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MINI REVIEW ARTICLE

Comparative response of nanometric forms of Zn and Fe to promote crop biofortification

Steffanny Sánchez-Portillo[1], Hipólito Hernández-Hernández[2], Álvaro Javier Pastrana-Pastrana[3], Laura Castro-Rosalez[1] & Fabián Pérez-Labrada[4*]

[1]Doctorado en Ciencias en Agricultura Protegida, Universidad Autónoma Agraria Antonio Narro Antonio Narro, Saltillo 25315, Coahuila, Mexico
[2]Instituto de Agroingeniería, Universidad del Papaloapan. Loma Bonita 68400, Oaxaca, Mexico
[3]Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Saltillo 25280, Coahuila, Mexico
[4]Departamento de Botánica, Universidad Autónoma Agraria Antonio Narro Antonio Narro, Saltillo 25315, Coahuila, Mexico

*Correspondence email - fabian.perezl@uaaan.edu.mx

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Abstract

Micronutrient deficiency or malnutrition, particularly iron (Fe) and zinc (Zn), is a serious global health problem and represents a challenge for the agri-food sector. Crop biofortification has emerged as a strategy to increase the mineral content of the target organs. To improve and maximize biofortification, alternatives, such as nanotechnology (mainly nanoparticles), have been proposed and studied. The objective of this minireview was to highlight the responses of nanometric forms of Zn and Fe used in crop biofortification, which can guide the design and/or selection of the most appropriate form to efficiently promote the bioaccumulation of these nutrients. Studies have shown that Fe and Zn applied as nanoparticles (NPs), in different shapes (including spherical, irregular and wurtzite crystals), sizes and doses significantly influence their absorption, translocation rates and ion bioavailability in plants. Spherical NPs are the most common Fe (1-100 nm) and Zn (12-81 nm) nanoforms used. The supplementation routes include foliar application, drench and seed priming, with responses depending on the dose applied, which ranged from 20-500 mg L^{-1} for Fe NPs and 10-1000 mg L^{-1} for Zn NPs. Biofortification with Zn NPs increased the Zn content up to 230 % in wheat grains and 56.61 % in melon fruits. Similarly, Fe contents in cucumber fruits and rice grains increased by 71.37 % and 155.91 %, respectively, when Fe NPs were applied. Different forms of Fe and Zn NPs offer promising strategies to enhance the synthesis and accumulation of bioactive compounds as well as the accumulation of Fe and Zn in plants. However, their use in a wider range of crops requires further study.

Keywords: nanoparticles; nanobiofortification; spherical; transporters; zeta potential

Introduction

The global nutritional deficit of Fe and Zn is a major challenge for the agri-food and health sectors and must be solved through comprehensive strategies. Crop biofortification is a reliable alternative for increasing the nutrient content of plants. Biofortified crops can be produced in two ways: conventional breeding and the use of fertilizers. The second pathway has become relevant in recent years because of its sustainability and because the ion of interest can be applied at the nanometer scale (1). The use of nanomaterials (NMs), material with any external dimension in the nanoscale has an internal structure in the nanoscale, allows a new opportunity to enhance nutrient bioaccumulation in crops. These NMs include nanoparticles (NPs), three external dimensions at the nanoscale, nanofibers (two external dimensions at the nanoscale), carbon nanotubes, nanowires, quantum dots, nanocapsules, graphene and fullerenes (2). Nanoforms are characterized by particles with a defined composition and specific structural parameters such as size and shape within a given range. These NMs have different functionalities depending on their structure (3, 4). Specifically, NPs possess distinctive physicochemical properties, such as high surface-to-volume, specific zeta (ζ) potential and degree of reactivity (5, 6). The spherical shape of NPs is the most common, followed by irregular, hexagonal and star shapes (2).

Surprisingly, nanometric forms of Fe and Zn represent a promising area within nanotechnology as alternatives to traditional bulk fertilizers (7, 8). Therefore, the different nanometric forms of these elements seem to have direct implications for their behavior and efficiency (9). Among the main Fe NPs, nano-zerovalent (nZVI or Fe^0), oxides and bimetallic types can be cited, whereas ZnO NPs form in those structures (e.g., wurtzite is a ZnO crystal structure) and nanoencapsulated forms (10-12).

Regardless of the type of NPs (whether applied through soil or foliar spray), the first contact with the plant is the cell wall, where the interaction can cause selective absorption through pores, stomata, hydathodes or entry by endocytosis (13). Otherwise, NPs may aggregate and block the apoplastic pathway (14). Next, they interact with the plasma membrane, causing oxidative damage. At this point, their entry can be mediated by the activation of specific transporters, such as ferric-chelate reductase (FRO2), natural resistance-associated macrophage protein (NRAMP3), iron-related transcription factor 2 (IRT2), heavy metal ATPases (HMA), zinc iron permease (ZIP) family, metal tolerance protein (MTP) family, vacuolar iron transporter (VIT) family and cation diffusion facilitators (CDF) family (15-17).

Several studies have demonstrated the potential of NPs in cereals, legumes and oilseeds to promote crop biofortification (18, 19). For example, α-Fe_2O_3 and Fe_3O_4 crystalline NPs promote efficient Fe distribution in maize, whereas in *Triticum aestivum* L., the application of FeO NPs, ZnO NPs and Fe NPs by seed priming or foliar application increased grain Fe content up to 121 %, in addition to improving Fe recovery (20-22). Similarly, ZnO NPs (wurtzite crystals, spherical) applied to the soil in *Phaseolus vulgaris* L., *T. aestivum* L. and *Zea mays* L. managed to significantly increase the Zn content in the edible parts (pods and grains) (23-25). Considering the variability of the ways in which Fe and Zn nanoparticles can be applied and the dependence of the impact (positive or negative), the objective of this work is to highlight a mini -review of the main responses presented according to the nanometric form in which Zn and Fe are applied and the impact they have on crop biofortification, which will allow the design of future studies aimed at improving the bioaccumulation of these nutrients.

Nanomaterials

Nanomaterials are structures smaller than 100 nm that possess physicochemical properties different from those of conventionally sized materials (5). The characteristics and properties of NMs strongly depend on their material, conditions and synthesis methods (26, 27). According to their nanoforms, NMs can be classified as NPs, nanofibers, carbon nanotubes, nanowires, quantum dots, nanocones, nanohorns, nanocapsules, graphene, nanoliposomes, nanomicelles, fullerenes and mesopores (2). The functionality of such NMs is strongly associated with their structure, with elongated hollow tube-shaped coiled carbon structures–1-2 nm in diameter (3). Hollow spherical particles formed by pentagonal and hexagonal carbon units between 8.2-36 nm, rectangular or square cross-sections of 1-100 nm, elongated and thin with diameter of 20-80 nm, fluorescent and <10 nm and tubular and acute-angled cone-shaped at the ends, with diameters between 2 and 5 nm (4, 28-30).

NPs, which are chemically or biologically synthesized, have been widely studied in agriculture. NPs can be spherical, irregular, cylindrical, conical, spiral, octagonal, triangular, star-shaped, flower-shaped, cubic, tetrahedral, pentagonal and hexagonal (2). Recently, it was found that in the synthesis process (biological or chemical), the shape and size of the NPs respond to the ultrasonication time, longer time duration is 60 min, smaller particle size becomes and they tend to agglomerate into spherical particles (31). In this context, it is necessary to consider this factor when biofortifying cultures.

Nanometric forms of iron

Some of the Fe NPs investigated are nano-zero-valent Fe NPs (nZVI or Fe^0), iron oxide NPs and bimetallic NPs (Fe/Pd, Fe/Ni, Fe/Ag and Fe/Cu) (10). nZVI has a typical Fe^0 core-shell and iron oxide layers and is characterized by high susceptibility to redox reactions (32, 33). However, their production poses a risk because they tend to aggregate rapidly owing to intense attractive forces, which impact physicochemical properties and decrease effectiveness, which may negatively impact their bioaccumulation rate in the tissue of interest (32, 34). Crop biofortification represents a sustainable and effective strategy to improve human nutrition, especially in regions where access to varied foods is limited, as it allows an increase in the content of essential micronutrients such as iron, zinc, vitamin A and folate in different crops, contributing to the reduction of chronic nutritional deficiencies such as iron deficiency anemia and hypovitaminosis A, strengthening the immune system and improving cognitive development in children (35, 36). In addition, by integrating these nutrients directly into commonly consumed foods, bioaccumulation through biofortification avoids the high costs of industrial supplementation and fortification programs (37).

Some Fe oxides, such as hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4), can be applied in nanoscale form (38). The α-Fe_2O_3 NPs show Fe^{3+} ions, on the other hand, the γ-Fe_2O_3 NPs present a cubic crystalline structure with high chemical stability (38, 39). In the case of Fe_3O_4 NPs, present inverted spinel structure containing Fe^{2+} and Fe^{3+}, so they tend to oxidize rapidly to Fe_2O_3 (7). Another form of NPs is β-Fe_2O_3 (cubic crystal structure) and ε-Fe_2O_3 with an orthorhombic crystal configuration, however, Fe_3O_4 NPs smaller than 20 nm exhibit paramagnetic or superparamagnetic magnetization (6, 39). Bimetallic NPs exhibit synergy between two different metals (40). For example, Fe is susceptible to corrosion in an aqueous media (41). While some bimetallic Fe NPs improve reducing ability, magnetism is not universally enhanced and depends on the second metal used (41).

Nanometric forms of zinc

ZnO NPs are the most used Zn-based nanomaterials owing to their symmetrical shape, high foliar and root absorption and high solubility (42). Another type of NPs is their hexagonal form (hexagonal wurtzite ZnO NPs), which is due to their stability in the hexagonal wurtzite phase, which provides defined edges and an anisotropic crystal structure that promotes higher surface reactivity, piezoelectric properties and slower release of Zn^{2+} (43). Another form of NPs is rod- and star-type type which have a greater exposed surface area but less symmetry, notably influencing their transport in the soil or within plants and have not been studied extensively in agricultural areas (11). In contrast, nanoencapsulated zinc sulfate allows for the sustained release of nutrients, reducing leaching losses (12). Similarly, Zn nanocomposites that include combinations of polymeric materials or biostimulants improve the stability and bioavailability of nutrients (44).

Interaction of Fe/Zn nanometric shape with the cell

The interaction of NPs with plant cells represents a critical phase for efficient biofortification. Although Fe and Zn NPs share certain physicochemical properties, they differ in certain characteristics, such as surface charge, reactivity, solubility and subcellular localization. These characteristics determine their effects on plant systems and, thus, their bioaccumulation in the tissue of interest.

Fe and Zn NPs directly interact with various cellular structures, thereby influencing their functionality and physiological processes. The first contact of NPs with the cell wall acts as a selective physicochemical barrier. Its main components, such as cellulose, hemicellulose and pectin, are negatively charged, which favors binding with positively charged NPs, according to the reactivity of the NM surface (45). In *Arabidopsis thaliana*, Fe NPs have been shown to cause OH radical-induced cell-wall loosening, which facilitates the entry of NPs by endocytosis (13). However, if the concentration of Fe NPs is very high, they can cause aggregation of NPs on cell walls and block the apoplastic pathway (14). Similarly, at optimal concentrations, Zn NPs modified the cell wall structure by inducing processes such as lignification, pectin accumulation and lignin-suberin deposition. In contrast, excessive levels of these NPs inhibit symplastic transport due to the accumulation of callose (46). Under suitable conditions, 30 nm ZnO NPs tend to accumulate in the apoplast, followed by transport to mesophyll cells (47).

In addition to the cell wall, NPs also interact with the plasma membrane, although their effects vary among species. In rice, for example, it has been observed that Zn NPs can alter membrane integrity, leading to increased electrolyte leakage and lipid peroxidation (48). In contrast, in lettuce, these NPs reduced electrolyte leakage, indicating improved cell membrane stability (49). Similarly, Fe NPs have been shown to strengthen membrane integrity by decreasing lipid peroxidation and electrolyte leakage (50). These findings show that the effects of NPs on the plasma membrane are highly dependent on plant species and, to a lesser extent, on the form of NPs, which should be considered when promoting biofortification.

However, optimal concentrations of Fe and Zn NPs can favor chloroplast structure and functionality in plant cells. In *Capsicum*, Fe NPs increased the number of chloroplasts, promoted granule stacking and contributed to the proper development of vascular bundles (14). In contrast, Zn NPs stimulated an increase in the chlorophyll content in lettuce (51). In addition, Fe NPs (10 and 20 nm) have been shown to maintain the integrity of chloroplasts and mitochondria, whereas 5 nm NPs cause deformations in chloroplasts and loss of integrity of thylakoid membranes (52).

Mechanisms of absorption, translocation and assimilation of Fe/Zn nanometric forms

Nanometric Fe and Zn ions can present several routes of entry into the plant, regardless of their form (root or foliar) (Fig. 1). In soil substrates beyond the form of NPs, the pH and root response seem to condition the effectiveness and uptake of NPs applied as a hormetic response (53). For example, metal NPs release Fe and Zn ions or are converted into soluble salts (54). NPs in the soil encounter a series of barriers before being internalized.

Root exudates

Amino acids, organic acids, sugars, phenolics, mucilage and proteins can interact with the surface charge of NPs, inducing aggregation or altering their uptake;

The epidermis

Where the NPs may follow either apoplastic or symplastic route;

The Casparian strip

Rich in suberin and lignin deposits, which appears as a barrier to the NPs so that these NMs can be distributed along it (55).

The small size of the NPs can promote their entry through discontinuities in this barrier, regardless of the shape. However, the interaction with NPs may also alter the band properties of the Casparian strip, enabling further ingress (55). Therefore, the highest absorption rate could be at the point of root growth, where the Caspari band is absent or less suberized and the shape of the NPs affects the degree of reactivity with the cellular components (54).

These NPs can be easily mobilized through the pores of the cell membrane and through the apoplastic pathway outside the plasma membrane, as the size of the plant cell wall ranges between 1 and 100 µm. Thus, the specific shape according to the

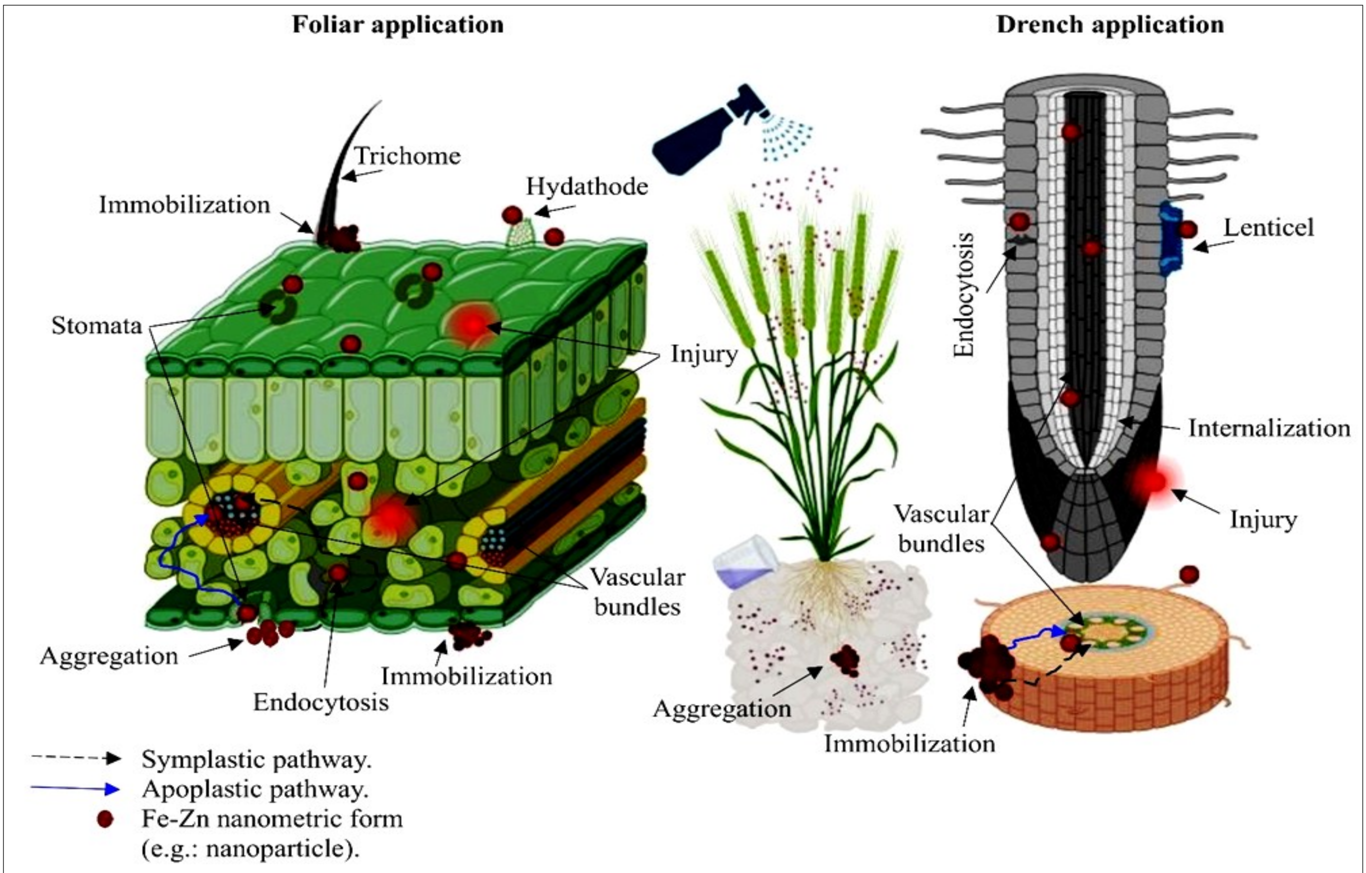

Fig. 1. Absorption, translocation and assimilation of Fe/Zn nanometric forms into leaves and roots.

size of the NPs affects their uptake (16). Some NPs can enter and mobilize through the symplast pathway through the plasmodesmata because the pore size of the cell wall is <20 nm, as reported in maize, in which ZnO NPs of various sizes (9 and 40 nm) were applied (17, 56). Surprisingly, owing to the characteristics of these NMs when interacting with the cell wall, there may be distortion/deformation that causes larger openings (54). Endocytosis is another route of entry (17, 54).

To cross the Casparian strip, Fe and Zn NPs can enter through the apparent free spaces of the cell membrane or through specific transporters such as IRT2, HMA, ZIP family, MTP family, VIT family and CDF family and be mobilized in the pericycle through the symplastic-apoplastic pathway to load the phloem and xylem where they are complexed with organic acids, asparagine, histidine amine nicotianamine and peptides (16, 17). Notably, transpiration rate and xylem loading are more critical than pore size in determining NP translocation (55, 57).

When NPs are applied foliarly, their absorption can occur via two routes: i) polar openings (such as stomata, hydathodes and trichomes) and ii) nonpolar (leaf cuticle and its pores) (58). The cell wall, particularly the cuticle, acts as the first barrier and regulates NPs uptake depending on their size and shape. For instance, it allows the passage of NPs smaller than 5 nm, as the effective cuticle size range from 0.6 to 4.8 nm (59). Therefore, the major route of NPs entry is through stomata, which typically have openings of 3-10 µm (17, 59). To a lesser extent, NPs may also enter via hydathodes.

Once inside the cells, NPs can enter and compartmentalize using several pathways (symplastic-apoplast), apparent spaces, modifications in the cell membrane by the oxidative burst, use of transporters or the electrochemical gradient of the membrane, which will provide the driving force for their entry (55). Otherwise, be loaded into the phloem and translocated to different parts of the plant (17). In the symplastic pathway, NPs are mobilized through the plasmodesmata typically have diameters ranging from 20 and 50 nm. These channels exhibit spatiotemporal variations that limit or enhance the translocation capacity of NPs (13). In *Citrus maxima* the spraying of γ-Fe_2O_3-NPs (20.2 ± 2.7 nm, spherical) alters the differential expression of genes associated with ferric nutrition (*FRO2* and *NRAMP3*) and cuticular wax synthesis (wax inducer1, *WIN1* and ATP-binding cassette sub-family G member protein, *ABCG12*) evidencing the strong interaction with foliar components (15). Recently, it was proposed that foliar application of (ZnO NPs) to rice plants under heat wave stress promotes the phyllosphere and enhances NPs dissolution and translocation to the mesophyll, thereby improving the content of this ion (60).

Biofortification with nano-Fe/Zn

The use of NPs in crop biofortification is increasing, although it has mainly focused on cereals, legumes and oilseeds and there is great potential for biofortification in other crops (18, 19). The use of NPs in biofortification allows precise dosing of nutrients, reduces the amount needed and minimizes the environmental impact associated with excess fertilizer. This is particularly important for elements essential for human health, such as Fe and Zn, because excess can be toxic. Several studies have shown that the use of NPs in agriculture can have phytotoxic effects on both the environment and human health. In plants, excessive accumulation of NPs, such as metal oxides (ZnO, CuO, TiO_2), can induce oxidative stress, inhibit root growth, alter photosynthesis and damage cell structure. These effects vary according to the type, concentration, size and surface area of NPs, as well as the characteristics of the soil and plant species (61). In the environment, its release can affect the soil microbiota, alter biogeochemical cycles and accumulate in the food chain (62). In humans, long-term exposure to agricultural NP by inhalation, ingestion or dermal contact has been associated with inflammatory responses, DNA damage and lung or liver dysfunction, although more toxicological and epidemiological studies are still required to assess their long-term risks (63).

In addition to improving the nutrient delivery efficiency, NPs can be designed to protect minerals from oxidation or leaching, ensuring greater nutrient incorporation into the organs of interest (64, 65). In this sense, ion accumulation may be due to the activation of specific transporters, such as the application of crystalline NPs of α-Fe_2O_3 (20.31 nm) and Fe_3O_4 (11.63 nm), which induced differential expression of *ZmYS1* and *ZmFER1* transporters as a strategy for efficient Fe uptake and distribution in maize (20). Similarly, biofortification with FeO NPs can be enhanced in genotypes with different Fe-uptake capacities (66).

Nanobiofortification with Fe and Zn NPs showed encouraging results because, in addition to increasing the mineral concentration in the parts of the crop of interest, they increased morphological parameters such as height, stem diameter and dry biomass (Table 1). Enhanced mineral content (P, Ca, Mg, S, Mn) and physiological (chlorophyll, photosynthesis) and biochemical (enzymatic and non-enzymatic antioxidant activity) parameters of the plant, improved the nutraceutical quality of the fruit and reduced oxidative damage (25, 34, 67, 68, 71-76, 86). These secondary responses may be due to the fact that Fe and Zn ions, in addition to bioaccumulating in the organ of interest, act as enzyme cofactors associated with photosynthesis and maintenance of cell membrane integrity (87).

However, some studies have indicated that high doses can induce stress and toxicity in plants, negatively affecting biomass accumulation and photosynthetic efficiency (88). Therefore, it is necessary to develop studies aimed at discerning the ideal doses and ways to promote biofortification without negatively impacting the plant. In this context, spraying of ZnO NPs (spheres) in wheat shows a homogeneous distribution in the leaf, but with a greater presence in the stomata and the positive charge of the NPs tends to be immobilized in the mesophyll, reducing the charge in the phloem (57). The authors pointed out that the size of the NPs can limit the influx, while the surface charge limits their distribution, in addition to the zeta potential role, thus the shape of NPs influences uptake, internalization and reactivity, especially in foliar applications. Fe NPs with a two-line nanocrystalline ferrihydrite shape allow further lattice distortion by releasing Fe (76). It should be noted that the distribution and average shape of the NPs are derived from the sonication time, which causes a reduction in the toxicity of the NPs (31).

Soil applications of various ZnO NPs (10-30 nm, quasi-spherical; 80-200 nm, spherical, rectangular and rod-shaped; 300 nm irregular structures) show Zn bioaccumulation (1.3 mg g^{1}) in *Brassica chinensis* L. (13). In green beans, the application of ZnO NPs (wurtzite crystals) promotes an increase in the Zn concentration in fruit in a higher proportion than in the roots, stems and leaves (23, 77). In the particular case of Fe_3O_4 NPs (spherical to oblong, 22 ± 5 nm), it seems that they tend to may

Table 1. Effects of nanometric forms of Fe and Zn on plant biofortification.

Application route and crop	NPs	Size	Dose	Response	Reference
Seed priming, *Triticum aestivum* L.	Fe_3O_4 NPs	50 to 100 nm	20 mg L^{-1}	Grains: ↑ Fe (121 %)	(21)
Seed priming, *Triticum aestivum* L.	ZnO NPs	20 to 30 nm	100 mg L^{-1}	Grains: ↑ Zn (105 %)	(21)
Seed-priming and sprayed, *Triticum aestivum* L	Fe NPs (irregular)	Not reported	30 mg L^{-1}	Grain flour (*in vitro* digestion flour): ↑ Fe release	(22)
Drench, *Phaseolus vulgaris* L.	ZnO NPs (wurtzite crystals)	50 nm	100 mg kg^{-1}	Pods (fruit): ↑ Zn (43 mg kg^{-1})	(23)
Soil, *Triticum aestivum* L.	ZnO NPs (spherical)	20 ± 5 nm	45 mg kg^{-1}	Glume: ↑ Zn (43 %)	(24)
Soil, *Zea mays* L.	ZnO NPs (spherical)	12 nm	10 mg L^{-1}	Grains: ↑ Zn (65- 72 %)	(25)
Soil, *Triticum aestivum* L.	ZnO NPs (spherical)	70 nm	150 mg L^{-1}	Grains: ↑ Zn 58. 6 %	(34)
Seed, *Triticum aestivum* in salinity	Fe_3O_4 NPs	80 to 110 nm	500 mg L^{-1}	Leaves: ↑ Fe	(66)
Sprayed, *Cucumis sativus* L.	Fe_2O_3 NPs (γ-Fe_2O_3)	50 nm	100 mg L^{-1}	Leaves: ↑ Fe (52.30 %) Fruits: ↑ Fe (71.37 %)	(67)
Vigna radiata L.	ZnO NPs	Not reported	32 µM	Leaves, roots and stems: ↑ Zn	(68-70)
Sprayed and drench, *Lactuca sativa L.*	ZnO NPs (quasi-spherical)	16.49 nm	17 mg Zn $plant^{-1}$	Leaves: ↑ 50-75 % Zn	(71)
Cucumis melo L.	ZnO NPs	50 nm	250 mg L^{-1}	Fruits: ↑ Zn (56.61 %)	(72)
Seed, *Triticum aestivum*	Fe_3O_4 NPs	80 to110 nm	500 mg L^{-1}	Shoot (all leaves): ↑ Fe (20 %)	(73, 74)
Foliar, *Lactuca sativa L.*	ZnO NPs (spherical, tubular, rod and polygonal)	81 nm	100 mg L^{-1}	Leaves: ↑ Zn (4-33 %)	(75)
Sprayed, *Triticum aestivum* L.	Fe-EDTA NPs	1 to 7 nm	10 mM Fe	Leaves: ↑ Fe (280 %)	(76)
Sprayed, *Phaseolus vulgaris* L.	ZnO NPs (wurtzite crystals)	50 nm	150 mg L^{-1}	Pods (fruit): ↑Zn (66.29 mg kg^{-1})	(77)
Vigna radiata L.	ZnO NPs (spherical)	24.4 ± 1.8 nm	10 mg L^{-1}	Leaves, roots, stems and seeds: ↑ Zn	(78)
Nanopriming, *Oryza sativa* L.	Fe_3O_4 NPs (spherical)	12.98 ± 5.71 nm	150 mg L^{-1}	Grains: ↑ Fe (155.91 %)	(79)
Oryza sativa L.	Zero valent nano-iron loaded *Spirulina* biomass. (spindle-shaped)	24.77 ± 5.264 nm	30.75 g kg^{-1}	Grains: ↑ Fe (28 %)	(80)
Soil, *Beta vulgaris* L.	ZnO NPs (spherical)	70 nm	125 mg Zn kg^{-1} soil	Root: ↑ Zn (44.1 %)	(81)
Drench, *Ocimum basilicum* L.	ZnS Quantum Dots (cubic-face-centered crystal structure zinc blende)	2.4 ± 0.7 nm	500 mg L^{-1}	Leaves: ↑ Zn (58.77 %) ↑ Fe (268.91 %)	(82)
Sprayed, *Triticum aestivum* L.	ZnO NPs (spherical to oblong)	20 ± 5 nm		Grains: ↑ Zn (~37.7 mg kg^{-1})	(83)
Soil, *Oryza sativa* L.	ZnO NPs (spherical)	20 to 50 nm	30 kg hm^{-2}	Grains (milled): ↑ Zn (37.15 %)	(84)
Seeds imbibition and suspension to the soil, *Triticum aestivum* L.	ZnO NPs (elliptical and cuboid)	20 ± 5nm	1000 mg L^{-1}	Grains: ↑ Zn (230 %) Stem: ↑ Zn (356 %) Leaves: ↑ Zn (204 %) Roots: ↑ Zn (433.54 %)	(85)

accumulate in vegetative tissues but limited translocation to grains has been observed (31, 89). In *Linum usitatissimum*, the combination of ZnO NPs (1000 ppm) and zinc sulfate increased the Zn content to 70 ppm in crop seeds (78). In contrast, in *Arachis hypogaea* L. and *Z. mays* L., spraying Fe_3O_4 NPs (50 and 100 nm, cubic shape) induced a higher Fe content in the leaves (59).

However, it is pertinent to mention that biofortification seems to respond in greater proportion to the doses applied than to the form of NPs. For example, the application of 400 mg L^{-1} ZnO NPs induced oxidative stress and ultrastructure damage to the organelles, whereas 160 mg L^{-1} ZnO NPs was the optimal dose to promote biofortification with Zn in *Dracocephalum moldavica* L. developed under semi-arid conditions (90).

Crop biofortification appears to respond to factors such as the plant's ability to exude low-molecular-weight compounds, the rate of nutrient uptake and mobilization and the degree of element compartmentalization of commercial interest (54). Transpiration rate can condition the efficient delivery of NPs; therefore, it should be considered at the time of biofortification. For example, transpiration rates are significantly higher in dicots than in monocots (55). Furthermore, regardless of the form applied (bulk or NPs), ion speciation leads to a more stable form in the tissue, which is stored as Zn^{2+}, Fe^{2+}, or Fe^{3+} (24, 91). However, in rice plants, Zn occurs as ionic Zn and particulate ZnO when ZnO NPs (30-80 nm) are used (60).

Conclusion

Nanoparticles are potential tools with encouraging results in promoting and enhancing the biofortification of crops with Fe and Zn. However, the plant response and bioaccumulation rate of the micronutrient of interest depend on the shape of these nanoparticles by modifying their reactivity. In addition to the shape, the mode of application, plant species, dose, frequency, type and size of nanoparticles affect the absorption and transport of the nutrients of interest. Future studies should emphasize efforts to discern how the shape of nanoparticles can improve the accumulation, bioavailability, bioaccessibility and phytoremediation potential of the micronutrient of interest in a wider range of crops. The lack of information on the impact of the application of nanoparticles of different shapes, sizes or doses on the environment and human health is evident. Therefore, it is an opportunity for future research to focus on nanosafety, in addition to elucidating the toxic effects they can cause depending on their chemical composition to establish policies to prevent the potential effects of nanomaterials.

Authors' contributions

SSP, HHH and FPL were responsible for conceptualization, writing the original draft and conducting the review and editing. AJPP and LCR contributed to writing, reviewing and editing the manuscript. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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

For grammatical corrections, the authors used Paperpal software. Following the use of this tool, the authors meticulously reviewed, revised and edited the content to ensure its accuracy, coherence and integrity. The authors take full responsibility for the final content and its validity.

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