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Iron fortification of food crops through nanofertilisation

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ABSTRACT

Micronutrient deficiencies are a significant cause of malnutrition worldwide, particularly in developing countries, affecting nearly 1.8 billion people worldwide. Agriculture is the primary source of nutrients for humans, but the increasing population and reducing arable lands areas are putting the agricultural sector under pressure, particularly in developing and less developed countries, and calls for intensive farming to increase crop yield to overcome food and nutrients deficiency challenges. Iron is an essential microelement that plays a vital role in plant and human growth, and metabolism, but its deficiency is widely reported and affects nearly one-third of the world population. To combat micronutrient deficiency, crops must have improved nutritional qualities or be biofortified. Several biofortification programs with conventional breeding, biotechnological and agronomic approaches have been implemented with limited success in providing essential nutrients, especially in developing and under-developed countries. The use of nanofertilisers as agronomic biofortification method to increase yields and nutrients, micronutrient availability in soil and uptake in plant parts, and minimising the reliance on harmful chemical fertilisers is essential. Using nanoparticles as nanofertilisers is a promising approach for improving the sustainability of current agricultural practices and for the biofortification of food crop production with essential micronutrients, thus enhanced nutritional quality. This review evaluates the current use of iron nanofertilisers for biofortification in several food crops addressing critical knowledge gaps and challenges that must be addressed to optimise the sustainable application.

Keywords: biofortification, conventional fertiliser, food crop, iron deficiency, micronutrients, nanofertiliser.

Introduction

Iron is fundamental for human well-being, as a pivotal component of cytochromes and hemoglobin. Iron deficiency is a well-reported problem, particularly in developing countries (Bouis and Saltzman 2017), affecting about one-third of the global population, particularly children and pregnant women. Anaemia caused due to Fe deficiency accounts for around 8 million deaths per year (Stoltzfus *et al.* 2004). The fundamental reason behind Fe deficiency is attributed to the lack of diversified diets and intake of Fe supplements (White and Broadley 2009). Deficiency of dietary Fe affects about 14% of world population (Matres *et al.* 2021). People with severe anaemia are at high risk of cardiovascular disease and tissue hypoxia in pregnant women and young children. Iron deficiency in females during pregnancy can cause irreversible damage to fetal brain development (Gordon 1997). Overall, Fe intake by humans is lower than the daily recommended dietary allowance of 10–18 mg day^{-1} (Trumbo *et al.* 2001). Typical human diets today contain fewer nutritionally rich foods than contained in the last century due to declining soil health, which is affecting human well-being, and causing malnutrition in many developing countries, particularly in southern Asia and sub-Saharan Africa (Barrett 2010). Plants are the primary source of sustenance for people, with food quality determining the health of many individuals. Research programs have developed hybrid high-yielding varieties of essential crops, such as wheat and rice (Prasad *et al.* 2013). However, there is usually a lower content of fundamental micronutrients in these staple foods. For example, Fe content in rice after milling is

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around 1.5–6.1 μg g^{-1} against the target of 13 μg g^{-1} set by HarvestPlus program (Hoa and Lan 2004; Bouis *et al.* 2011). A sustainable solution to this problem is the consumption of diverse food sources, but this is an expensive option for poor people in danger of hunger and malnutrition. Nanotechnology could be the most sustainable way to enhance food productivity by promoting crop production, crop protection, and improving crop agronomic traits and food security eradicating micronutrient deficiencies in humans (Elemike *et al.* 2019; Chugh *et al.* 2021). This review focuses on application of nanobiotechnology for enhanced micronutrient availability in food crops through the use of nanofertilisers for successful agronomic biofortification of important crops.

Iron plays a pivotal role in plant growth and metabolism for different physiological, developmental and biochemical processes (Kasote *et al.* 2019; Afzal *et al.* 2020; Fakharzadeh *et al.* 2020). Iron deficiency causes chlorosis and necrosis in plants, restricts crop productivity and yield, and lowers the nutritional quality of grain (Phattarakul *et al.* 2012; Chen *et al.* 2017). Soil Fe contents range from 20–40 g kg^{-1} (Cornell and Schwertmann 2003) but plant available Fe is low in most alluvial soils (Mahender *et al.* 2019). Plant Fe contents range from 100–500 mg kg^{-1} of dry weight and is present in two distinct oxidation states such as Fe^{2+} (ferrous) and Fe^{3+} (ferric). Iron deficiency is a common issue in various crops due to its poor transformation into insoluble Fe(III) oxides and oxyhydroxides, making it inaccessible to plants (Cantera *et al.* 2002; Pérez-Labrada *et al.* 2020). Furthermore, nutrient bioavailability in plants relies on their relocation into edible parts and nutrient retention during downstream postharvest processing. While additional handling of food can deplete nutrients, it can also deplete anti-nutrients and improve micronutrient bioavailability (Hotz and Gibson 2007). The global decline in soil quality represents a challenge for improving grain Fe contents (Bouis and Welch 2010; Cakmak *et al.* 2010). Iron-deficient soils in cereal-growing zones cause inherently low grain Fe concentrations and are considered as a fundamental deficient source for Fe intake by dietary means (Alloway 2009). Rice and wheat cultivars, two main staple crops worldwide, which are widely used for human consumption have low amount of Fe as most is lost in processing due to removal of outer bran layers (Ludwig and Slamet-Loedin 2019).

Biofortification of staple food crops with fundamental nutrients is a practical and sustainable approach. Biofortification refers to the increase in the amount and bioavailability of micronutrients in plant parts consumed by humans, using nutritional management techniques and plant biotechnology (Bouis *et al.* 2011), to improve human well-being and nourishment. Fortified crops enter the market for further post-harvest processing to benefit the population. Fortified foods lower the incidence of illnesses related to malnutrition, such as poor maternal well-being, low intelligence, and diminished work capacity (Bouis *et al.* 2011; Hossain and Mohiuddin 2012). Several global biofortification projects are underway to deliver micronutrient-rich staple food sources and combat micronutrient deficiency. HarvestPlus is a worldwide test program of the Consultative Group on International Agricultural Research (CGIAR) (Bouis and Saltzman 2017) promoting biofortification as a favoured technique for micronutrient enhancement in grains. This worldwide research partnership includes a broad range of specialists in numerous fields, including agronomy, plant genomics, plant breeding, food and nutrition, social behaviour, acceptance, and policy analysis.

There are two potential biofortification strategies: genetic and agronomic biofortification (Fig. 1). Genetic biofortification includes conventional plant breeding and

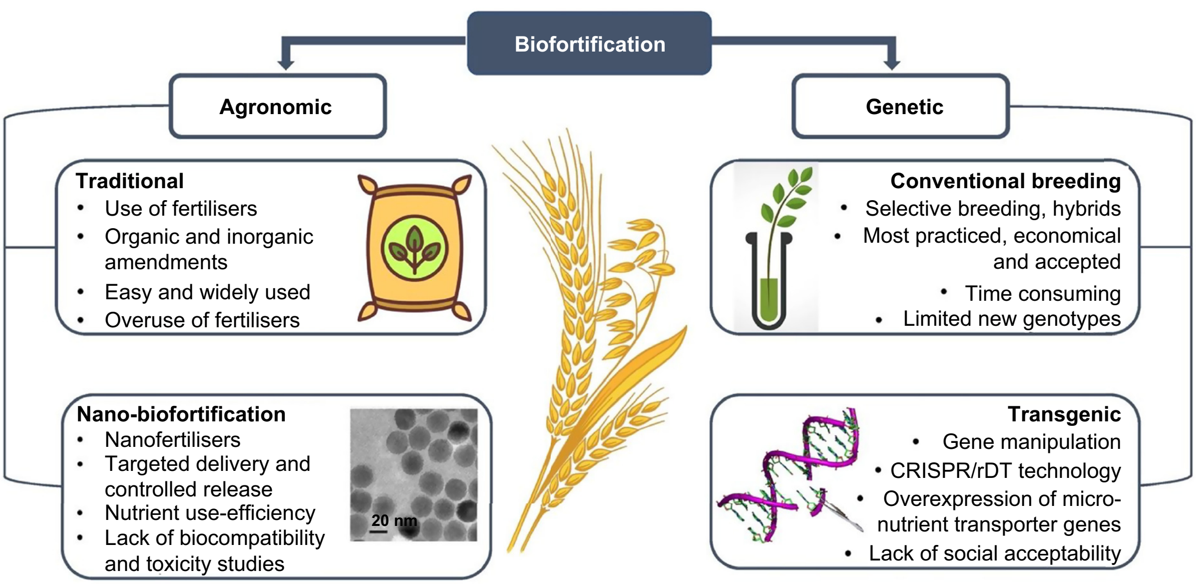

Fig. 1. Biofortification strategies for micronutrient enhancement of food crops.

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transgenic methods to develop crop varieties accumulating high concentrations of essential micronutrients in grains (Cakmak 2008). The main goal of such plant breeding methods is to produce staple food crops with lower levels of anti-nutrients, higher levels of micronutrients, and increased nutrient accessibility (Bouis 2003).

While plant breeding is the most practiced economical and sustainable technique for nutrient fortification (Murgia *et al.* 2012; Melash *et al.* 2016), creating new genotypes is time-consuming (Prasad *et al.* 2014). Also, the amount of accessible micronutrients in the soil is a limiting factor for micronutrient uptake by plants (Velu *et al.* 2014). Moreover, a transgenic strategy requires known genes with desired functions to influence the trait of interest. Genetically altered micronutrient-rich crops may not be widely accepted due to lack of awareness and many regulatory difficulties in various countries regarding-transgenic plants, making this innovation economically unviable (Dixit *et al.* 2018). Additionally, limited resource availability, in terms of Fe rich staple crops germplasms, pose a limitation on the success of conventional breeding techniques (Ludwig and Slamet-Loedin 2019). Despite various successes in using plant breeding and transgenics, it usually takes 8–10 years from lab to market release due to the involvement of careful selection of variety, as well as stringent and time consuming environmental and biosafety clearances (Matres *et al.* 2021).

Agronomic biofortification is a suitable alternative for enhancing micronutrient content in staple food crops to overcome the limitations of plant breeding methods for biofortification. The agronomic biofortification of crops is quick and sustainable. Compared to plant breeding methods, agronomic biofortification is a temporary solution for this issue (Cakmak *et al.* 2010). However, the agronomic biofortification strategy could be more valuable for overcoming malnutrition in developing countries (Velu *et al.* 2014).

Agronomic biofortification uses fertilisers application to the soil to enhance grain micronutrient concentrations. The type of fertiliser and the developmental stage of crop plants when it is applied varies (Cakmak 2008). Several forms of micronutrients, including inorganic and chelated forms, are used as fertilisers. Agronomic biofortification of staple crops such as wheat and rice, through soil, foliar, or combined fertiliser application is well-reported (Yilmaz *et al.* 1997; Khan *et al.* 2008; Zhang *et al.* 2010; Mathpal *et al.* 2015). Certain soil conditions, including good drainage, slightly acidic pH, and sufficient organic matter, increase fertiliser use efficiency and reduce ecological contamination. While fertiliser use efficiency has increased in recent times, it has created a net negative soil nutrient balance as nutrient removal is greater than the addition of fertilisers (Solanki *et al.* 2015). Therefore, it is essential to investigate advanced methodologies to ensure effective fertiliser delivery, appropriate doses, and controlled release in a plant accessible form without causing ecological concerns. Nanotechnology offers sustainable solutions for modern farming by providing the targeted and controlled delivery of nutrients in nanofertilisers. Nanofertilisers benefit over the use of conventional fertilisers as they are used in small quantities with slow and controlled release capabilities ensuring efficient uptake and minimising waste and overuse, thus improving soil and plant health with enhanced productivity and efficiency (Chugh *et al.* 2021).

Why nanofertilisation over conventional fertilisation?

Conventional fertilisers are used in large amounts to improve crop productivity, but nearly 50% of the applied fertiliser is leached into waterways beingunavailable for plants, thus increasing soil, water, and air pollution. In addition, most nutrients are insoluble (nutrient immobilisation) in the soil, making them inaccessible for plant use (Connor *et al.* 2011). Hence, chemical fertiliser application can result in short-term gains in productivity but prove deleterious to soil health in the long-term, disrupting plant nutrient homeostasis and nutritional status, which hinders plant growth and productivity (Solanki *et al.* 2015; Kumar *et al.* 2020). Therefore, there is a requirement for an alternative to chemical fertilisers for efficient plant nutrient use and sustainable crop production (Assainar *et al.* 2020).

Nanotechnology can be used to incorporate nanonutrients and carriers into fertilisers with improved and targeted formulations that minimise nutrient loss due to their high use efficiency (Chugh *et al.* 2021). Nanoparticles can be used as smart delivery systems for targeted controlled-release kinetics due to their large surface area, size, shape, high surface mass ratio, zeta potential, crystallinity, porosity, hydrophobicity/hydrophilicity, and surface functionalisation (DeRosa *et al.* 2010; Solanki *et al.* 2015). These properties facilitate nutrient retention, and allow slow and controlled-release of nutrients to improve nutrient use efficiency, and thereby crop productivity. Moreover, nanofertilisers can be synthesised biologically using biological materials as reducing, capping, and stabilising agents, which can be an added advantage in terms of biocompatibility and toxicity concerns associated with chemically synthesised nanofertilisers. Therefore, nanotechnology offers a platform for a novel and sustainable delivery system of nanonutrients to plants using nanoporous plant surfaces with increased efficiency and accessibility in nutrient availability and uptake. Metal nanoparticles and nanoforms of many plant nutrients as nanofertilisers are promising alternative methods to the existing, expensive, environmentally damaging conventional chemical fertilisation techniques, with long-term sustainability in terms of applicability and acceptability.

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Synthesis and characterisation of nanoparticles

Generally, nanoparticles can be synthesised by three methods: physical, chemical and biological methods. Several reviews have discussed various methods of nanoparticle synthesis (Iravani *et al.* 2014; Khodashenas and Ghorbani 2014; Pantidos 2014; Ali *et al.* 2016). Fe nanoparticles have been synthesised using various mechanochemical methods such as, combustion, laser ablation arc discharge, pyrolysis, electrodeposition. Physical methods present with the difficulty to control the size of nanoparticles in the range (Cuenya 2010). Chemical methods include sol–gel synthesis, reverse micelle, template-assisted synthesis, coprecipitation, hydrothermal, etc. These methods are easy, efficient and tractable, and efficient in managing the size, composition, and shape of the nanoparticles primarily depending on the type of salt used, but are not environmentally friendly (Wu *et al.* 2008). On the other hand, biologically synthesised nanoparticles are reported to be comparable with their physical or chemically synthesised nanoparticles and ensure biocompatibility with high reproducibility (Wiley *et al.* 2004). The methods of biosynthesis of nanoparticles can be intracellular or extracellular. Intracellular synthesis wherein nanoparticles are generated within the cells (of plants, fungi, bacteria, etc.), whereas extracellular synthesis is when the synthesis happen outside the organism generally aided by several biomolecules and extra-cellular metabolites (such as proteins and peptides) (Hulkoti and Taranath 2014; Chugh *et al.* 2021). The biosynthesis machinery relies on the self-assembling capacity and reduction capability of biomolecules, including proteins, amino acids and peptides, which governs the nucleation and capping that help in stabilisation and growth of the nanoparticle (Goswami *et al.* 2011). In metallic nanoparticles, nucleation is aided by electron transfer from the host protein to metal ion. The nucleation and growth steps largely depend on the type and structural conformation of the protein involved (Thanh *et al.* 2014). Peptides react with nuclei of the metal preformed nanostructure, forming a reducing environment in the solution, producing reduced metal ions, and aiding crystal growth (Pantidos 2014). Biologically synthesised nanoparticles provide a suitable alternative to existing chemical or physical methods of nanoparticle synthesis, and are reported be less toxic, energy efficient to produce resulting in lower levels of hazardous by-products that can damage the environment. Biosynthesised nanoparticles can be biocompatible and produced with high reproducibility. Biological nanoparticle synthesis provides a sustainable alternative to existing expensive, environmentally hazardous conventional synthesis techniques (Chugh *et al.* 2021). After the synthesis of nanoparticles, thorough characterisation is vital for safe and effective application. Physicochemical characterisation is required to comprehend the material properties and functionalities because these factors govern the synthesised material's functional attributes, such as solvency, dispensability, and stability of nanomaterials (Nair *et al.* 2008). Modern visualising techniques, for example, scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), etc. are the most commonly used techniques for determining the size and shape topology of nanoparticles (Rong *et al.* 2004, 2006). Besides these microscopic analyses, biophysical and mechanical attributes are essential to comprehend the behavioural pattern of synthesised nanoparticles. Various techniques such as, energy-dispersive X-ray (EDAX), X-ray powder diffraction (XRD), Fourier transform infrared (FTIR), X-beam photoelectron, Raman spectroscopy, and vitality dispersive X-ray (EDAX) spectroscopies helps in studying the chemical and physical characteristics, surface functionalities, thermal stability and elemental composition of nanoparticles. These studies are extremely valuable to understand the particulate nature of the synthesised nanoparticles (Arshad *et al.* 2011; Janaki *et al.* 2015). Furthermore, nontoxic behaviour and biocompatible applications of synthesised nanoparticles can be further enriched by coating them with organic or inorganic molecules, including surfactants, drugs, proteins, starches, enzymes, antibodies, nucleotides, non-ionic detergents, and polyelectrolytes to form biomoleculs, which govern the nanoparticle interaction with cell. However, the interaction and behaviour at the molecular level between nanoparticles and biological systems are to a great extent unknown (Gupta and Gupta 2005). A complete knowledge of the role of nanosised materials on plant physiology at the molecular level is still lacking (Khodakovskaya *et al.* 2011).

Use of nanoparticles as nanofertilisers for Fe biofortification

Nanofertilisers can be used to deliver nutrients to plants in several ways, e.g. in the nano-nutrient (particle) form, coated in a layer of thin protective polymer, or nanoemulsion, or encapsulated in nanotubes or nanoporous materials. Several studies have demonstrated the beneficial impacts following the application of Fe nanofertilisers (Amuamuha *et al.* 2012; Mir *et al.* 2015; Lemraski *et al.* 2017) through enhanced agronomic traits such as seed germination, yield, and Fe concentration in various plant parts, with effective relocalisation of Fe from the nanoparticles.

Fe nanoparticles have been used to increase the Fe content in plant parts of various crops (Table 1). The pre-treatment of various leguminous seeds with α-Fe_2O_3 nanoparticles increased root growth (Palchoudhury *et al.* 2018). Application of Fe nanoparticles improved nutritional quality, biomass, yield, N and P metabolism, and Fe fortification in peanuts (Rui *et al.* 2016). The use of Fe nanoparticles improved the enzyme function of heme protein which is responsible for cytochrome functioning (Zahra *et al.* 2015; Rui *et al.* 2016). The exposure of γ-Fe_2O_3 nanoparticles

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Table 1. Use of Fe nanofertilisers in several food crops.

Nanoparticle	Plant species	Nanoparticle size (nm)	Application method	Concentration (mg/kg)	Effect on plant	References
Fe_2O_3	Maize (*Zea mays*)	17.7–21.2	Hydroponic	20	Increased root elongation, germination index and vigor index	(Li *et al.* 2016)
	Peanut (*Arachis hypogaea*)	20	Root	250, 1000	Increased Zn and Fe in roots and shoots, root length, plant height, biomass	(Rui *et al.* 2016)
	Soybean (*Glycine max*)	Not specified	Foliar	500, 750	Increased biomass, yield	(Sheykhbaglou *et al.* 2010)
	Various legumes	16	Seed	0.005	Increased root growth	(Palchoudhury *et al.* 2018)
	Pomelo (*Citrus maxima*)	20.2	Foliar	20, 50, 100	Increased shoot Fe concentration	(Hu *et al.* 2017)
	Watermelon (*Citrullus lanatus*)	9–18	Seed	20	Increased root activity, ferric reductase activity, root apoplastic Fe content, biomass	(Li *et al.* 2013)
	Rapeseed (*Brassica napus*)	<300	Irrigation water	0.5, 0.8, 1, 2	Enhanced growth, chlorophyll content	(Palmqvist *et al.* 2017)
	Wheat (*Triticum aestivum*)	20–30	Seed	100	Increased germination rate, root biomass	(Feizi *et al.* 2013)
	Wheat (*Triticum aestivum*)	80	Seed	25, 200, 400	Enhanced shoot length, germination rate, grain Fe content	(Sundaria *et al.* 2019)
	Wheat (*Triticum aestivum*)	20–40	Hydroponic	500	Enhanced root and shoot lengths, biomass, chlorophyll content	(Al-Amri *et al.* 2020)
	Wheat (*Triticum aestivum*)	20–40	Foliar	100, 200, 300, 400	Improved yield, shoot and root Fe content, fresh and dry weight	(Rostamizadeh *et al.* 2021)
Fe_3O_4	Rice (*Oryza sativa*); Maize (*Zea mays*)	50–100	Hydroponic	2000	No effect on germination, rate or root elongation	(Yang *et al.* 2015)
	Lettuce (*Lactuca sativa*)	12–20	Soil	250	Increased shoot and root lengths, P availability and uptake	(Zahra *et al.* 2015)
	Perennial ryegrass (*Lolium perenne*); Pumpkin (*Cucurbita mixta*)	100	Hydroponic	30, 100, 500	No significant effect, relative to control or bulk-Fe	(Wang *et al.* 2011)
	Wheat (*Triticum aestivum*)	5–20	Seed	0.125, 0.5	No effect on germination rate, shoot length	(Lee *et al.* 2018)
	Wheat (*Triticum aestivum*)	6.85	Seed	2000	Growth inhibition and oxidative stress	(Konate *et al.* 2017)
	Wheat (*Triticum aestivum*)	10	Hydroponic	5, 10, 15, 20	No effect on lipid peroxidation, growth, germination, or chlorophyll content; increased root Fe content	(Iannone *et al.* 2016)
	Pumpkin (*Cucurbita maxima*)	20	Root (growth medium)	500	No difference in growth, increased Fe in roots and Fe translocation to other plant parts	(Zhu *et al.* 2008)
$FeCl_3.6H_2O$	Wheat (*Triticum aestivum*)	20–30	Applied with irrigation water	25	Increased spike length, grain number per spike, grain weight, proteins related to starch degradation, glycolysis, tricarboxylic acid	(Yasmeen *et al.* 2017)
$Fe^{3+}{}_{10}O_{14}(OH)_2$ (Ferrihydrite)	Maize (*Zea mays*)	3	Hydroponic	1000, 2000, 4000	Increased seed germination, chlorophyll content, root growth	(Pariona *et al.* 2017)

(*Continued on next page*)

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Table 1. (*Continued*).

Nanoparticle	Plant species	Nanoparticle size (nm)	Application method	Concentration (mg/kg)	Effect on plant	References
Carbon coated Fe nanoparticles	Pumpkin (*Cucurbita pepo*)	43–46	Foliar	Not specified	Increased Fe translocation from leaves to other plant parts, no effect on growth	(Corredor *et al.* 2009)
	Wheat (*Triticum aestivum*)	10	Root	Not specified	Translocated into the cortex, leaf petioles, internodes, within and outside vascular tissues; strongly accumulated in leaf trichomes	(Cifuentes *et al.* 2010)
	Tomato (*Solanum lycopersicum*); Sunflower (*Helianthus annuus*); Pea (*Pisum sativum*)	10	Root	Not specified	Translocated into the cortex, leaf petioles, internodes; within and outside vascular tissues	(Cifuentes *et al.* 2010)
SPIONs (FeO_x)	Soybean (*Glycine max*)	9	Hydroponic	60	Diffused toward interior of stem parenchyma; detected in stem and leaves, vascular and parenchyma tissues; increased chlorophyll levels	(Ghafariyan *et al.* 2013)
$NiFe_2O_4$ (magnetic nickel ferrite)	Barley (*Hordeum vulgare*)	12.5	Hydroponic	125, 250, 500	Enhanced Ni and Fe content in leaves; increased Ca, Mg, K, Na and Mn contents	(Tombuloglu *et al.* 2019*b*)
$MnFe_2O_4$ (manganese ferrite)	Barley (*Hordeum vulgare*)	14	Hydroponic	125, 250, 500, 1000	Enhanced Mn and Fe content; no significant change in chlorophyll or carotenoid content	(Tombuloglu *et al.* 2018)
SrMgCaFeO (nano-hexaferrite)	Barley (*Hordeum vulgare*)	42.4	Hydroponic	125, 250, 500	Enhanced Sr, Mg, Ca and Fe contents; germination, biomass, soluble protein and chlorophyll contents	(Tombuloglu *et al.* 2019*a*)

increased shoot Fe concentration in *Cucurbita maxima* but no such increase was observed in controls or plants treated with Fe(II)-EDTA (Hu *et al.* 2017). In the same study, root Fe concentration did not significantly increase, suggesting adequate transportation and localisation of Fe from roots to other plant parts (Hu *et al.* 2017). Foliar application of carbon-coated Fe nanoparticles in pumpkin plants helped translocate Fe from leaves to other plant parts but did not affect plant growth and function (Corredor *et al.* 2009). The application of Fe-chelated nanofertilisers improved growth parameters such as yield and nutrient concentrations (especially NPK) in basil (Peyvandi *et al.* 2011) and rice (Fakharzadeh *et al.* 2020). Pre-treatment of spinach seeds with iron pyrite (FeS_2) nanoparticles enhanced the growth (Srivastava *et al.* 2014). Leaf Fe and K accumulation increased in spinach supplied with 4 kg ha^{-1} Fe-chelated nanofertiliser (Moghadam *et al.* 2012). Application of Fe-chelated nanofertiliser improved NPK absorption and rice grain quality (Fakharzadeh *et al.* 2020).

Several other nanofertilisers with multiple elements have increased elemental contents in plant tissues. For example, $NiFe_2O_4$ enhanced nickel and iron contents (Tombuloglu *et al.* 2019*b*), $MnFe_2O_4$ nanoparticles enhanced manganese and iron contents (Tombuloglu *et al.* 2018), and SrMgCaFeO (magnesium-substituted strontium nano-hexaferrite) enhanced strontium, magnesium, calcium, and iron contents (Tombuloglu *et al.* 2019*c*) in barley leaves. Wheat plants (*Triticum aestivum* L. cv. L15) were used for foliar application of $FeHO_2$ nanoparticles at 1–10 mM with humic substances as stabilisers and urea as an adjuvant. Nanoparticle application increased Fe accumulation by about 75% in leaves relative to the control, but did not increase plant growth due to the lag period in plant response to Fe supply (Zimbovskaya *et al.* 2020). Foliar application of nano-fed Fe fertilisers significantly affected biological yield, grain yield, harvest index, grain weight, spike number, and plant height in wheat (Harsini *et al.* 2014). However, plant and grain Fe contents were not reported. Seed priming with γ-Fe_2O_3 nanoparticles enhanced shoot length, germination rate, and grain Fe content in wheat (Sundaria *et al.* 2019). Application of Zn and Fe nanoparticles improved plant growth in cadmium-stressed wheat plants by reducing oxidative stress and cadmium concentration, and increasing Zn and Fe concentrations in wheat shoots, roots, and grains (Rizwan *et al.* 2019). A recent study investigating the effect of Fe_2O_3 (iron (III) oxide) nanoparticles on wheat in a hydroponic system reported enhanced root and shoot lengths, biomass, and chlorophyll content (Al-Amri *et al.* 2020). This increase in chlorophyll content could be attributed to the increased leaf Fe content due to Fe nanoparticles application. The maximum amount of applied nanoparticles was taken up by wheat's roots and leaves, and Fe was translocated from roots to leaves. In another study, foliar application of iron oxide nanoparticles

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improved yield, shoot and root Fe contents, and fresh and dry weights in wheat relative to the bulk form of Fe (Rostamizadeh *et al.* 2021).

Mode of action (application, uptake and translocation)

Different physiological and biophysiochemical variables regulate the uptake, translocation and distribution of nanoparticles in plants. Physiological factors include plant age (stage at which nanoparticle is applied), plant species, and biotransformation pathway of the nutrient. Other factors, such as mode of application (aerial/foliar, root and seed), and interactions with other environmental components (microbiota, soil water, soil surface and soil structure), also play a defining role. Biophysiochemical properties (size, shape, net charge, surface functionalisation and surface coating), which characterise the function of nanoparticles when introduced to plant cells and their mode of application, cumulatively affect the fate of nanoparticles (Raliya *et al.* 2015). Concentration and size of nanoparticle are the principle parameters that can influence their uptake and translocation. Nanoparticles of the same metal core having different sizes used in various concentrations might have different physiological behavior in the plant system (Prerna *et al.* 2021). This could be attributed to distinction in several contact sites on nanoparticles with different sizes and shapes, which are accessible for interaction between nanoparticle and cell membrane, thus affecting the free energy accessible for nanoparticles to interact with cell (Chithrani *et al.* 2006).

Mode of application

Nanofertilisers are delivered to plants through three main techniques: soil application, foliar application, and seed treatment (Fig. 2). The most common strategy for fertilisation is the soil application. Soil is a dynamic and heterogeneous mixture of many biotic and abiotic factors. Soil texture and pH can affect the fertilisers fate and life span in the soil. Foliar application involves directly spraying fertilisers onto aerial plant parts, primarily leaves, to limit nutrient loss. Most foliar-applied fertilisers are readily accessible for plant use, circumventing the difficulties faced with soil application. Comparative studies have shown that the foliar method of nanoparticle delivery has significant benefits for nano-nutrient uptake because of direct absorption compared with soil application (Alidoust and Isoda 2013; Wang *et al.* 2013), in crops such as rice (Wei *et al.* 2012), wheat (Aciksoz *et al.* 2011) soybean (Alidoust and Isoda 2013), and black-eyed peas (Delfani *et al.* 2014). Despite many advantages, the foliar application requires proper optimisation considering the role epidermal cells and stomatal pores play in nutrient take-up, owed to their diurnal physiological reactions (Alidoust and Isoda 2013; Salehi *et al.* 2018). It is also important that the nutrient formulation does not block stomatal pores, as this can impact normal stomatal function.

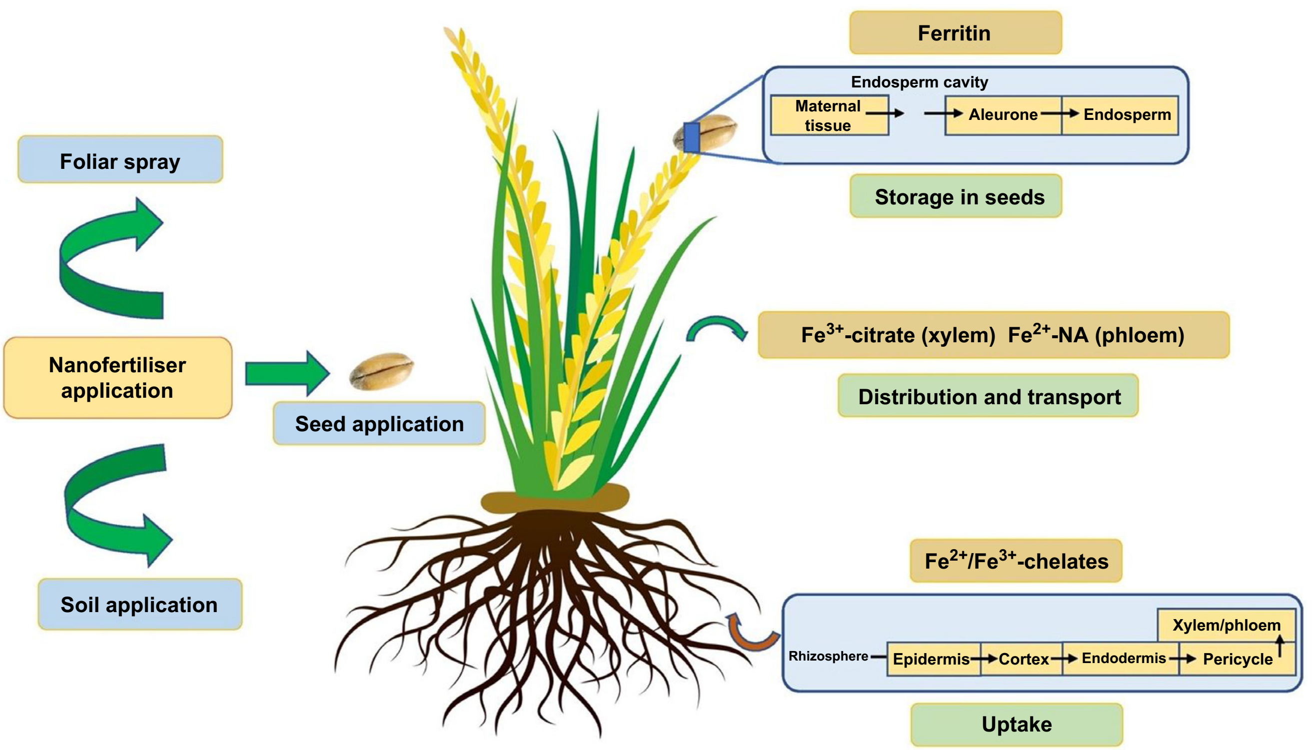

Fig. 2. Application methods, uptake, translocation, and mobilisation of Fe nanoparticles in crop model.

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Furthermore, nanoparticles on the leaf surface can interact with various functional groups at the interface such as carboxyls, hydroxyls, methylene, amines and aromatics which can be controlled by altering the surface chemistry of nanoparticles (Avellan *et al.* 2021).

Mode of entry

Potential modes of entry for nanoparticles in aerial plant parts include passive uptake through openings such as stomata and hydathodes, with specific size exclusion (Kurepa *et al.* 2010). Other suitable routes for nanoparticle uptake involve wound and injury on the plant surface (Al-Salim *et al.* 2011). Lateral root junctions provide easy access for nanoparticle uptake from roots, especially near the rhizodermis root tip (Chichiriccò and Poma 2015). Microorganisms (symbiotic and/or parasitic) and organic matter with other exudates in the soil affect nanoparticle uptake dynamics. When nanoparticles are applied to the soil, the sudden and excessive exposure of these nanoparticles may affect the soil microbial communities and may agglomerate due to complex soil physicochemical properties, which could limit nanoparticle uptake by plants (Cao *et al.* 2016; Anderson *et al.* 2017; Raliya *et al.* 2018). Therefore, delivery of nanoparticles by foliar application is more beneficial for nano-nutrient uptake than the soil application (Raliya *et al.* 2015, 2016). Lab-scale studies revealed that foliar spray through aerosol formulation can deliver monodisperse nanoparticles that are not prone to agglomeration (Raliya *et al.* 2018).

Mode of uptake and translocation

Upon application, the further uptake, movement and accumulation of nanoparticles depend upon the plant species, and the size, chemical properties, concentration and stability of the nanoparticles (Lv *et al.* 2015). The cell wall behaves as a semi-permeable membrane, enabling selective movement (size-specific) through pores (Miralles *et al.* 2012). Upon entering external defensive layers, nanoparticles have two main modes of mobilisation: apoplastic and symplastic pathways. Apoplastic transport propels radial movement, transporting nanoparticles to the central cylinder of the root and vascular bundle to enter symplastically into the stele for further translocation upward to leaves (Zhao *et al.* 2017; Tombuloglu *et al.* 2019*a*). Apoplastic movement is indispensable for applications requiring systemic delivery of nanoparticles. The Casparian strip forestalls the radial movement of nanoparticles in the root endodermis, which can be overcome by changing from the apoplastic into a symplastic pathway. The symplastic pathway is a more regulated and coordinated pathway for nanoparticles movement in plants (Palocci *et al.* 2017; Zhang *et al.* 2018).

Further, nanoparticles tend to accumulate in cytoplasm, vacuoles or lysosomes after different processes like phagocytosis, pinocytosis or endocytosis, which facilitate the entry of nanoparticles into the cell (Cho *et al.* 2011; Lesniak *et al.* 2012). Several interfacial interactions between nanoparticle bound protein ligands or epitopes and cell membrane bound receptors determine the contact and entry site for nanoparticles at adhesion sites (Decuzzi and Ferrari 2007). Several iron transporters have been extensively studied in various plant models such as wheat, rice, maize and finger millet, etc. (Anuradha *et al.* 2017; Boonyaves *et al.* 2017; Chandra *et al.* 2021). When the nanoparticle enters the cytoplasm, further cell-to-cell movement occurs with the help of plasmodesmata (Lin *et al.* 2009; Geisler-Lee *et al.* 2012; Zhai *et al.* 2014). Several studies have demonstrated that metal nanoparticles can infiltrate seeds and translocate into seedlings, with no adverse effect on germination rate or viability, suggesting the effective use of functional nanoparticles for stimulating plant growth using seed priming (Racuciu 2012; Pokhrel and Dubey 2013; Sanzari *et al.* 2019).

Varying reports exist concerning the uptake and translocation of Fe nanoparticles in plants. In a hydroponic study with pumpkin seedlings, Fe_3O_4 nanoparticles were present in root, stem and leaves, whereas no uptake was reported in soil grown seedlings, reiterating the important role of growth medium in nanoparticle uptake (Zhu *et al.* 2008). Another study on pumpkin and ryegrass did not observe the translocation of Fe_3O_4 nanoparticles in shoots (Wang *et al.* 2011). In maize, Fe_2O_3 nanoparticles were reported moving into the endodermis through the exodermises via apoplastic pathway. Some nanoparticle accumulation was also observed in root cell vacuoles. But, no root to stem transfer was observed with majority of nanoparticles localised around the epidermis of the root systems (Li *et al.* 2016). In another study on maize, ferrihydrite and hematite nanoparticles were observed in vascular bundles (xylem, phloem and cell wall) using confocal laser scanning microscopy (Pariona *et al.* 2017). While most studies evidence the uptake and movement of Fe nanoparticles into vascular bundles, further translocation to shoots and subsequent aerial parts could be dependent on the type of nanoparticles used (Gillispie *et al.* 2019).

Challenges for biofortification using nanofertilisers

The complex and uncertain properties of nanoparticles make it challenging when determining their biocompatibility and fate in the soil-plant system. Generally, nanoform is considered more toxic than its bulk (non-nano) form, but this claim needs extensive examination supported by toxicological evidence (Das *et al.* 2016). Rational science-based methods are required in order to deal with the toxicological impacts of nanoparticles on biological and environmental systems (Nel *et al.* 2006). There are few studies on plant-nanoparticle interactions due to technological inefficiencies in the strategies involved. Comprehensive biocompatibility

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and risk assessment studies are required to determine the fate of nanoparticles in the soil-plant system to garner wider acceptance. In addition, little information is available on the effects of Fe nanoparticles on transcription factors. Advances in proteomics and transcriptomic techniques will increase our knowledge on plant responses to nanoparticle stress, providing insight into the molecular mechanisms involved, and revealing links between plant metabolism and gene expression.

Several studies have reported physiological injury to plants in response to nanoparticles. Confocal analysis revealed the injury to root tip cells due to Fe_2O_3 nanoparticles (Al-Amri *et al.* 2020), which can be attributed to the generation of reactive oxygen species disrupting the cell membrane. It is not clear if nanotoxicity is directly linked to the nanoparticles and their interactions with cells or the defence mechanisms activated in response to nanoparticle stress at the biomolecular level. Therefore, it is crucial first to understand the chemical and physical properties of nanofertilisers and then to investigate their effect on plants to assess any risks to humans and the environment (Pradhan and Mailapalli 2017).

It is vital to understand how plants take up nutrients associated with nanoparticles in relevant physiological processes. More comprehensive studies are needed, with relevant parameters measured, such as photosynthetic activity and specific nutrients in particular biomolecular pathways (Zimbovskaya *et al.* 2020). Moreover, the translocation of nanoparticles in plants requires further examination for their effect on end-users. Despite many studies examining the impact of nanoparticles on various plants, there is a lack of knowledge on nanoparticle size (varied 1–100 nm), which is a crucial factor affecting their movement in the plant body, determining plant growth and development (Al-Amri *et al.* 2020). Agronomic biofortification aims to improve nutritional qualities without hampering crop yield, which however, more comprehensive laboratory to field studies are required to confirm. There is a persistent need to assess the efficacy and robustness of nanoformulations in the field, particularly over the long term (Dapkekar *et al.* 2018). There are concerns about the transfer of nanoparticles to edible plant parts and further to animals and humans through the food chain that requires examination before the large-scale application of nanoparticles. A comprehensive life cycle assessment (LCA) is fundamental to assess the effect of nanofertilisers on the environment, and designing appropriate dosages of nanofertilisers (Hasler *et al.* 2015).

Conclusions and future perspective

Fe deficiency in human beings is widely reported, primarily in developing and less developed nations. Lack of nutrient-rich diverse diets (lacking dietary Fe) is a major cause of malnourishment. The problem is pronounced in poor population with lack of adequate supply for food and resources. Agronomic biofortification through fertilisation is a decisive strategy for increasing seed nutrient contents and potential yield of staple food crops. Indeed, agronomic biofortification would be essential and it is significant, particularly to their cultivation in nutrient-poor soils. Genetic and agronomic biofortification could be the best way to obtain nutrient-rich crop varieties (Burchi *et al.* 2011; Das *et al.* 2013; Tam *et al.* 2020). Biofortification using fertilisation is a suitable methodology for fighting hidden hunger in the global population. Nano-enabled technologies could be beneficial for reducing the dependence on chemical fertilisers and biofortifying staple crops. Use of Fe nanoparticles and Fe nanocomposites as nanofertilisers can prove to be a sound and sustainable method to achieve the goal of increasing micronutrient content and crop yield. Increase in yield is also an important factor to be considered as this growth and yield parameter is of direct benefit to the farmer, and will in turn ensure for easy acceptance of nanofertilisers. Fe nanoparticles have the ability for nutrient biofortification, but it is important to address the gaps in knowledge pertaining to the transport and behaviour of nanoparticles in plants to facilitate the rational design of nanoparticles for nutrient delivery with controlled kinetics and minimal risk. Nanofertilisers can bring innovation in agriculture for nutrient-rich crops with economic advantage, if the products are environmentally and economically sustainable.

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