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ADVANCING NUTRITIONAL SECURITY THROUGH ZINC-ENRICHED WHEAT, RICE, AND MAIZE

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Abstract

he Green Revolution significantly increased the yield of staple food crops, helping to meet the nutritional needs of a rapidly growing global population. However, this advancement came at a cost: a decline in the micronutrient content of foods, leading to widespread deficiencies–most notably zinc (Zn). Today, nearly 30% of the world's population suffers from Zn deficiency, predominantly in developing regions, especially South and Southeast Asia.Given the critical role of Zn in crop productivity and human health, this review provides a comprehensive synthesis of current strategies for Zn biofortification in three major cereal crops: wheat, rice, and maize. It focuses on four key approaches: (i) agronomic biofortification through fertilizer application, seed priming, organic amendments, and microbial interventions; (ii) genetic enhancement via conventional breeding; (iii) transgenic methods; and (iv) nano-based Zn delivery systems. By critically comparing these strategies across crops, the review highlights research gaps and identifies future directions for optimizing Zn biofortification.While existing studies often address individual crops or approaches, no previous review has examined Zn deficiency across all three major cereals within a unified framework. This comprehensive analysis offers valuable insights for improving global food and nutritional security and supports progress toward achieving the United Nations Sustainable Development Goals (SDGs) 2030.

INTRODUCTION

Zinc (Zn) is a micronutrient essential for the normal and healthy metabolisms of their biological systems in all living beings. As a cofactor in enzymatic activities, Zn is essential to synthesize proteins, including vitamin A metabolism, and insulin regulation (Agnew & Slesinger, 2021; Imran *et al.*, 2016; Katayama, 2020). Zinc regulates diverse metabolic activities; such as photosynthesis and sugar formation, protein synthesis, fertilization, seed creation, and plant growth (Noulas *et al.*, 2018; Olsen *et al.*, 2016). Additionally, it modulates physiological and molecular mechanisms in response to drought stress, hormone regulation, signal transduction, and pest disease resistance (Cabot *et al.*, 2019; Hassan *et al.*,2020). Globally, acute Zn deficiency has taken in account after the past 50 years due to the introduction of new high-yielding crop varieties after "Green Revolution" era. These new crop varieties were Zn-inefficient compared to locally adapted (landrace) varieties. Apart from crops exhibiting obvious deficiencies, and disappointing yields; Zn deficiency became increasingly prevalent concern worldwide among scientists (Cakmak *et al.*, 2008b).

The global issue of micronutrient deficiency particularly Zn deficiency necessitates the coordinated efforts of the international community, policymakers, agricultural/plant scientists, dieticians, physicians and others. Zinc biofortification of cereal crops (wheat, rice, maize) with Zn biofertilizers and the development of Zn-efficient crops are among the most important strategies to improve the micronutrient deficiency. Briefly, utilizing Zn biofertilizers is one of the most efficient, sustainable, and cost-effective strategies to increase the Zn status of the soil to cultivate cereals crops such as wheat, rice and maize. (Khan & Khan, 2022). It is noteworthy that staple foods cannot provide the same levels of minerals and vitamins per day as supplements or food by products (Bouis *et al.*,2011). In spite of numerous attempts and amazing progress in recent years toward plant breeding and typically 8-20 years required for novel and traditional varieties in agricultural (Van Der Straeten *et al.*,2020). However, its appropriate application demands a comprehensive understanding of the target plant and soil system. Keeping in view, in agriculture sector, a new paradigm for fertilizer development and sustainable fertilization solutions has arisen in recent years (Bindraban *et al.*, 2015). The development of innovative approaches, products, and strategies are enhanced the understanding of plant physiology, soil processes, and plant-soil interactions. These advancements aim to optimize nutrient utilization, reduce fertilizer consumption, and improve overall nutrient efficiency (Agbenin, 1998; Macintosh *et al.*, 2019). Among many methods, the employment of microbes to boost the Zn content of food is another novel approach. Microbial activity improves soil nutrient status by enhancing Zn solubility and plant uptake are recent approach (Yahaghi *et al.*, 2019). Primarily the low bioavailability of micronutrients particularly Zn is associated with phytic acid in grains, and excessive intake of phytic acid caused the Zn deficiency

(**Figure1**). To address this challenge, better understanding of phytic acid at physiological and molecular level is necessary. In wheat, maize and rice grains, phytic acid is present in phosphorous form, accounting total of 65% to 85% of total seed phosphorous content.

In the prevailing worst global situation associated human health risk (two billion humans suffer from the deficiency of one or more micronutrients especially Zn deficiency), it is the dire need for time to take efficient as well as reliable steps to combat this hidden hunger. Recently many approaches are used to increase the micronutrient in cereals crops. Biofortification has emerged as a promising approach to enchase the Zn contents in plants, improve nutritional level and mitigate the Zn deficiency in humans as well. However, many studies revealed that by Zn biofortification controls Zn deficiency. The importance and significance of Zn in both human and plant life cycles particularly its biofortification raises a serious concern to address its eminent threat along with its possible mitigation by sustainable and environmentally friendly way. However, no review paper has yet evaluated the optimal method of Zn biofortification in Wheat, Rice, and Maize simultaneously. Therefore, the aim of review article is to address the Zn biofortification of Wheat, Rice and Maize by all possible strategies like agronomic biofortification (fertilization, seed priming, organic amendment, microbial assisted Zn and foliar spray), developing Zn efficient strains, transgenic approaches and application of nano chemistry for Zn biofortification. Moreover, this review also elucidates the research gap regarding the optimum application of Zn to avoid its toxicity as well as conserve precious resources to ensure sustainable and precision agriculture. The biofortification methods could be effective especially in addressing the hidden huger associated with Zn micronutrient than artificial supplementation.

2 Zinc as a micronutrient for plants

2.1 Zinc Chemistry in soil plant systems

Zinc is a group IIB transition element named after the German word Zinke by the Swiss physician and alchemist Paracelsus (Mir *et al.*, 2015). There are five stable isotope forms of Zn found in nature: ^{64}Zn (48.63%), ^{66}Zn (27.90%), ^{67}Zn (4.90%), ^{68}Zn (18.75%) and ^{70}Zn (0.62%) (Broadley *et al.*, 2007). Zinc being an isomorph for magnesium (Mg), as both have +2 oxidation state and a similar size. The ion Zn^{+2} have strong binding affinities for nitrogen (N), oxygen (O), or sulphur (S) of amino acid residues in proteins/enzymes, with N of histidine being the most prevalent, followed by S of cysteine, O of aspartate/glutamate and carbonyl O of peptide bond, glutamine/asparagine, and hydroxyl of tyrosine (Leuci *et al.*, 2020). Additionally, at least 6% of the bacterial proteome and 9% of the eukaryotic proteome use Zn, making it the second most abundant metal cofactor after Fe. The majority of cellular Zn is connected with proteins, with total cellular Zn concentrations in eukaryotic cells estimated in the hundreds of micromolar against pico- and -nanomolar concentrations of labile pools of free or loosely bound Zn (Bellomo *et al.*, 2011; Lanquar *et al.*, 2014; Maret, 2015; Qin *et al.*, 2013).

2.2 Zinc Essentiality

Normally Zn requirements of most crops range from 30 to 200 mg kg^{-1} dry weight for optimum growth (Marschner & Rengel, 2012). Zinc, a critical micronutrient, plays structural and catalytic roles in numerous processes, including cell division, cell growth, and protein synthesis (Jain *et al.*, 2010). In addition, it is essential for chromatin structure, gene expression and regulation, metabolism of nucleic acids, carbohydrates, lipids, proteins, and photosynthetic carbon fixation (Gai *et al.*, 2017; Noulas et al., 2018). Additionally, Zn is essential for synthesizing tryptophan, an amino acid precursor of auxin (Tsonev & Cebola Lidon, 2012). Zinc is also essential for the functioning of over 300 enzymes, including carbonic anhydrase, aldolases, carboxypeptidases, alkaline phosphatases, superoxide dismutase, phospholipase, and alcohol dehydrogenase (Gupta *et al.*, 2016; Lin *et al.*, 2016).

Numerous molecules involved in the production of DNA and RNA, such as RNA polymerases and reverse transcriptase, are Zn metalloenzymes that are involved (Choi *et al.*, 2018). In numerous plants, Zn finger transcription factors contribute to the growth and function of flower tissues, such as pistil, anthers, pollen, and tapetum (Hafeez *et al.*, 2013). Zinc is also implicated in the defense mechanism of plants; necessary for the salicylate defense signaling pathway (Zwiesche *et al.*, 2015). It is an essential component of carbonic anhydrase and a stimulant of aldolase, two enzymes involved in carbon metabolism (Tsonev & Cebola Lidon, 2012). It has been demonstrated that Zn application improves agricultural output and quality (Chattha *et al.*, 2017; Hassan *et al.*, 2019), whereas Zn shortage, diminishes crop yield and degrades crop quality (Mousavi *et al.*, 2007).

2.3 Zinc Toxicity

Zinc has been reported to have many advantages in plants however its toxicity is another aspect that remained partially explored under various soil and environmental conditions. The alterations in antioxidant capacity and an increase in reactive oxygen species generation have been reported in response to excessive Zn in plants (Feigl *et al.*, 2015; Jain *et al.*, 2010) (**Figure 2**).

Only sufficiently high Zn rates (> 10 kg ha^{-1}) are anticipated to have a positive impact on soil Zn availability and crop yield (Liu *et al.*, 2019; Sánchez-Rodríguez *et al.*, 2021). The growing environmental concern and the tiny gap between Zn essentiality and toxicity in plants have drawn the scientific community's attention to its impacts on plants and its important role in ensuring agricultural sustainability (Kaur & Garg, 2021). More than 3000 mg kg^{-1} dry soil Zn concentrations have been found in polluted agricultural fields (Audet & Charest, 2006; Long *et al.*, 2003). Smelting and mining for Zn have contributed significantly to soil Zn content. Burning fossil fuels, phosphate fertilizers (often 50- 1450 mg Zn kg^{-1}), manure (15-250 mg Zn kg^{-1}), limestone (10-450 mg Zn), fungicides and rubber mulch are also sources of Zn in soils that are artificially generated (Audet & Charest, 2006).

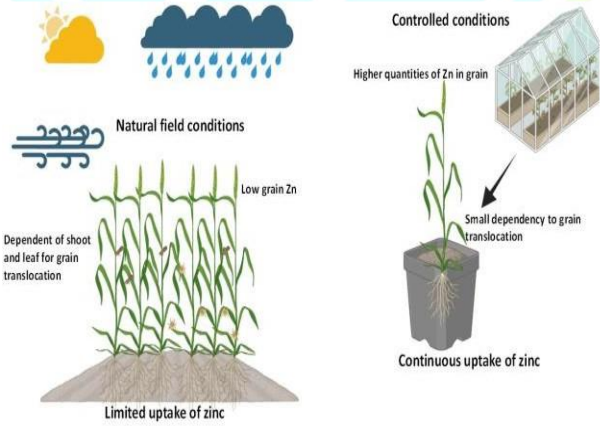

Figure 1: Difference of Zinc uptake by plants under field and controlled condition

Zinc accumulation, transport and sequestration in plant-soil system

Phosphorous (P) contents and P fertilization impacts soil available Zn its bioaccumulation and translocation (Zhang *et al.*, 2015; Sánchez-Rodrguez *et al.*, 2017; Liu *et al.*, 2020). Soil solution contains only a small fraction of total Zn concentration. Mineral composition (CaCO3, iron oxides), texture, pH, moisture, and organic matter are all factors that influence soil Zn bioavailability and govern its biogeochemistry (Liu *et al.*, 2020; Liu *et al.*, 2020; Sánchez- Rodríguez *et al.*, 2017; Zhang *et al.*, 2015). Compared to cereals, legumes have higher Zn contents like *Amaranthaceae*, *Brassicaceae*, and *Salicaceae* accumulate high Zn, while *Poaceae*, *Solanaceae*, and *Linaceae* accumulate least Zn (Akhtar *et al.*, 2019; Gregory *et al.*, 2017). As a result, cereals unable to absorb Zn, resulting in stunted plant development and decreased crop yields (Akhtar *et al.*, 2019) (especially with P fertilizers applied in the absence of Zn) (Bindraban *et al.*, 2020; Sánchez-Rodríguez *et al.*, 2021) **(Figure 1)**. In addition, the effects of soil and foliar Zn treatment on Zn accumulation in plants are still debatable.

Global cereals production and consumption

Studies have indicated that cereals account for 50% of the calories consumed globally, despite being the most traded agricultural crop on the global market. This encourages the requirement to evaluate its past, present, and future applications (Olugbire *et al.*, 2021). Traditional cropping of rice-wheat and maize-wheat systems in the Indo-Gangetic Plains has resulted in a negative nutritional balance and a deficit of micronutrients due to adopting an intensive cropping system, uneven agricultural practices, and reduced usage of organic manures (Nadeem & Farooq, 2019). FAO's latest projection for global cereal production in 2021 has been increased by 2.2 million tons and is now estimated to reach 2,796 million tons, a 0.7% increase over the previous year.

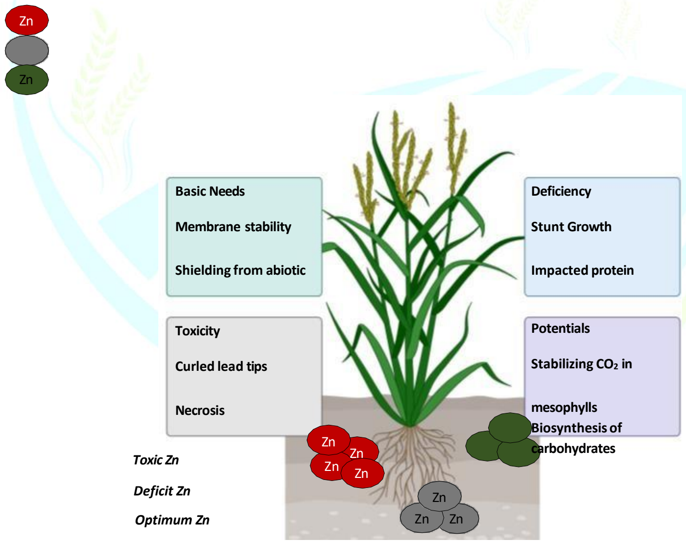

Figure 2: Role of zinc in the cereals owing to its deficiency, optimum and toxic applications

2.4 Wheat

Wheat is known to be major staple food for the developing countries after rice and maize. Improper application of fertilizer in wheat cultivation might be impact the soil eco-biodiversity via acidification. Though, wheat plant roots uptake Zn from the soil directly as Zn2þ via ZRT-/IRT-like proteins (ZIPs) or as complexes with phyto-siderophores (substances in the mugineic acid family) produced by wheat roots. These complexes are subsequently delivered to the roots by yellow stripe-like (YSL) transporters from the rhizosphere (Borrill et al., 2014).

Once within the root epidermal cells, Zn2þ or Zn-phyto- siderophore complexes migrate through simplistic pathways, from the cortex to the epidermis and then to the pericycle, before being loaded into the xylem (Gupta *et al.*, 2016). Zinc is largely located in the aleurone layer and embryo of the whole wheat grain (Singh *et al.*, 2014) (**Table 1**). Specifically, the Zn concentration in the aleurone layer of the wheat crop can reach up to 432 mg kg^{-1}, but starchy endosperm has just 14 mg kg^{-1} and accounts for 20-25% of Zn in the total grain (Table 1). Contrastingly, endosperm Zn

concentration is often less than 10 mg kg^{-1} (Table 1). Recently it was reported that the Zn concentration in endosperm must be at least 30 mg kg^{-1} to satisfy nutritional needs (Borrill *et al.*, 2014; Cakmak & Kutman, 2018; Menguer *et al.*, 2018; Wang *et al.*, 2011).

Accumulation of Zn in the shoot is required more than the physiological requirements of a plant, therefore, Zn transfer from roots to shoots must be stimulated via sustainable approach. Zinc translocation from leaves contributes more to overall Zn allocation to wheat grains than Zn absorption concomitant with grain filling. In cereals, the xylem at the base of each seed is discontinuous; therefore, Zn must be transported from the xylem to the phloem before entering the grain. This causes a major bottleneck for Zn buildup in grain (Stomph *et al.*, 2009). Consequently, it is essential for plant growth and significantly impacts crop yield and quality (Cakmak & Kutman, 2018).

During the grain-filling stage in semiarid wheat planting regions, the topsoil is typically dry, limiting soil moisture and decreasing Zn migration to wheat roots (Cakmak & Kutman, 2018; Noulas *et al.*, 2018). Optimal leaf Zn concentrations vary between 30 to 100 mg Zn kg^{-1} DW (White & Broadley, 2011). Under high pH circumstances, the negative charges of soil particles, such as carbonates, might rise and resulting in the significant adsorption and hydrolysis of Zn ions (Alloway, 2009). Numerous crop species, such as wheat, rice, and corn have been extensively studied to determine the Zn route in grain. Despite numerous obstacles, including the root-shoot barrier and grain filling (Palmgren *et al.*, 2008).

2.5 Rice

Major cash and stable crop for the half's world population is rice and being an important crop its role in providing essential nutrient poses equal importance. In rice crop, micronutrient especially Zn is not required to accumulate in rice root vacuoles immediately (Palmgren *et al.*, 2008). However, reports regarding rice crop indicate that excessive Zn is also accumulated in the shoots, particularly the stem (Jiang *et al.*, 2008). Whereas, Zn levels in roots and stems are equivalent across a broad range of plant Zn mass concentrations (ZnMC mg Zn kg^{-1} biomass) (Jiang *et al.*, 2008). In rice, Zn transport via xylem during grain filling may be more significant than phloem-transported. It is important to elucidate that radioactive Zn was administered to either leaves or roots at flowering, the majority of Zn was found in the grains via root application (Jiang *et al.*, 2007). It is still uncertain to interpret these tissue distinctions as physiological barriers. The endosperm is predominantly composed of starch, which is not actively involved in determining the accumulation of minerals during grain metabolism. Therefore, the physiological regulation of

Table 1. Concentrations of Zn in different parts of wheat grains (mg/kg)

Genotype	N	Endosperm	Bran	Embryo	Whole grain	Reference
T. longissim	1	8.03±0.21	98±1.27		111.85±2.61	(Kumar et al., 2016)
T. speltoides	1	25.6±0.32	118.7±0.15		99.9±0.14	
T. kotchyi	2	7.2-11.97	85-130.2		47.7-63.4	
T. monococcum	3	5.23-14.5	73.8-82.2		45.647.9	
T. peregrina	3	7.36-10.9	50-130		48-58	
T. kotchyi substitution	3	8.32-14.9	50.5-162.4		30-36.4	
T.asetivum	10	27.4	76-180	183	50.87	(Cardoso et al., 2018)
T. asetivum	1	6.5-59	9.9-125.6	41.5-243.3	-	(Persson et al., 2016)
T. asetivum	2	5-20	42-157	69-132	-	(Ismail Cakmak, Pfeiffer, & McClafferty, 2010)
T. asetivum	6	5-13	59-96	-	26.32	(Eagling et al., 2014)
T. asetivum	1	7.8-8.7	54-105	-	-	(Brier et al., 2015)
T. asetivum	4	16-25	111-238	-	-	(Xue, Drenth, & McIntyre, 2015)
T. asetivum	4	4.5-12.9	24.8-54.5	-	-	(Z. Liu et al., 2008)
T. asetivum	2	8-20	-	179-193	-	(Sieprawska et al., 2014)
T. asetivum	2	7.5-9.3	15-236	-	18.2-21.7	(Xue et al., 2015)
T. asetivum	1	4-38	-	27-172	9-80	(Kutman, Yildiz, & Cakmak, 2011)
T. asetivum	2	10-15	76-180		32.6-73.7	(Qi et al., 2019)
T. asetivum	2	11.2-25.9	62.9-124.7		-	(Hui Liu et al., 2014)
T. asetivum	1	3.0-11.3	19.2-354.6		50.1-140.7	(Y. Wang et al., 2011)
T. asetivum	4	4.46-9.23	59.4-122.7		27-35.21	
T. tauschill	2	4.56-6.03	79		31.6-38.1	
T. asetivum	1	8-10	10-138	-	11.2-26.8	
T. asetivum	1	14±1	432±15	292±34	64±1.6	

mineral concentrations and their gradients should be investigated using starch-free dry matter instead of total dry matter. Re-analysis of the ZnMC for rice reveals that endosperm ZnMC increases by 87% of the increase in bran ZnMC when additional Zn is stored in the grain, as opposed to the 37% previously reported (Jiang *et al.*, 2008).

2.6 Maize

Besides wheat and rice crop, maize crop has its suitable place in fighting food security and providing essential nutrition to the human. Since, most of Zn is located in the maize embryo and pericarp, the majority of Zn is absorbed by the embryo (Cheah *et al.*, 2020). One of the limiting variables of zinc bioavailability is the accumulation of phytate (a dietary inhibitor that chelates Zn), which accounts for 75- 80% of the total phosphorus in corn grains (Prasanna *et al.*, 2020). Improved maize shoot biomass in response to a rise in Zn availability suggested that the available Zn was assimilated (Liu *et al.*, 2017). Due to sterility and limited translocation of resources, maize grains positioned in the apical portion of the ear typically develop poorly. They are classed as inferior grains, whereas those located in the middle and lower portions of the ear are fine grains

(Zhao *et al.,* 2018). Maize grain production could be increased by enhancing the growth of these inferior grains, which typically have fewer kernels and less biomass. It has been found that there is a positive correlation between kernel number and the Zn concentration of maize stems, whereas the absence of Zn causes barren ear tips (Potarzycki, 2010). Insufficient absorption caused by abiotic stress, such as Zn deficiency, shortened the linear grain-filling time in later-growing kernels (Serrago *et al.,* 2013). Improved maize shoot biomass in response to a rise in Zn availability suggests that the available Zn was assimilated (Liu *et al.*, 2017). Managing Zn is essential for enhancing pollen viability and Zn uptake in maize and ensuring grain development, particularly in the apical section (Liu *et al.,* 2020).

3 Zinc interaction in Plant and soil system

proteins (Costello et al., 2011). The rest of intracellular Zn is loosely bound to various ligands, such as nicotinamide, histidine, glutathione, phytochelatins, phosphate ions, etc., and the aggregate of these complexes comprises the labile Zn pools in the cellular structure (Clemens, 2019; Krämer, 2018). Binding of Zn with these ligands, intracellular stored Zn content in plant tissues is buffered to around hundreds of PPM under typical Zn supply rates (Lanquar et al., 2014; Zlobin et al., 2019). These lower cytosolic Zn2+ contents are enough to activate native Zn proteins but are less than the harmful low-nanomolar free Zn2+ values that inhibit cytosolic proteins and cause injury (Vinkenborg et al., 2009). Altering the Zn supply can significantly affect the accessibility of the cell's unbound Zn2+ content. Under conditions of abundant Zn, accessible Zn2+ levels in plant

The total Zn concentration in plant cells is quite high, ranging from 0.3 to 3 mM depending on the cell type (Blindauer & Schmid, 2010). Due to the significantly complex capability of Zn ions as described by the Irwing- Williams series, the total concentration of Zn does not influence its biological activity (Haase et al., 2015). Most of the total cellular Zn is firmly and irreversibly linked to Zn

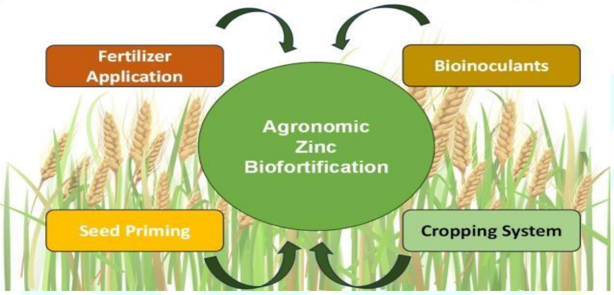

Figure 3: Different approaches for zinc fortification in cereals

cells rise to nanomolar levels. Still, Zn deficiency can entirely exhaust the usable Zn in the plant's cytosolic portion, and variation in Zn availability has a clear detrimental impact on plant tissue formation (Lanquar *et al.,* 2014; Zlobin *et al.,* 2019). Plant cells must therefore be able to detect changes in free Zn^{2+} and respond appropriately to recover Zn homeostasis. It is believed that transcription factors that detect cytosolic Zn levels are the primary regulators of Zn homeostasis in all three domains of life, from bacteria to animals (Choi & Bird, 2014).

The phytoavailability and leaching potential of Zn may differ depending on the type of soil, especially if the soil contains a significant proportion of organic matter. The clay fractions greatly influence Zn availability in the soil. Increased Zn adsorption and a corresponding decrease in available Zn can be found in soils with high portion of clay, lower P and Mn concentrations, higher Fe and Al oxide contents, and higher levels of organic matter (Małecki *et al.,* 2016). Sand and acidic soils have less organic matter because of the high Zn content in the soil (Moreno-Lora & Delgado, 2020). Phyto available Zn was higher in non-calcareous soils with high clay content, however, it decreased with increasing Fe oxide levels (Moreno-Lora & Delgado, 2020). Because of the high CaCO3 content and pH of calcareous soils, chemisorption increases Zn retention in these soils (Wang et al., 2017). The phytoavailability of Zn^{2+} in soil solution is inversely correlated with soil pH (Salinitro *et al.*, 2020).

4 Mechanisms and pathways of Zn biofortification via multiple sustainable approaches

4.1 Agronomic biofortification

Biofortification of Zn is an emerging field that might be regulated by the application methods, agronomic practices, fertilizer types, and role of plant native ability to develop transgenic Zn fortified mechanisms (Figure 3).

4.1.1 Impact of fertilization type, dosage and application methods

In agriculture, a new paradigm for fertilizer development and sustainable fertilization solutions has arisen in recent years (Bindraban *et al.*, 2015). Depending on the type of fertilizer, soil parameters and crop species, the application technique and rate was varied. Crops susceptible to Zn deficiency or calcareous soils with a higher pH require higher application rates (Alloway, 2008b). Fertilizers can be administered via seed treatment, broadcasting topsoil, seed bed banding and foliar spray. Before sowing rice seedlings, the roots can also be immersed in fertilizer. However, the most commonly used method is soil application. Several studies indicate that foliar treatment is superior to soil application and results in greater grain accumulation (Kopittke *et al.*, 2019). However, foliar Zn application, which provides comparatively less Zn than soil application, avoids the element's complex dynamics in soil and is more effective for crop biofortification (Ismail Cakmak, 2008a; Zia *et al.*, 2020).

One reason may be that foliar sprays circumvent soil conditions that inhibit nutrient absorption by roots. Zinc sulfate ($ZnSO4$) may be applied at rates between 5 and 25 kg Zn ha^{-1} (Cakmak, 2008a; Cakmak & Kutman, 2018; Liu *et al.*, 2020). For foliar spray, the dosage is typically five times less than that of soil treatment (1 kg ha^{-1}). The spray solution typically contains between 2 and 5 grams of zinc sulphate heptahydrate ($ZnSO4. 7H2O$) per liter (Boonchuay *et al.*, 2013; Cakmak, 2010). When the nutrient is administered to leaves, certain physiological characteristics such as leaf penetration and subsequent translocation of Zn play a significant effect (Rehman *et al.*, 2021). Plants, including grains, vary in their susceptibility to Zn shortage and response to Zn fertilization (Cakmak & Kutman, 2018). Despite their high cost, chelated forms of Zn, such as Zn- EDTA, are used as foliar sprays for high-value crops (Alloway, 2008b). Some compounds used as fertilizers may contain substantial quantities of Zn. For example, superphosphate is the fertilizer with the largest quantities of Zn (600 mg Zn kg^{-1}) (Alloway, 2008b). The increase in Zn enrichment of urea from 0.5 to 2.0% boosted rice and wheat grain yield significantly (equivalent to an application of 1.3– 5.2 kg Zn ha^{-1}). Zn enrichment of urea at 2% Zn as Zn sulphate enhanced the rice grain yield by 29.4% and the wheat grain yield by 19.1% (Prasad *et al.*, 2013).

Furthermore, the Zn application of 30 kg $ZnSO4.7H2O$ hm^{-2} increased chlorophyll content in summer maize leaves, which enhanced photosynthesis and boosted grain yield (Liu *et al.*, 2016).

Yang et al., (2011) reported that application of Zn decreased the wheat grain phytate content and phytate Zn molar ratio. Actual values for phytic acid were 8.13 g kg^{-1} grain, compared to 7.01 g kg^{-1} for an unfertilized crop. Zinc fertilization decreased the phytate Zn molar ratio from 23.38 in unfertilized crops to 13.74 in Zn-fertilized wheat, bringing it below the level of 15, over which the bioavailability of Zn is lowered (Graham, 2008). The timing of fertilizer application is also crucial for cereal grain accumulation. It was noticed that foliar application of Zn during blooming time led to the greatest rise in grain Zn content (Cakmak, 2010). Soil/foliar Zn fertilization has been proposed to alleviate Zn shortage to some degree; nevertheless, fertilizer treatment may not be successful due to the low nutrient utilization efficiency of a crop that is regulated by its genetic features (Sciacca *et al.*, 2018).

4.1.2 Seed priming

To develop and advanced understanding the role of seed priming is also a recent approach in which cereal seeds are treated with various solutions to achieve a high degree of biofortification. The priming solution enhanced crop performance. Wheat (Cultivar Lasani-2008) responded better to seed priming with 0.05 and 0.1 M $ZnSO4$, whereas wheat (Cultivar Faisalabad-2008) responded better to seed priming with 0.5 M

Zn solution of ZnSO4 and 0.1 M solution of ZnCl2. As a result of the seedlings' speedy and uniform emergence, there was an increase in their shoot length, root length, and dry weight. Higher Zn priming concentrations than 0.5 M Zn using ZnSO4 or 0.1 M ZnCl2 did not further boost germination and seedling growth (Rehman *et al.*, 2015). A study was done both at the NWFP Agricultural University research station in Peshawar (AUP-Farm), and Farmer-Managed Participatory (FAMPAR) trials were conducted in the Risalpur area of Nowshera District. Authors found that when 2.75 kg Zn ha^{-1} was added to the soil grain average production was greatly boosted of maize in four trials, but there was no further advantage from applying double this amount **(Table 2).** The effect of adding 5.5 kg Zn ha^{-1} did not differ substantially from the control without Zn. After applying 2.75 kg ha^{-1} of Zn, an additional 720 kg of maize grain (25%) was produced as a result. Cob production and cob weight all rose considerably following the application of 2.75 kg Zn ha^{-1} however the response to adding 5.55 kg Zn ha^{-1} was either the same or worse than adding 2.75 kg Zn ha^{-1} (Table 2) (Harris *et al.*, 2007).

Rice germination and early seedling growth may be improved if Zn seed priming is treated at the optimal dose. Improved germination and development of early seedlings, the time it took to reach 50% emergence and the mean emergence time were all lowered by seed priming with Zn solution at 0.1% and 0.5% concentrations. 0.1% Zn solution improved tailoring, leaf emergence, extended length of leaves, and increased amount of chlorophyll in plants. As a result, we advocate priming rice seeds with Zn concentrations as low as 0.1% (Abbas *et al.*, 2014).

4.1.3 Organic amendment

In addition to the direct administration of Zn, studies have shown that the presence of organic amendments like biochar, cow dung manure and composted natural organic materials or ligands additions to increase the Zn uptake by plants **(Table 3**). The use of organic ligands results in the formation of organic Zn complexes in soil, which may increase or reduce Zn solubility in soil and plant uptake. Recently, it was discovered that humic acid application increased soil Zn sorption capacity by 73-95%, whereas citric acid application lowered it by 52-68% (Piri *et al.*, 2019). They hypothesized that citric acid forms soluble Zn complexes and reduces soil Zn sorption, whereas humic acid forms insoluble Zn complexes. Similarly, the use of citric acid (20 mmol kg^{-1}) increased Zn desorption in soil from 17 to 30 g kg^{-1}, hence increasing Zn bioavailability (do Nascimento *et al.*, 2020). Changes in soil pH may also boost the effectiveness of organic ligands in enhancing Zn phytoavailability. Specifically, 0.1 M citric acid increased Zn leaching by up to 42%. (Cheah *et al.*, 2020). Ethylenediaminetetraacetic acid has also been commonly documented to boost the Phyto availability of Zn and other metals (Chen & Cutright, 2001; Shahid *et al.*, 2014; Turgut *et al.*, 2005). EDTA increased soil Zn Phyto availability by up to 377%, consequently increasing straw and grain Zn levels by up to 120% and 61%, respectively (Wang *et al.*, 2017). Compared to ZnSO4, Zn-EDTA fertilization resulted in more Zn accumulation in wheat (Zhao *et al.*, 2018).

4.1.4 Microbial-assisted Zinc

Among numerous micronutrients, Zn is the most essential. It is frequently found in the soil as insoluble Zn (smithsonite; ZnCO3, sphalerite; ZnS, Zincite; ZnO, franklinite; ZnFe2O4, and willemite; Zn2SiO4), which is unavailable to plants. Diverse microbes possess the innate ability to transform this fixed form of Zn into the labile Zn form, making it accessible to plants. These microorganisms are known to solubilize Zn. Although the ability to solubilize fixed Zn is not a frequent trait among bacteria and fungi, it does exist. Several investigations have shown that bacteria and fungi might solubilize Zn in vitro using an agar plate or liquid medium (Khan, 2021) **(Table 4**).

Many of the microbial strains are known to create organic acids, some were demonstrated to produce organic acids and others were aimed to produce siderophores, among them a few strains were discovered to produce both. Numerous studies have indicated that a modest reduction in pH dramatically enhances the absorption of micronutrients. It

Table 2. Results of a combined of four field trials (no. 5, 6, 7 and 8). Within rows, values with the same letters are not significantly different at $P< 0.05$; *= treatment effect significant at $P< 0.05$ (Harris et al., 2007).

Variable	Treatment			Significance	LSD(0.05)
	No added Zinc	2.75 kg ha^{-1}	5.50 kg ha^{-1}		
Total dry matter (t ha^{-1})	10.92 a	13.37 b	13,157 b	*	2.0
Stover dry matter (t ha^{-1})	6.90	8.31	8.46	Ns	
Cob number (ha^{-1})	44,797 a	54,820 b	47,224 a	*	7204
Cob yield (t ha^{-1})	4.01 a	5,066 b	4,699 b	*	0.68
Grain yield (t ha^{-1})	2.83 a	3.55 b	3.14 a	*	0.49
Shelling percentage	70.1	69.7	68.8	Ns	

is known that microorganisms produce a variety of siderophores, including catecholates (*enterbactin*), carboxylates (*rhizobactin*), and hydroxamates (*Pyoverdine*) (Ahmed & Holmström, 2014). Therefore, soil bacteria are associated with increased Zn content of wheat, maize, and rice grains (Khande *et al.*, 2017; Mumtaz *et al.*, 2017).

4.1.5 Developing Zn- efficient strains

Plant breeding and marker-assisted screening can be used to boost the micronutrient content of the world's most important staple crops (Bouis & Saltzman, 2017). Although numerous efforts and remarkable progress have been made in recent years toward plant breeding for crop biofortification (Van Der Straeten *et al.*, 2020). For optimal yield and Zn concentration, preliminary research is required to identify Zn-efficient and responsive crop genotypes under low-Zn environments. Zinc-efficient genotypes efficiently utilize Zn, while Zn-responsive genotypes demonstrate a significant response to exogenously applied Zn (Singh *et al.*, 2019). It is important to note that less use of fertilizers may help economical screening, eco-friendly and towards sustainable approach (Jhanji *et al.*, 2013; Singh *et al.*,2019). Due to its vital physiological significance in living organisms, researchers have devoted considerable efforts to producing crops with optimal Zn levels in low-Zn soils (Alloway, 2008a). Biofortified wheat varieties created at The International Maize and Wheat Improvement Center produced up to 5% higher yields than commercial controls and were released in India, Pakistan, Nepal, and Bangladesh, which are the target nations for Zn-enriched wheat. In 2014, the aim for breeding Zn was increased to 12 ppm above commercially grown checks, based on a revised estimated recommendation from nutritionists and the finding of lower frequency lines, such as 'Zn Shakti, with Zn levels above the updated target. The focused breeding for increased Zn resulted in the release of over 20 biofortified wheat cultivars in target nations (**Table 5**). All of these varieties have a good grain yield (at least as high as the conventional varieties released in the same locations) and contain 8–10 ppm (25– 40%) more Zn on average than conventional varieties (Velu *et al.*, 2020).

Marker-assisted breeding can take advantage of the many genetic studies that have been done to uncover Quantitative Trait Loci (QTLs) for high Zn in grains. Several genes in rice have been linked to Fe and Zn uptake and accumulation, and these genes have been exploited to successfully create transgenic rice strains with high Zn levels. In the event of successful breeding, the Zn concentration could rise by 6–8 mg kg^{-1} (TO, 2014). Low-Zn rice varieties with donors with good yield potential and Zn testing in early segregating lines from the F4 generation onwards (Fig. 7) (Swamy *et al.*, 2016). Improving the kernel Zn bioavailability is the difficulty of biofortification in maize breeding. Several quantitative trait loci (QTL) mapping research revealed that four to twenty genes per population control kernel Zn accumulation (Goredema-Matongera *et al.*, 2021). Characterization of exclusively available maize germplasm across the world and the development of diverse heterotic pools are prerequisites for the derivation of nutritionally improved parental lines and to acceleration of the biofortification breeding programs (Maqbool & Beshir, 2019).

4.2 Transgenic approaches

Genetic biofortification refers to the process of developing crop cultivars through the use of genetic engineering with a high concentration of micronutrients in the edible section of the plant. This procedure is difficult and time-consuming (Sow & Ranjan., 2022). Because the uptake and accumulation of micronutrients in edible sections of plants are controlled by polygenes that have minimal impacts, the standard breeding-based biofortification procedures have only met with limited success (Naqvi *et al.*, 2009). In addition, the success that may be obtained on the natural variation in the gene pool. Genetic engineering will be a viable alternative for increasing micronutrients at targeted levels without adequate genetic variability and fixable major gene effects (Bhullar & Gruissem, 2013; Dunwell, 2014). However, to produce transgenic for nutrient biofortification. It is desirable to consider the following two criteria: (1) the selection of a

idely suited genotype of an economically

Table 3: Effect of organic amendments on Zn accumulation in different plant parts.

Amendment	**Dose**	**Zn dose**	**% Change**	**Organ**	**Plant species**	**Reference**
Cattle manure	500 Mg ha^{-1}	92.8 mg kg^{-1}	-34	Shoot	*Oryza sativa*	(Saengwilai & Meeinkuirt, 2021)
				Root		
Leonardite			-36	Shoot		
			-12	Root		
Compost	1%	243 mg kg^{-1}	-24	Root	*Brassica rapa*	(Li *et al.*, 2021)
	5%		90			
	1%		358	Shoot		
	2%		498			
	5%		838			
Sewage Sludge	30 t ha^{-1}	56.2 mg kg^{-1}	13	Leaf	*Zea mays*	
	75 t ha^{-1}		25			(Yu *et al.*, 2021)
	150 t ha^{-1}		57			
	300 t ha^{-1}		59			
Medical residue	30 t ha^{-1}		-11			
	75 t ha^{-1}		17			
	150 t ha^{-1}		11			
	300 t ha^{-1}		-26			
Cattle manure	30 t ha^{-1}		4			
	75 t ha^{-1}		7			
	150 t ha^{-1}		-14			
	300 t ha^{-1}		-34			
Press mud	50%	6.6 mg kg^{-1}	-34	Plant	Capsicum annuum	(Ugulu *et al.*, 2021)
Poultry waste			-36			
Farmyard Manure			-12			
Fly ash	5%	101.3 mg kg^{-1}	-51	Root	*Oryza sativa*	(Lee *et al.*, 2019)
	10%		-57			
Zeolite	5%		-73			
	10%		-83			
Fly ash	5%		-57	Shoot		
	10%		-63d			
Zeolite	5%		-62			
	10%		-59			
Fly ash	5%		-35	Grain		
	10%		-30			
Zeolite	5%		-66			
	10%		-56			
Fly ash	5%		-57	Whole plant		
	10%		-54			
Zeolite	5%		-52			

	10%		-55			
Salicylic acid	5 mM	5 mM	-30	Cotyledons	Fenugreek	(Mabrouk *et al.*, 2019)
			-74	Radicles		
Lime	900 kg hm^{-2}	200 mg kg^{-1}	-4	Leaf	*Oryza sativa*	(Duan *et al.*, 2015)
Poultry manure	30 g kg^{-1}	1352 mg kg^{-1}	-50	Root	*Helianthus annuus*	(Xiu-Zhen *et al.*, 2012)
			-21	Stem		
			-41	Leaves		

			-36	Flower		
Leonardite	20%	97.6 mg kg^{-1}	-25	Shoot	*Oryza sativa*	(Saengwilai *et al.*, 2017)
			-72	Root		
			10	Panicle		
Cow manure	20%		36	Shoot		
			59	Root		
			6	Panicle		
Sheep manure	10%	9641 mg kg^{-1}	-14	Root	*Medicago sativa*	(Elouear *et al.*, 2016)
	10%		-20	Shoot		
Humic acid	2 g kg^{-1}	146 mg kg^{-1}	5		*Chrysopogon zizanioides*	(Vargas *et al.*, 2016).
	10 g kg^{-1}		-50			
	20 g kg^{-1}	147 mg kg^{-1}	-50	Shoot		
	2 g kg^{-1}	146 mg kg^{-1}	-2	Root		
	10 g kg^{-1}		-35			
	20 g kg^{-1}		-57			
	2 g kg^{-1}	6617 mg kg^{-1}	-24			
	10 g kg^{-1}		-52			
	20 g kg^{-1}		-57			
Poultry manure compost	20 g kg^{-1}	616.6 mg kg^{-1}	30		*Brassica juncea*	(Huang *et al.*, 2020)
	40 g kg^{-1}		83			
	60 g kg^{-1}		80			
	80 g kg^{-1}		41			
	100 g kg^{-1}		9			
	20 g kg^{-1}		2			
	40 g kg^{-1}		21			
	60 g kg^{-1}		23			
	80 g kg^{-1}		8			
	100 g kg^{-1}		-5			
Peat	10%	2061 mg kg^{-1}	-4	Root	*Paulownia fortune*	(Zhang *et al.*, 2019)
				Stem		
				Leaves		
	20%		-7	Root		
			-13	Stem		
			-12	Leaves		
	30%		-16	Root		
			-31	Stem		
	30%	2061 mg kg^{-1}	-16	Leaves		

the accumulation of nutrients in the edible region of the crop plant without having an unfavorable effect on plant physiology or growth as well as economic yield (Vanderschuren *et al.*, 2013).

Important to improving grain Zn content is the overexpression of genes involved in Zn translocation and mobilization, which results in higher Zn bioavailability and does not negatively affect grain production (Borrill *et al.*, 2014). Over-expression of nicotianamine (NA) synthase by adding 35S enhancer elements led to increases in Zn content in paddy that were two to three times higher (Lee *et al.*, 2009). Similarly, transgenic rice that expressed the barley nicotianamine synthase gene HvNAS1 under the control of the rice actin1 promoter accumulated two to three times the normal amount of Zn in polished rice grains (Lee *et al.*, 2009). At the International Rice Research Institute, many thousand transformants of IR64 and IR69428 are created with soybean or rice ferritin and rice nicotianamine synthase (NAS2) overexpressed genetic constructs. The Zn content in those lines has exceeded the level targeted from the field testing. As a result, nicotianamine is an interesting target for Zn biofortification as it has an upregulation of NAS genes. In addition, biofortifying cereals with NAS on their own or in conjunction with ferritin have a significant potential for reducing the prevalence of mineral deficiencies among humans worldwide (Lee *et al.*, 2009; Zheng *et al.*, 2010).

Table 4. Zinc solubilizing microorganisms and their plant growth-promoting activities

Organism	Identification	Zinc solubilization (mm)			PGPR activities	References
		ZnO	ZnCO3	Zn3(PO4)2		
Bacteria						
***Acinetobacter* sp.**	Polyphasic		-		PS, Sid, IAA	(Rokhbakhsh-Zamin *et al.*, 2011)
***Bacillus* sp. AZ6**			-		-	(Hussain *et al.*, 2020)
***Burkholderia lata* ZnSB2**	16S, biochemical	15.3	19.3	11.8	NH3, PS, Amy	(Dinesh *et al.*, 2018)
***Burkholderia cenocepacia* KNU17BI2**	16S	25.2	19.6	-	PS, NH3, IAA, Sid, AF	(Tagele *et al.*, 2019)
***Burkholderia contaminans* KNU17BI3**	16S	22.4	21.8	-	PS, NH3, IAA, Sid, AF	
***Curtobacterium* sp. Strain 81**	16S, MALDI- TOF	-			-	(Costerousse *et al.*, 2018)
***Plantibacter* sp. Strain 5**	16S, MALDI- TOF				-	
***Pseudomonas sp.* Strain 24**	16S, MALDI- TOF				Sid	
***Streptomyces* sp. Strain 68**	16S, MALDI- TOF				Sid	
***E. cloacae* PBS-2**	16S	1	1	0.5	-	(Kamran *et al.*, 2017)
***Peudomonas fragi* strain EPS-1**	16S	9	8	3	Phos, IAA, Sid	
***Pantoea dispersa* strain EPS-6**	16S	11	10	4	Lip, Cell, IAA	
***Rhizobium* sp.**		18	10	6	-	

LHRW1						
Gluconacetobacter diaotrophicus	-		28	12	Nematicidal	(Saravanan *et al.*, 2007)
Pseudomonas aeruginosa **(CMG 823)**	API test kit	+	-	+		(Fasim *et al.*, 2002)
Serratia **sp. (TM9)**						(Othman *et al.*, 2017)
Fungi						
Beauveria caledonica						(Fomina *et al.*, 2004)

Grain protein content b1(gpc-B1) is a quantitative trait locus in wheat that is related to higher levels of grain protein and also higher levels of Zn (Zheng *et al.*, 2010). After the introgression of the Gpc-B1 locus from the wild tetraploid wheat *Triticum turgidum ssp. dicoccoides* into various recombinant chromosome substitution lines, an increase of 10-34% in the concentrations of grain Zn was observed in cultivated wheat, indicating the role of Gpc-B1 in the remobilization of Zn from the leaves to the grains (Distelfeld *et al.*, 2007). Transgenic approaches that inhibit phytatic biosynthesis and over-express phytase enzymes in seeds can also improve Zn concentrations by lowering phytic acid buildup in wheat grains (Raboy, 2003). However, genetic engineering is a long-term solution that requires tedious processing processes before human use (Abid *et al.*, 2017). The genetic variety of Zn accumulation in kernels has been examined and considerable differences in Zn concentrations have been identified. In maize, Quantitative trait locus mapping was performed to determine the chromosomal areas linked with Zn accumulation (Maqbool & Beshir, 2019). Mapping Binary Trait Loci in the F2:3 Design generation of maize was created by crossing two parents with different kernel and cob Zn contents. In these mapping populations for the kernel, Zn content with high heritability, a vast genetic variety and transgressive segregation were identified. QTLs were identified using genetic analysis across solo and combined contexts, and 15 and 16 QTLs were detected under both environments, with some QTLs being identical under combined analysis. The majority of detected QTLs were located on chromosomes 2, 7, and 9 in both mapping groups. This research also demonstrated that QTLs for kernel Zn concentrations were co-localized on chromosomes 2, 7, and 9. Co-localization of quantitative trait loci (QTLs) for Fe and Zn concentrations demonstrated that the concentrations of these minerals might be increased simultaneously by targeting the same chromosomal areas with marker-assisted selection (Qin *et al.*, 2012).

4.3 Application of nano-chemistry for zinc biofortification

In contrast to bulk materials, nanoparticles possess unusual qualities and the influence of nanoparticles can be observed in virtually area of research and development in the scientific and technological fields (Mirza *et al.*, 2019). It concludes that biogenic ZnO nanoparticles can be employed as an effective nano-priming agent for seed treatment to boost both Zn nutrient and plant growth for sustainable agricultural development (Sharma *et al.*, 2022). Nanoparticles are gaining popularity in today's world because of the variety of advantages that make them more desirable. These characteristics include a high ratio of surface area to volume, high stability, high adsorption, increased surface reactivity. Therefore, an application of a low dosage can have a visible impact on enhanced crop growth, yield, and biofortification (Mittal *et al.*, 2020; Rastogi *et al.*, 2017). When it comes to designed metal nanoparticles, ZnO nanoparticles (ZnO-NPs) are the ones that are most commonly used as a substitute for Zn fertilizer. ZnO has been given the designation of a "Generally Recognized as Safe" (GRAS) substance by the Food and Drug Administration of the United States. It also has a low level of toxicity to people (Mokammel *et al.*, 2022). ZnO-NPs has been proven to be superior to

ZnSO4 in terms of its use for addressing the Zn deficiency that exists in agricultural crop plants (Singh *et al.*, 2021). ZnNPs, frequently cause plants to exhibit varying degrees of physiological reactions linked with Zn (Sturikova *et al.*, 2018).

In *Phaseolus vulgaris*, excessive applications of ZnSO4 and Zn-NPs are deleterious rather than advantageous, although these elements are essential for the regular growth and development of plants (Salehi *et al.*, 2021). When it comes to cereal crops like maize, one of the most common methods for treating Zn deficiency is the foliar application of bulk ZnS. Specifically for maize, foliar application of ZnO- NPs could reduce cadmium accumulation (Rizwan *et al.*, 2019) and priming the seeds could considerably minimize the risk of pathogens infecting the seeds (Estrada-Urbina *et al.*, 2018). Nano-priming is a relatively new method of seed priming that involves the use of manufactured nanoparticles. It has recently garnered notice for its potential to improve crop yield and protection due to the distinctive physicochemical features that it possesses. When compared to the usage of engineered nanomaterials in open environments, priming seeds in a controlled setting offers several benefits that cannot be matched by the alternative. Although it is possible to permeate seed coats with nanoparticles to increase seed germination and growth characteristics, the mechanism behind this process is still mostly unclear (Dasgupta *et al.*, 2017). The production of ZnO-NPs can be accomplished using a variety of processes, including direct precipitation, combustion, hydrothermal, sono-chemical, solvothermal, vapor phase, microwave-aided, wet chemical and micro emulsion (Sangeetha *et al.*, 2011). According to our study, there is no reliable data available for nano-technology use for the Zn biofortification of wheat and rice.

Conclusion

Zinc (Zn) is consistently acquired by people from plant sources; hence, Zn deficiency in plants might result in Zn deficient human bodies. There is a large population that relies on wheat (*Triticum astevium*), maize (*Zea mays*) and rice (*Oryza sativa*), as their primary source of nutrition. Cereal- based foods account for sixty percent of the total amount of food consumed on the planet. Because of this reality, a significant portion of the human population is currently afflicted with harmful diseases. The only way out of this terrible scenario is to strengthen these basic foods with additional nutrients. It is necessary to collaborate with all possible fields that can work for biofortification to improve Zn levels in the human body while simultaneously avoiding the toxicity of Zn. These fields include agronomy, genetics, crop physiology, soil science, microbiology, human nutrition, chemistry, medicine and the newly emerging field of nanotechnology. The following future research directions are necessary to address the Zn deficiency:

- ➢ Collaborative research combines several field professionals to biofortify grains rapidly and reliably.
- ➢ COVID-19-positive patients are also Zn deficient; this idea necessitates rigorous scientific investigation to mitigate the negative impacts of this global scourge.
- ➢ Identification of cultivars with the capacity to store Zn rapidly in their grains. Then, these variations have utilized in breeding operations to generate Zn deficiency- specific genotypes for areas where people are deficient.
- ➢ Creation and distribution of kits for on-farm testing of soil Zn status to adopt effective remediation measures to prevent negative effects.
- ➢ Raise awareness of Zn insufficiency in underdeveloped communities and set the ground for the simple assessment of Zn-enriched crops.

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7. Conflict of Interest

The authors declare no conflict of interest.

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