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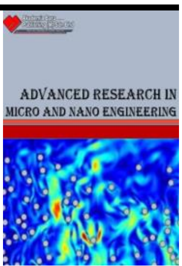

Enhancing Agricultural Productivity Through Iron Oxide Nanoparticle Priming: Opportunities, Challenges, and Surface Modification Strategies

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ABSTRACT

The global food crisis is exacerbated by various challenges, including agricultural restrictions and environmental stresses, necessitating innovative approaches to enhance crop development and productivity. One promising technique is seed priming with iron oxide nanoparticles (FeNPs), which has shown significant potential in improving seed germination, crop growth, and stress resistance. This mini-review explores the synthesis, application, and physiological impacts of FeNPs in agriculture, emphasizing their role in addressing iron deficiency in plants and promoting robust plant development under challenging environmental conditions. Despite their benefits, the practical use of FeNPs faces critical challenges, notably nanoparticle agglomeration in biological media, which can diminish their effectiveness and lead to phytotoxicity. This review highlights advanced surface modification strategies, including the use of biocompatible polymers like chitosan and silica encapsulation, to enhance the colloidal stability, reduce agglomeration, and ensure the safe delivery of FeNPs. It discusses the mechanisms by which these modifications improve nanoparticle dispersion and interaction with plant systems, thereby optimizing their agronomic benefits. The review concludes with insights into the future directions of nanoparticle use in seed priming, particularly focusing on the implications of nanoparticle physicochemical properties on their agronomic efficacy and environmental safety.

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1. Introduction

Global food security is increasingly threatened by the agricultural trade restrictions imposed by nations seeking to augment domestic availability and reduce costs. Uniform germination, robust seedling vigour, optimal plant growth, and timely maturity are critical for ensuring high-quality crop development and productivity. In this context, the agricultural seed industry focuses on enhancing these vital yield-defining stages of crop cultivation by improving the germination and vigour indices of seeds. However, challenges, such as weak seed vigour and adverse environmental conditions, can lead to delayed germination and poor seedling establishment.

Crop development faces significant hurdles, especially in the low-input agricultural systems prevalent in underdeveloped nations. Factors such as limited water availability, soil crusting, and high temperatures severely impair crop productivity in semiarid regions. These challenges are exacerbated by climate change, which intensifies the detrimental effects of high temperature on agroecosystems. Many studies have focused on static agricultural management strategies, such as adjusting irrigation volume and frequency and optimizing fertilizer and pesticide dosages, to mitigate negative impacts on crop production and enhance agronomic traits. Among the various technologies employed to maximize crop yield, seed priming has emerged as one of the most effective, low-risk, and cost-efficient. Modern priming techniques are known to significantly improve germination rates and enhance resilience to both abiotic and biotic stressors. Seed priming methods range from standard techniques, such as hydro-priming, osmo-priming, bio-priming, chemical priming, halo-priming, solid matrix priming, and the use of plant growth regulators (PGRs), to advanced methods, including nano-priming and priming with physical agents, as illustrated in Figure 1 [1,2].

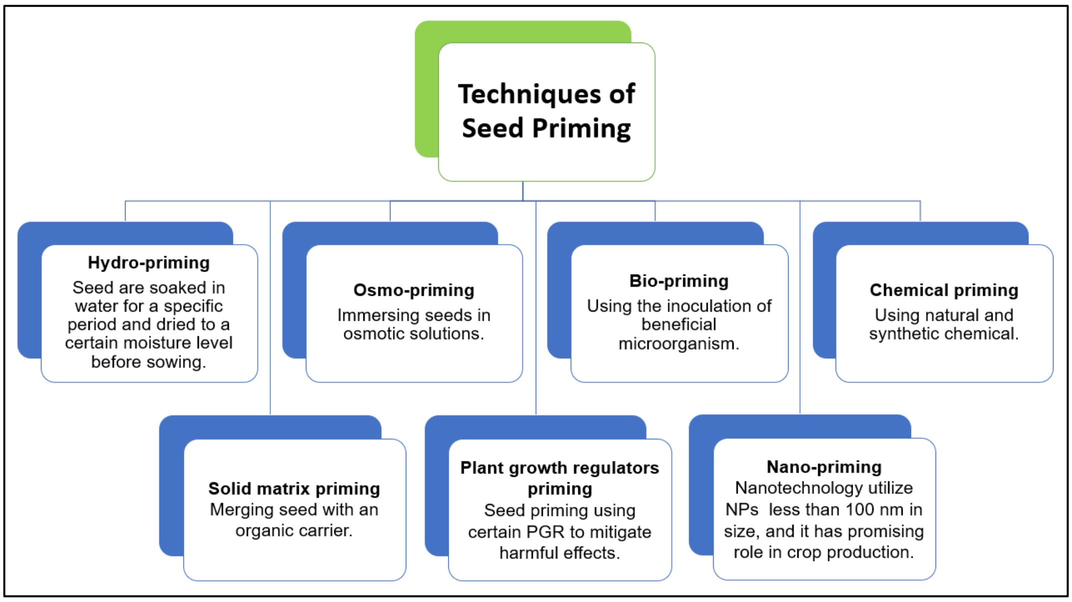

Fig. 1. Schematic diagram of the different seed priming methods

Nano-priming has proven to be particularly effective, outperforming traditional priming methods in promoting sustainable agricultural production [3,4]. Recent studies have suggested that nano-priming of seedlings can activate a range of genes associated with germination and stress resistance. Nanoparticles, such as silver, gold, copper, and titanium oxide, have been successfully used in these applications.

Iron (Fe), the fourth most abundant element in the Earth's core, is also recognized for its non-toxic properties and is essential for various cellular functions in plants, including respiration and photosynthesis [5,6]. Research has shown that iron enhances plant resistance to biotic stresses, whereas iron deficiency leads to abiotic stress. Iron oxide nanoparticles (FeNPs) have been shown to significantly enhance seed germination and crop growth [3,7-10]. Despite the abundance of iron in the soil, its availability to plants is often limited, especially in calcareous soils where solubility is poor. Iron deficiency remains a significant global agricultural productivity issue, often resulting in chlorosis and reduced photosynthesis in plants, thereby diminishing crop quality and yield [11,12]. Additionally, iron is a component of oxidase enzymes, including catalase and peroxidase. Its deficiency causes both chloroplast structural deterioration and chlorophyll loss to occur at the same time [6]. Since iron in plants has a very low mobility chlorosis first manifests in the interveinal areas of the youngest leaves. If the deficit is severe enough, chlorosis may spread to the veins and even cause the little leaves to turn white. Hence, iron is indeed an important element for nutrient uptake in plants. The performance of iron seed priming on various crops is shown in Table 1.

Table 1
Effect of iron seed priming on grain yield, production, and growth of various crops

Materials	Crop	Concentration	Parameters	References
Fe solution	Groundnut (*Arachis hypogaea L.*)	-	Improves the growth and yield of groundnut.	[13]
	Bread Wheat	1mg/L to 8mg/L	Improves germination, mitosis, and yield of bread wheat.	[14]
Iron oxide nanoparticles	Sorghum (*Sorghum bicolor (L.) Moench*)	10, 50, 100, and 500 mg/L	Increased biochemical activity and biomass, improved water content in leaves.	[15]
	Watermelon (*Citrullus lanatus*)	20, 40, 80, 160 mg/L	Increase nonenzymatic antioxidant potential and seedling development.	[5]
Iron nanoparticles	Wheat (*Triticum aestivum* L.)	25, 50, 100, 200, 300, 400, 500, and 1000 µg/mL	Improved seed vigour and plant morphology.	[16]
Iron coated seed	Rice	-	The primed rice seeds considerably outperformed the unprimed rice seeds in terms of seed germination, seedling development, establishing plant height, dry weight, a-amylase activity, and glucose concentration throughout time. The a-amylase activity and soluble sugar concentration in the primed rice seeds were greater under anoxia.	[17]

FeNPs have attracted considerable attention because of their unique properties, including superparamagnetic, high surface-to-volume ratio, and large surface area, which facilitate easy separation [18]. However, despite their potential benefits to agriculture and the agro-sector economy, challenges such as nanoparticle agglomeration remain [19,20]. Studies by Vollath have noted that nanoparticles tend to agglomerate due to weak van der Waals forces, forming submicron-sized entities [21-23]. In contrast, covalent or metallic bonds within the nanoparticle aggregates are not easily disrupted [24]. In addition, as noted by Wulandari and Mardila, the high surface-to-volume ratio of metallic nanoparticles makes them prone to instability and aggregation [25]. This necessitates surface modification to stabilize the magnetic nanoparticles and to prevent oxidation. The resulting increase in size, uneven sedimentation, and restricted mobility can lead to phytotoxic effects, resource loss due to runoff, and decreased efficiency, ultimately reducing plant performance and productivity [26].

Therefore, a key challenge in the use of FeNPs for seed priming is their tendency to agglomerate in biological and physiological media. Surface functionalization can be achieved by introducing polymers that are less toxic and can maintain the FeNPs in smaller clusters over the long term. This review discusses the highlights and challenges of this approach, compares colloidal stability based on zeta potential and hydrodynamic size, evaluates crop performance, and assesses how the materials used to functionalize the surface of FeNPs can mitigate agglomeration issues in seed priming.

2. Translocation Behavior of FeNPs onto Plant Cell Walls

Transpiration rate is a primary factor regulating the movement of nanoparticles (NPs) within plant systems. The uptake and internalization of NPs are influenced by various plant structures and functions, including the nature of the cell wall, mucilage properties, stomatal openings, and symbiotic relationships with the host plant. The interaction of NPs with the soil, along with their inherent stability and physiological architecture of plant cells, significantly affects the translocation and accumulation of NPs within plants.

The plant cell walls serve as specialized barriers that regulate NPs entry. They determine whether NPs can be solubilized or allowed to pass through, based on the size of the pores in the cell wall; typically, NPs must be between 40-50 nm to traverse from the plant surface into the cells [27,28]. The physicochemical nature of NPs also affects their ability to penetrate these barriers or adhere to radical surfaces and exudates, with positively charged NPs showing improved adherence to the cell wall.

Research highlights the significant increase in photosynthetic pigments such as chlorophyll a, b, and carotenoids in moringa plants exposed to foliar applications of FeNPs at concentrations of 40 ppm, demonstrating potential enhancements in plant physiological processes [29]. However, studies on the specific translocation behaviour of FeNPs are limited and warrant further exploration, especially when compared with other nanoparticles used in agriculture. Regardless, for instance, in the related agriculture field, studies have shown that zinc oxide nanoparticles (ZnONPs) in various forms, such as zinc phosphate, exhibit maximal absorption in the roots and shoots of *Z. mays* when exposed hydroponically, likely because of increased dissolution in the rhizosphere, enhanced plant uptake, or efficient ionic zinc translocation [30]. Similar speciation of Zn accumulation has been observed in wheat grown in soil environments [31]. Still, ZnONPs have become one of the most preferred metal oxide nanoparticles in biological applications due to their high biocompatibility, low cost, and low toxicity [32].

The effect of nanoparticle size on translocation is also evident in studies involving titanium dioxide (TiO_2) NPs in *Triticum aestivum*, where it has been suggested that particles exceeding a threshold diameter of 140 nm fail to accumulate in the roots. Moreover, a threshold diameter of 36 nm allows accumulation in the root parenchyma but restricts translocation to the shoot or stele [33].

3. Methods for Enhancement of FeNPs Colloidal Stability

The colloidal stability of nanoparticles (NPs) in a solution is indicative of their equilibrium state and is crucial for numerous industrial and manufacturing processes. Nanoparticles are typically stabilized by the formation of a dispersion layer around their surface. The effectiveness of this stabilization, particularly in suspensions with high nanoparticle concentrations, depends crucially on achieving the optimal thickness of this dispersion layer (adlayer). Thin adlayers tend to promote particle aggregation, whereas excessively thick adlayers restrict the mobility around the particles, creating a constrained volume [34,35]. Suitable surface-coating strategies have been employed to counteract challenges such as precipitation and aggregation. These strategies were designed to provide sufficient repulsive forces to counterbalance the attractive van der Waals (VDW) forces and magnetic interactions present on the surfaces of the FeNPs.

Nanoparticles can interact with various biomacromolecules upon contact with physiological fluids. Extensive research has investigated these interactions, focusing on the inherent properties of NPs and their effects on cellular responses in vitro [36]. However, the colloidal resilience of NPs within biological media remains relatively underexplored. Agglomeration in such complex environments is common, leading to the formation of large, irregular clusters, an irreversible process known as aggregation. This aggregation alters the cytotoxic impact and cellular uptake of nanoparticles, potentially skewing results and impacting experimental reliability [37]. Traditional in vitro systems frequently overlook the potential alterations in colloidal properties when NPs are introduced into physiological fluids, despite evidence suggesting that NP aggregation significantly influences cellular responses, both directly through increased particle size and indirectly through altered diffusion and sedimentation rates [36].

Understanding the factors that influence NPs colloidal stability and aggregation in biological media is essential for the development of safe and effective nanotherapeutics. These factors directly affect the biodistribution, pharmacokinetics, and toxicity profile of NPs in vivo. For example, Barrow *et al.,* reported that nanoparticle aggregation likely caused capillary occlusion in the lungs, leading to morbidity in toxicity studies involving high-dose intravenous administration of nano-hydroxyapatite to Wistar rats [38]. Additionally, achieving efficient NPs diffusion during the seed germination phase in agriculture requires steric stabilization to maintain the colloidal stability. This stability allows for enhanced diffusive capacity within plants, as corroborated by Curtis *et al.*, which is vital for predicting transport behaviours within plant systems [39].

The colloidal stability in aqueous solutions was assessed in the presence of basic electrolytes. Stability is governed by inter-particle behaviour, which is influenced by intermolecular and surface interactions, VDW forces, the repelling electrostatic double layer (EDL), and structural factors, such as depletion attraction [35,36,40]. The balance of these forces determines the colloidal stability of the NPs in suspension. The EDL typically counteracts the VDW forces, helping stabilize the dispersion. In aqueous conditions, the surface charge on NPs, resulting from the ionization or dissociation of surface groups or the adherence of charged molecules or ions, plays a crucial role in maintaining stability [36].

Effective strategies to prevent NP aggregation include particle coating with capping agents, the use of coupling agents or compatibilizers, and the application of electrostatic repellents to charge the filler surface and keep particles separate [41]. Additionally, optimal manufacturing settings can enhance the aggregate disruption. For example, using the right screw speed and feed rate in an extruder can exert sufficient shear to break particle clusters [41]. The stability of the colloidal system can also be inferred from the zeta potential measurement; particles with a high negative or positive zeta potential are likely to repel each other and maintain the suspension stability. Ideally, a zeta potential beyond -30 mV to +30 mV is indicative of a sufficient repulsive force to ensure colloidal stability. Despite these measures, FeNPs often show poor stability and a propensity to agglomerate, with an average hydrodynamic particle size of 317.53 nm and a zeta potential of -14.33 mV, highlighting the need for improved stabilization techniques [42].

Ensuring the colloidal stability of FeNPs is crucial for their effective application in agricultural practices. Colloidal stability can be assessed by measuring the zeta potential, whereas stated, values beyond -30 mV to +30 mV indicate a sufficient repulsive force to prevent aggregation. However, as

FeNPs exhibit poor stability resulting in the tendency to agglomerate, researchers have explored various methods to enhance the colloidal stability of FeNPs to address these challenges. Indicated in Fawiza's inquisition that surface modification using organic molecules such as surfactants, polymers, and small organic ligands has shown promising results in enhancing FeNPs stability [43-45]. Besides, using biopolymers to interact with the nanoparticle surface through hydrogen bonding and electrostatic interactions is also one of the common approaches to enhance stability in diverse media based on previous studies [46-48]. The following section provides a concise overview of the various strategies used to enhance the colloidal stability of FeNPs, aligning with the review's main objectives and offering detailed examples from the literature.

4. Chitosan-stabilized FeNPs

Chitosan (CS) is one of the most effective biopolymers, enhancing crop yields and mitigating the adverse effects of saline conditions owing to its bioactive properties derived from natural sources such as crab shells and shrimp [49-51]. Its environmentally friendly and biodegradable characteristics make it extensively useful in agriculture for enhancing plant resistance to various stresses, especially drought [52-54]. Chitosan is nontoxic, biodegradable, and naturally occurring. In addition to that, chitosan is an indigestible ingredient due to its fiber content. In an acidic solution, chitosan can be diluted and dissolved, such as can be diluted in 0.1 M acetic acid, but it does not dissolve in water or any other organic solvent [55]. Stabilizing FeNPs with chitosan not only minimizes agglomeration during synthesis but also enhances their functional efficacy. According to Shinde, chitosan-coated nanoparticles exhibit persistent zeta potentials that significantly reduce aggregation due to repulsive forces between particles [56,57]. Appu *et al.,* reported that chitosan application results in FeNPs with an average particle size of 396 nm and zeta potential of +76.9 mV, substantially improving their colloidal stability [58].

This arrangement provides chitosan with an abundance of amino groups that can interact effectively with the negatively charged surfaces of FeNPs via electrostatic attraction, elevating the stability of the colloidal suspension. By forming a strong bond with the iron oxide surface when chitosan is applied to adhere to FeNPs, these amino groups successfully prevent aggregation by establishing an electrostatic and steric barrier. This dual stabilisation process improves FeNP dispersion in diverse media, resulting in a lower hydrodynamic size and a more stable zeta potential, which frequently shifts to positive values, preventing agglomeration. Beyond stability, chitosan can provide functional benefits such as increased biodegradability and tailored delivery capabilities, making chitosan-coated FeNPs ideal for agricultural applications requiring long-term release and low environmental effects. Studies have shown that chitosan-coated FeNPs are more stable in both aqueous and soil conditions, resulting in increased nutrient delivery and plant growth stimulation. Current research is focused on leveraging these properties to boost the efficiency of FeNPs for seed priming and crop protection, considering the molecular weight variations in chitosan affecting nanoparticle behaviour and distribution [59-61].

5. Silica-encapsulated FeNPs

Silica is a widely favoured material for nanoparticle encapsulation owing to its chemical stability and compatibility with a range of functional groups for applications in medicine, biosensing, and agriculture. The sol-gel method, originally developed by Stober *et al.*, was adapted to produce silica-coated FeNPs, enhancing their stability in aqueous environments [62]. This method involves the alkalinization of tetraethyl orthosilicate, followed by condensation in an ethanol-water solution, with subsequent modification to integrate the FeNP nuclei. However, FeNPs tend to form large aggregates if they are not properly treated before integration into a silica matrix. By immersing FeNPs in a dilute alkaline sodium silicate solution, Hashemi *et al.,* improved the compatibility and reduced the tendency of FeNPs to clump, enhancing seed stability when introduced into agricultural systems [62]. Teow *et al.,* noted that silica-coated FeNPs achieved a zeta potential of -20.93 mV and a smaller particle size of 228.23 nm, indicating moderate stability that mitigates aggregation issues [42].

6. Polyvinylpyrrolidone (PVP) as Capping Agent

The reduction of nanoparticle agglomeration has been the focus of limited, yet significant, studies. Seo *et al.,* demonstrated that polyvinylpyrrolidone (PVP) effectively reduced the agglomeration of nanoparticles, reducing the average particle size from 857 to 225 nm. Additionally, PVP plays a crucial role in inhibiting the formation of iron oxide crystals and managing oxidation processes [63]. As a capping agent, PVP uses steric effects facilitated by its hydrophobic chains to prevent nanoparticle aggregation. Its non-toxic, biodegradable, biocompatible, and temperature-resistant properties make PVP one of the preferred polymeric materials for encapsulating iron oxide nanoparticles (FeNPs). PVP also serves as a carrier medium in medication delivery systems and iron-containing nanocomposites.

However, a significant challenge with PVP is its lengthy decomposition. If the decomposition period is too short, it does not allow the PVP structure is not fully developed, whereas a lengthy period risks undesirable hydrolysis [64]. PVP's effectiveness of PVP is also influenced by the interaction of its functional groups with the surfaces of FeNPs. Specifically, the CH_2 groups contribute to stability and flexibility, facilitating movement across surfaces, whereas the C=O and C-N groups create strong bonds with the nanoparticles, enhancing stability and preventing aggregation.

Research on sol-gel synthesis modified for FeNPs indicates that PVP concentration affects both the nanoparticle behaviour and magnetic properties. Studies have shown that higher PVP concentrations eliminate the Morin transition observed in samples with lower concentrations and enhance the saturation magnetization across various Fe^{3+}: PVP monomer ratios (1:6, 1:12, 1:18, and 1:24), suggesting an interaction that affects the magnetic properties of FeNPs [65]. Identifying the optimal Fe^{3+}: PVP ratio depends on the specific application needs, that is, whether the focus is on harnessing the Morin transition or achieving higher magnetization.

Further research revealed that PVP with higher molecular weights can bind more ions on the FeNP surface, thus increasing its zeta potential. This is evident in PVP-FeNPs, where molecular weights of 10,000, 25,000, and 40,000 mol/g correspond to zeta potentials of -10.2, -18.4, and -42.5 eV, respectively [66]. These findings suggest that higher-molecular-weight PVP establishes a more robust electrostatic interaction, which significantly enhances the colloidal stability of FeNPs. Therefore, with the highest molecular weight tested, the increased electrostatic repulsion among the molecules led to improved steric stability.

7. Conclusion and Future Outlook

This review underscores the transformative potential of nanotechnology in agriculture, particularly the use of iron oxide nanoparticles (FeNPs) for seed priming. The unique physicochemical properties of nanoparticles, such as high reactivity and increased surface area-to-volume ratio, enable more effective interactions with the environment, significantly enhancing seed germination and crop establishment. The use of nanomaterials in seed treatment represents a cutting-edge approach that meets the increasing demands of modern agriculture by facilitating efficient use of resources and uniform crop development.

However, the practical application of FeNPs faces challenges, particularly the tendency of nanoparticles to aggregate under biological and physiological conditions, which can impede their effectiveness and safety. To address this, the incorporation of less toxic biocompatible polymers such as chitosan has proven to be beneficial. Chitosan not only stabilizes FeNPs, reducing aggregation but also enhances crop yields and mitigates the adverse effects of environmental stressors, such as salinity. The high zeta potential of chitosan-coated FeNPs promotes dispersibility and stability, making them more effective for seed-priming applications.

Despite these advances, significant gaps remain in our understanding of how modifications of the molecular weight of chitosan affect the behaviour and efficacy of FeNPs. Future research should focus on the following aspects.

i. Systematic Evaluation of Molecular Weight Variations: Investigating how different molecular weights of chitosan influence the physicochemical properties of FeNPs, particularly their stability, reactivity, and biocompatibility.
ii. Long-term Agricultural Studies: Field trials to be conducted to assess the long-term impacts of chitosan-coated FeNPs on crop yield, soil health, and ecosystem dynamics to ensure that these nanotechnologies do not inadvertently cause environmental harm.
iii. Mechanisms of Nanoparticle Uptake and Translocation: Deepening the understanding of how nanoparticles are absorbed and transported within plant systems is crucial for optimizing their design and application routes.
iv. Regulatory and Safety Assessments: Developing comprehensive guidelines and safety assessments to govern the use of nanoparticles in agriculture. This includes evaluating potential toxicity and environmental impact and ensuring compliance with international standards for sustainable farming practices.
v. Innovations in Nanoparticle Synthesis: Exploring new synthetic methods that can further enhance the functionality of FeNPs, such as targeted delivery systems for nutrients or genetic material, which could revolutionize precision agriculture.

By addressing these areas, researchers can not only enhance the efficacy of nanoparticle-based seed treatments but also ensure their sustainability and safety for widespread agricultural use. The integration of advanced nanomaterials into farming practices promises to sustainably meet future agricultural demands, supporting global food security in the face of changing environmental conditions.

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