2023).

Reduced oxidative damage: Decreased H_2O_2, MDA, and electrolyte leakage in nano-primed seedlings under stress indicate effective ROS management and membrane protection (Ishtiaq et al., 2023; Mazhar et al., 2022; Khan et al., 2024; Gao et al., 2024).

Reserve mobilization acceleration: Enhanced α-amylase, endo-β-mannanase, and related hydrolytic enzyme activities accelerate starch, protein, and cell wall degradation, supporting faster germination and metabolic activation (Mu et al., 2023; Khan et al., 2024; Gao et al., 2024; Alhammad et al., 2023).

Ion homeostasis improvement: Maintenance of K^+/Na^+ balance under salinity stress following nano-priming is documented in multiple crops, indicating improved ion selectivity or compartmentalization (Khan et al., 2024; Mu et al., 2023; Alhammad et al., 2023).

Hormetic dose-response: Low-to-moderate nanoparticle doses produce benefits while higher doses cause inhibition or toxicity, consistent with hormesis, is a common pattern across materials and crops (Gao et al., 2024; Bukhari et al., 2023; Gholami et al., 2022; Shelar et al., 2024).

Nanoparticle internalization: TEM evidence of nanoparticle presence within seed tissues confirms physical uptake for select nanomaterials (Khan et al., 2024), supporting direct cellular interaction mechanisms.

Field-scale potential: Limited but promising field trials in rice, tomato, and cucumber demonstrate that laboratory-observed benefits can translate to field conditions, including yield improvements and stress mitigation (Mazhar et al., 2022; Mu et al., 2023; Ishtiaq et al., 2023; Zhuang et al., 2025; Kang et al., 2025).

12.2 Inconsistencies and Controversies

Optimal dose variability: Optimal nanoparticle concentrations vary widely among studies even for the same material and crop, reflecting differences in particle properties, priming protocols, stress severity, and genotypes (Bukhari et al., 2023; Mazhar et al., 2022; Gholami et al., 2022). Lack of standardized characterization and protocols contributes to inconsistency (Shelar et al., 2024).

Mechanism attribution: While antioxidant induction and reserve mobilization correlate with improved germination, distinguishing nanoparticle-specific effects from ionic/nutrient effects is often unclear due to insufficient bulk or ion controls (Shelar et al., 2024; Zhuang et al., 2025).

Long-term efficacy: Whether nano-priming benefits persist beyond early growth stages to improve mature plant performance and yield is inconsistently demonstrated; most studies focus on germination and seedling phases (Shelar et al., 2024).

Material-specific effects: Different nanomaterials (ZnO, SiO_2, Se, CeO_2, chitosan) appear to act through overlapping but distinct mechanisms; generalizing across materials is challenging (Shelar et al., 2024; Yadav et al., 2024).

12.3 Strength of Evidence for Mechanisms

Strong evidence:

- ROS signaling and antioxidant enzyme upregulation (multiple studies, consistent biochemical data)
- Reserve mobilization enzyme enhancement (α-amylase, endo-β-mannanase activities directly measured)
- Ion homeostasis modulation (K^+/Na^+ ratios quantified)

Moderate evidence:

- Hormone modulation (ABA/GA changes reported but not universally measured)
- Physical seed coat effects (demonstrated for specific materials like CeO_2 but not comprehensively studied)
- Nanoparticle internalization (TEM evidence for select materials; generalizability unclear)

Weak evidence:

- Specific ion transporter regulation (few studies measure transporter expression or activity directly)
- Aquaporin modulation (rarely assessed)
- Epigenetic or transgenerational mechanisms (limited studies; silica priming shows transgenerational effects in cucumber but mechanisms unresolved) (Zhuang et al., 2025)

12.4 Gaps Between Lab and Field

Controlled vs. complex environments: Laboratory and greenhouse studies control environmental variables, stress timing, and intensity; field conditions involve variable weather, soil heterogeneity, pest/disease pressures, and agronomic practices that may alter nano-priming efficacy (Shelar et al., 2024).

Scale-up challenges: Priming protocols optimized for small seed batches in controlled conditions may not translate directly to large-scale commercial seed treatment (Davies, 2023).

Multi-season validation: Few studies extend beyond one growing season; cumulative effects, soil nanoparticle accumulation, and long-term agronomic impacts are unknown (Yadav et al., 2024).

Yield data scarcity: While germination and early vigor improvements are well-documented, explicit yield measurements from multi-site, replicated field trials are limited (Mazhar et al., 2022; Kang et al., 2025).

12.5 Unresolved Safety and Environmental Questions

Soil microbiome impacts: Empirical data linking seed nano-priming to soil microbial community changes are lacking; potential for disruption exists but is not quantified (Yadav et al., 2024).

Food safety: Nanoparticle residues in edible tissues are rarely measured; human and livestock exposure risks are unassessed (Shelar et al., 2024).

Environmental fate: Long-term persistence, transformation, and off-site transport of nanoparticles from seed priming are poorly characterized (Yadav et al., 2024).

Regulatory frameworks: Absence of clear regulations creates uncertainty for commercialization and adoption (Yadav et al., 2024; Shelar et al., 2024).

12.6 Overall Assessment

Promise and caution: Nano-priming shows clear potential for enhancing germination, early vigor, and stress tolerance across diverse crops and stressors. The consistency of laboratory findings and initial field validations support continued research and development. However, methodological heterogeneity, knowledge gaps in long-term safety and environmental fate, and lack of economic and socioeconomic data necessitate cautious, evidence-based advancement with rigorous risk assessment and stakeholder engagement (Davies, 2023; Shelar et al., 2024; Yadav et al., 2024).

13. KNOWLEDGE GAPS AND FUTURE RESEARCH AGENDA

Advancing nano-priming from promising research to safe, effective, and sustainable agricultural practice requires targeted research addressing identified gaps. This section outlines concrete, prioritized recommendations.

13.1 Standardization and Methodological Rigor

Priority: Critical

1. **Develop consensus nano-priming protocols:** Convene international expert working groups to establish standardized protocols for nanoparticle characterization, priming procedures, stress assays, and outcome measurements for major crops (wheat, rice, maize, soybean, tomato).
2. **Create reference nanomaterials:** Produce and distribute well-characterized reference nanoparticle batches (ZnO, SiO_2, Se, chitosan) for inter-laboratory method validation and reproducibility testing.
3. **Publish minimum reporting standards:** Develop and disseminate nano-priming-specific reporting guidelines (analogous to CONSORT for clinical trials) covering nanoparticle characterization, priming protocols, experimental design, statistical analysis, and data availability.
4. **Conduct round-robin studies:** Organize multi-laboratory experiments using identical nanoparticle batches and protocols to assess reproducibility and identify sources of variability.

13.2 Dose-Response and Toxicity Assessment

Priority: High

1. **Comprehensive dose-response studies:** Conduct systematic dose-response experiments spanning sub-optimal to toxic concentrations (minimum 6–8 dose levels) for major nanomaterials and crops, with statistical modeling to determine EC10, EC50, NOEC, and LOEC values.
2. **Include appropriate controls:** Routinely include unprimed, water-primed, bulk material-primed, and ionic form-primed controls to distinguish nanoparticle-specific effects.
3. **Assess mixture effects:** Evaluate interactions between nanoparticles and other seed treatments (fungicides, insecticides, fertilizers) or multiple nanoparticles to identify synergies, antagonisms, or additive effects.
4. **Genotype × dose interactions:** Screen diverse genotypes within species to identify genetic determinants of nano-priming responsiveness and toxicity susceptibility.

13.3 Mechanistic Elucidation

Priority: High

1. **Omics approaches:** Apply transcriptomics, proteomics, and metabolomics to nano-primed seeds and seedlings to comprehensively map molecular responses, identify key regulatory nodes, and discover novel mechanisms.
2. **Transporter and channel studies:** Directly measure expression and activity of ion transporters (HKT, NHX, SOS1), aquaporins (PIPs, TIPs), and nutrient transporters in nano-primed plants to elucidate ion homeostasis and water relations mechanisms.
3. **Hormone profiling:** Quantify endogenous hormone levels (ABA, GA, auxins, cytokinins, ethylene, brassinosteroids, salicylic acid, jasmonic acid) and hormone-responsive gene expression following nano-priming.
4. **Epigenetic and transgenerational studies:** Investigate DNA methylation, histone modifications, and small RNA changes to determine if nano-priming induces epigenetic memory; conduct multi-generation experiments to assess transgenerational stress tolerance.
5. **Nanoparticle-biomolecule interactions:** Use biophysical techniques (surface plasmon resonance, isothermal titration calorimetry, molecular docking) to characterize nanoparticle interactions with seed proteins, enzymes, membranes, and nucleic acids.

13.4 Uptake, Fate, and Environmental Impact

Priority: High

1. **Speciation and fate tracking:** Employ single-particle ICP-MS, X-ray absorption spectroscopy, and stable isotope labeling to track nanoparticle uptake, translocation, transformation (dissolution, aggregation, biotransformation), and persistence in seed-plant-soil systems over full crop cycles.
2. **Soil microbiome field studies:** Conduct multi-site, multi-season field trials measuring soil microbial community composition (16S/ITS amplicon sequencing, metagenomics), diversity indices, functional gene abundances (N-cycling, C-cycling), and enzyme activities in soils planted with nano-primed seeds.
3. **Non-target organism testing:** Perform standardized ecotoxicity assays (earthworms, collembolans, beneficial insects, soil nematodes) using environmentally relevant nanoparticle concentrations derived from seed priming scenarios.
4. **Lysimeter and leaching studies:** Use lysimeters to quantify nanoparticle and dissolved ion leaching to drainage water under realistic irrigation and rainfall regimes.

5. **Accumulation in food chains:** Assess nanoparticle transfer from soil to plants to herbivores (livestock, wildlife) and potential for bioaccumulation or biomagnification.

13.5 Field Validation and Agronomic Performance

Priority: High

1. **Multi-site, multi-season field trials:** Conduct replicated field experiments across diverse agroecological zones, soil types, and climates, measuring germination, growth, yield, quality, and economic returns over multiple growing seasons.
2. **On-farm participatory trials:** Engage farmers in co-designing and conducting nano-priming trials on working farms, incorporating farmer knowledge and priorities.
3. **Stress gradient experiments:** Test nano-priming efficacy across gradients of salinity and drought severity (mild, moderate, severe) to define stress thresholds where benefits are maximized.
4. **Integrated crop management:** Evaluate nano-priming in combination with other agronomic practices (irrigation management, fertilization, crop rotation, cover cropping) to optimize whole-system performance.

13.6 Food Safety and Residue Analysis

Priority: High

1. **Residue quantification in edible tissues:** Use spICP-MS, XAS, and other speciation techniques to quantify nanoparticle vs. ionic forms in grains, fruits, vegetables, and fodder from nano-primed crops at harvest.
2. **Dietary exposure modeling:** Integrate residue data with consumption patterns to estimate human and livestock dietary exposure to nanoparticles from nano-primed crops.
3. **Toxicology and bioavailability studies:** Conduct feeding studies with animals consuming crops from nano-primed seeds, measuring tissue accumulation, bioavailability, and potential adverse health effects.
4. **Post-harvest stability:** Assess nanoparticle stability during storage, processing (milling, cooking), and digestion to inform exposure and risk assessments.

13.7 Economic and Socioeconomic Research

Priority: Medium-High

1. **Cost-benefit analysis:** Conduct farm-level economic analyses comparing nano-priming costs (nanoparticles, labor, equipment) with benefits (yield increases, quality improvements, input savings, risk reduction) across farming systems.
2. **Life cycle assessment (LCA):** Perform comprehensive LCA of nano-priming systems, quantifying environmental burdens (energy, emissions, resource use) and benefits (yield, reduced inputs) from nanoparticle synthesis to crop harvest.
3. **Value chain analysis:** Map nano-priming value chains, identifying actors, costs, margins, and bottlenecks from nanoparticle production to seed treatment to farm use to markets.
4. **Adoption and diffusion studies:** Survey and interview farmers, extension agents, and seed companies to assess awareness, perceptions, adoption intentions, and barriers; model diffusion pathways.
5. **Equity and access research:** Evaluate how nano-priming adoption affects different farmer groups (smallholders vs. large-scale, men vs. women, different socioeconomic strata) and design inclusive access strategies.

13.8 Regulatory Science and Policy Development

Priority: Medium-High

1. **Risk assessment frameworks:** Develop nano-specific risk assessment frameworks for agricultural nanomaterials, integrating exposure assessment, hazard characterization, and risk characterization with uncertainty analysis.
2. **Testing guidelines:** Collaborate with regulatory agencies (EPA, EFSA, OECD) to develop standardized testing guidelines for nanoparticle efficacy, environmental fate, ecotoxicity, and food safety in agricultural contexts.
3. **Regulatory pathway mapping:** Analyze existing regulatory frameworks across countries, identify gaps and inconsistencies, and propose harmonized pathways for nano-priming product registration and approval.
4. **Stakeholder engagement:** Facilitate dialogue among researchers, farmers, industry, regulators, NGOs, and consumers to build consensus on safety standards, labeling, and governance.
5. **Policy instruments:** Evaluate policy options (subsidies, incentives, training programs, public-private partnerships) to support safe and equitable nano-priming adoption.

TABLE 4: Research Priority Matrix

Research Area	Priority Level	Rationale	Estimated Timeline	Key Stakeholders
Standardization & protocols	Critical	Essential for reproducibility and comparability	1–2 years	Researchers, standards organizations
Dose-response & toxicity	High	Needed for safety and efficacy optimization	2–3 years	Researchers, regulators
Mechanisms (omics, transporters)	High	Deepens understanding, enables rational design	3–5 years	Researchers, funders

Fate & environmental impact	High	Critical for risk assessment	3–5 years	Researchers, regulators, environmentalists
Multi-site field trials	High	Validates lab findings, informs recommendations	3–5 years	Researchers, farmers, extension
Food safety & residues	High	Essential for regulatory approval and consumer acceptance	2–4 years	Researchers, regulators, industry
Economic analysis	Medium-High	Informs adoption decisions	2–3 years	Economists, farmers, industry
Regulatory frameworks	Medium-High	Enables commercialization	2–4 years	Regulators, researchers, industry, NGOs
Socioeconomic & equity	Medium	Ensures inclusive benefits	3–5 years	Social scientists, farmers, NGOs

14. CONCLUSIONS

Nanomaterial-assisted seed priming represents an innovative approach to enhancing crop germination, seedling vigor, and stress tolerance under salinity and drought conditions. The body of evidence accumulated from 2014 to 2025 demonstrates that diverse nanomaterials—including metal oxides (ZnO, TiO_2, CeO_2, CuO), selenium nanoparticles, nano-silica, chitosan nanoparticles, carbon-based nanomaterials, and composite systems—can significantly improve germination metrics, antioxidant defenses, ion homeostasis, and metabolic activation across cereals, oilseeds, legumes, and vegetables.

14.1 Key Findings and Contributions

Agronomic benefits: Nano-priming consistently enhances germination percentage, germination rate, and seedling vigor under both optimal and stress conditions, with improvements ranging from 10% to over 100% depending on material, dose, crop, and stress severity. Field trials in rice, tomato, and cucumber confirm that laboratory-observed benefits can translate to real-world agricultural settings, including yield improvements and sustained stress resilience.

Mechanistic insights: Nano-priming operates through interconnected pathways involving controlled ROS signaling (hormesis), upregulation of antioxidant enzymes (SOD, CAT, POD, APX), acceleration of reserve mobilization (α-amylase, endo-β-mannanase), modulation of hormonal balance (ABA reduction, GA enhancement), improvement of ion homeostasis (K^+ retention, Na^+ exclusion), and protection of photosynthetic machinery. TEM evidence confirms nanoparticle internalization in seed tissues, supporting direct cellular interaction mechanisms.

Dose-response and hormesis: Nano-priming exhibits biphasic dose-response relationships, with optimal concentrations producing maximal benefits and higher doses causing inhibition or toxicity. Optimal doses are crop- and material-specific, reflecting differences in seed biology, nanoparticle properties (size, coating, dissolution), and stress conditions.

Material diversity: Different nanomaterial classes offer distinct advantages: metal oxides provide micronutrient delivery and ROS modulation; nano-silica offers low toxicity and eustress signaling; chitosan nanoparticles provide biocompatibility and elicitor activity; selenium nanoparticles enhance antioxidant functions; composite systems enable multifunctional effects.

14.2 Critical Knowledge Gaps

Despite promising findings, significant gaps remain:

Methodological heterogeneity: Inconsistent nanoparticle characterization, priming protocols, stress assays, and reporting practices limit reproducibility and cross-study synthesis. Standardization is urgently needed.

Long-term safety and environmental fate: Empirical data on nanoparticle persistence, transformation, and ecological impacts (soil microbiomes, non-target organisms) are scarce. Food safety assessments, including nanoparticle residue quantification in edible tissues and dietary exposure modeling, are largely absent.

Field-scale validation: Multi-site, multi-season field trials with comprehensive agronomic, economic, and environmental monitoring are limited. Scaling from laboratory to commercial agriculture faces technical, economic, and regulatory challenges.

Mechanistic depth: While biochemical correlates of nano-priming are well-documented, molecular mechanisms (specific gene targets, transporter regulation, epigenetic changes) require deeper investigation using omics and genetic approaches.

Socioeconomic considerations: Economic analyses, farmer adoption studies, and equity assessments are virtually absent, hindering translation to practice.

14.3 Recommendations for Stakeholders

For researchers:

- Adopt standardized characterization and reporting practices
- Prioritize multi-site field trials, fate studies, and safety assessments
- Engage in interdisciplinary collaborations integrating agronomy, nanotechnology, ecology, toxicology, and social sciences
- Share data openly to enable meta-analyses and reproducibility checks

For regulatory agencies:

- Develop nano-specific testing guidelines and risk assessment frameworks for agricultural nanomaterials
- Establish clear registration pathways, safety thresholds, and labeling requirements
- Support pre-competitive research on environmental fate and food safety

- Facilitate international harmonization of regulations

For industry and seed companies:

- Invest in scale-up technologies and quality control for nano-priming products
- Conduct rigorous safety and efficacy testing in collaboration with independent researchers
- Engage transparently with farmers, consumers, and regulators
- Explore sustainable, low-cost nanoparticle synthesis and formulation methods

For farmers and extension services:

- Participate in on-farm trials and provide feedback on practicality and efficacy
- Seek reliable information from extension services and independent sources
- Adopt nano-priming cautiously, starting with small-scale trials
- Advocate for accessible, affordable, and safe nano-priming technologies

For policymakers and funders:

- Fund interdisciplinary research addressing knowledge gaps identified in this review
- Support development of regulatory frameworks and testing infrastructure
- Promote equitable access to nano-priming technologies for smallholder farmers
- Encourage public-private partnerships for sustainable innovation

14.4 Future Outlook

Nano-priming holds promise as a tool for enhancing crop resilience in the face of climate change, soil degradation, and water scarcity. However, realizing this potential requires a science-based, precautionary approach that prioritizes safety, sustainability, and equity alongside efficacy. By addressing methodological gaps, conducting rigorous field and safety studies, engaging diverse stakeholders, and developing supportive regulatory and policy frameworks, the agricultural research community can guide nano-priming toward responsible innovation that benefits farmers, consumers, and the environment. The next decade will be critical for nano-priming research and development. Coordinated, well-funded, and transparent efforts to standardize methods, validate field performance, assess long-term impacts, and ensure socioeconomic inclusivity will determine whether nano-priming transitions from laboratory curiosity to mainstream agricultural practice. The evidence reviewed here provides a solid foundation; the research agenda outlined offers a roadmap. Success will require collaboration, rigor, and commitment to the principles of sustainable agriculture and responsible innovation.

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