Journal of Experimental Agriculture International

Volume 46, Issue 10, Page 625-642, 2024; Article no.JEAI.124948
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Unlocking Potential: The Role of Zinc Fortification Combating Hidden Hunger and Enhancing Nutritional Security

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LR, JR, NK and MK Conceptualized the study. Authors LR, SS, AK, SNS, CKJ, DS and AK Investigated the work. Authors LR, JR, NK, SK, SS, SR and JP wrote and prepared the original draft of the manuscript. Authors LR, SR, RS, MK and SK reviewed and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jeai/2024/v46i102986

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124948

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Cite as: *Rana, Lalita, Navnit Kumar, Jitendra Rajput, Sumit Sow, Shivani Ranjan, Sarita Kumari, Jyostnarani Pradhan, Anil Kumar, S. N. Singh, Ajeet Kumar, CK. Jha, Meenu Kumari, Devendra Singh, and Ritwik Sahoo. 2024. "Unlocking Potential: The Role of Zinc Fortification Combating Hidden Hunger and Enhancing Nutritional Security". Journal of Experimental Agriculture International 46 (10):625-42. https://doi.org/10.9734/jeai/2024/v46i102986.*

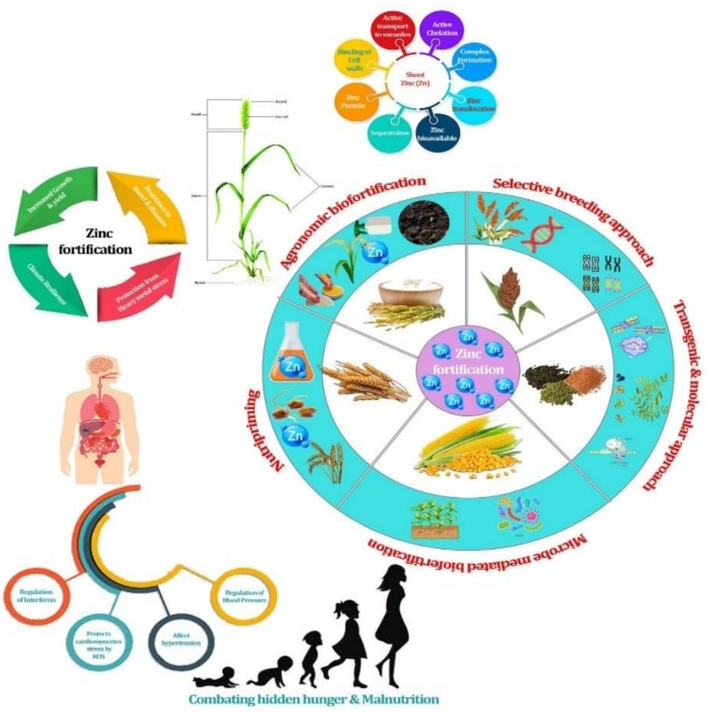
Received: 15/08/2024
Accepted: 17/10/2024
Published: 22/10/2024

Review Article

ABSTRACT

Micronutrient shortage is rapidly becoming apparent have drawn more attention in the cultivation of crops. The main causes of this deficit are the introduction of high-yielding varieties, an intensified cropping strategy, and advanced irrigation systems etc. A further aspect contributing to this issue is the increased use of high analysis chemical fertilizers instead of organic plant nutrition (composts, farmyard manure, etc.). Most countries have acute shortages of micronutrients due to the significant depletion of soil reserves caused by current agricultural production technologies. In order to increase both the quantity and quality of crops, micronutrients are crucial. Through the integration of agronomic, breeding and transgenic techniques, researchers seek to strengthen the zinc concentration in field crops, so improving their nutritional value and mitigating the risk of zinc deficiency in human diets. The availability and absorption of micronutrients in crops are improved by agronomic techniques such as foliar spraying, and soil fertilizer treatment including organic amendments. Meanwhile, biofortification of vegetable and fruit crops has also been achieved by transgenic and breeding strategies. In other hand, Rhizobacteria-based biofortification, Chelated Zn biofortification, nutri-priming are also important techniques in Zinc fortification programs to ensure food security and nutritional quality, bio-fortification of micronutrients in crops is vital. In addition, bio-fortification improved quality and crop output, reducing hidden hunger and demonstrating that it was a viable and economical approach. The present review addresses several aspects of zinc insufficiency in human populations, including public health and socioeconomic issues, bio-fortification and ferti-fortification studies, and future efforts to mitigate zinc deficiency in soil and the population at large.

GRAPHICAL ABSTRACT



Keywords: Micronutrient shortage; biofortification; transgenic and breeding strategies; food security; food security and nutritional quality; hidden hunger.

1. INTRODUCTION

Sufficient diets of zinc are necessary for human health. Daily requirement of Zn is 4-25, 8 and 13 mg/day for infants and children, women and man respectively [1] but unfortunately this requirement is not fulfilled due many factors contributing significantly to malnutrition, including a lack of availability to adequate quantities of nutrient-dense food, unbalanced diet, lack of nutritional variation, and other issues. In underdeveloped countries, zinc deficiency was the fifth highest illness risk factor and about one-third of the world's population lacks zinc [2]. Bio-fortification may be the most sustainable strategy to boost food yield by producing, protecting, and improving agricultural agronomic features and food security while eliminating human vitamin deficiencies. Zn is found in organic protein complexes in animal products and inorganic ions in plants [3]. Physiological factors affect Zn absorption and availability. Mother's tissues, developing fetus, and nursing child may require increased fractional Zn absorption in humans. Zinc levels in Indian soils ranges from 0.01 to 52.9 mg/kg. Among the micronutrients, Zn deficiency (Fig. 1.) prevails more after boron in Indian soils conditions [4].

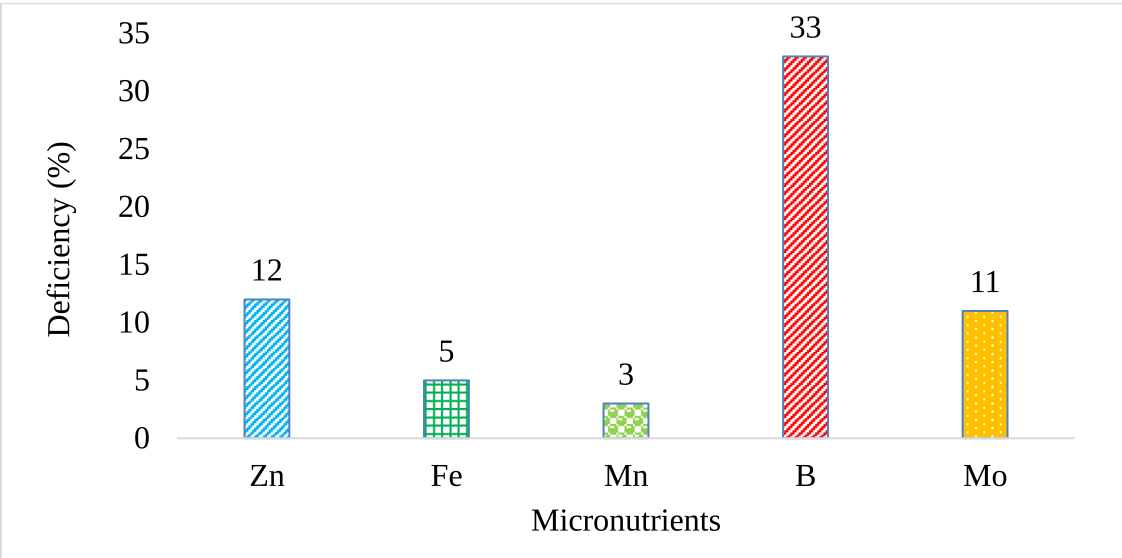


Fig. 1. Deficiency of micronutrients in the soil

Table 1. Causes of zinc deficiency in humans

Particulars	Comments	References
Inadequate dietary intake	Insufficient intake of zinc-rich foods (e.g., meat, shellfish, legumes, seeds, nuts).	[14]
Malabsorption syndromes	Conditions like Crohn's disease, celiac disease, and short bowel syndrome reduce zinc absorption.	[15]
Increased physiological needs	Higher zinc requirements during pregnancy, lactation, and childhood growth spurts.	[16]
Chronic Illnesses	Conditions such as diabetes, chronic kidney disease, and liver disease can affect zinc levels.	[17]
High phytate diet	Diets high in phytate (found in grains and legumes) inhibit zinc absorption.	[18]
Alcoholism	Excessive alcohol consumption can impair zinc absorption and increase zinc excretion.	[19]
Vegetarian or Vegan Diets	Plant-based diets can be low in bioavailable zinc, requiring careful planning to meet needs.	[20]
Genetic disorders	Conditions like acrodermatitis enteropathica, caused by mutations affecting zinc absorption.	[21]

In most Indian agricultural areas, multi-nutrient deficiencies reduce crop vigour, crop yield, quality, and monetary gain [5]. High-yielding crop cultivars and an acute shortage of organic resources like farmyard manure (FYM) and composts have exacerbated the problem [6]. Micronutrient management in agricultural systems must be improved to combat the widespread shortage of micronutrients (mainly zinc), as 49% of soils are zinc-deficient [1]. Zinc is found in soil as the divalent cation Zn^{2+}. Increasing the zinc content of food crops using various agricultural approaches is known as zinc bio-fortification. More and more people are opting to fortify or biofortify their diet with zinc. Nevertheless, micronutrient deficits have frequently been found in various crops despite the relatively high total levels because of the low availability of accessible micronutrients [7]. Zinc deficiency (Table 1) is a major public health concern worldwide but it is especially prevalent in underdeveloped nations where people eat a lot of staple foods and don't get enough variety in their diets. Food quality affects many people's health because plants are their main source of food. Research has produced more productive staple food crops. Essential micronutrients are frequently lower in staple foods. Diverse food sources are a sustainable solution, but they are expensive for impoverished people facing risk of hunger and malnutrition. Farmers primarily rely on macronutrients (N, P, and K) hence; zinc (Zn) and other micronutrient deficiencies are becoming more prevalent [8] in the soil. The country's areas are experiencing acute deficit (8%), deficiency (29%), and latent shortage (15%) of Zn. On the other hand, less than ten percent of the soils in states such as Mizoram, Uttarakhand, Tripura, Nagaland, Arunachal Pradesh, Meghalaya and Himachal Pradesh had zinc deficiencies [9]. Micronutrient content of soil [10] affected by numerous factors [11], including parent material, soil type, intrinsic soil properties like pH and soluble salt concentration (EC), the quality and quantity of soil organic matter and calcium carbonate content, trace elements provided by manures and fertilizers, the content of available macronutrients, micronutrient relations, and vegetation. The loss of micronutrients through leaching, liming of the soil, insufficient application of organics (green manuring and overuse or devoid of micronutrient fertilizers) exacerbates the depletion of accessible micronutrients in the soil. Zinc deficiency resulted in khaira disease in rice, rosetting in wheat, white buds in maize, small leaves and mottling in vegetables, and reduced fruit production in citrus fruits. The response to applied zinc received in a variety of crops and cropping systems across the country indicates the impact of zinc deficiency on agricultural productivity [12, 13]. This review focuses on the use of zinc bio-fortification for improved micronutrient availability in food crops through the use of fertilizers, organic amendments, Rhizobacteria-based biofortification, biological chelation of Zn, nutri-priming, nano and micro-encapsulation for successful agronomic, breeding, and transgenic fortification of important crops.

2. INNOVATIVE BIO-FORTIFICATION APPROACHES

There are two main categories of biofortification: agronomical approaches, which include traditional plant breeding techniques, and transgenic approaches, which involve biotechnological techniques (e.g., genetic changes). Both methods are utilized to improve the micronutrient levels in food crops. Important biofortification techniques, (transgenics, agronomic practices, and traditional plant breeding) have transformed staple foods (maize, rice, wheat, sorghum, chickpea and potatoes etc. [22].

2.1 Agronomical Bio-Fortification

Zinc treatment via foliar spraying has the potential to increase efficiency with which plants absorb zinc, which in turn can lead to increased crop development and production. The micronutrient zinc is an essential component for plants, as it plays a significant part in a variety of physiological processes. These processes include the activation of enzymes, the synthesis of proteins, and the metabolism of carbohydrates. The delivery of zinc through foliar spraying is particularly useful in circumstances in which the availability of zinc in the soil is restricted, or in which the pH of the soil, the amount of organic matter present, or other variables may prevent the uptake of zinc by the roots of the plant. Additionally, timing, concentration, and frequency of application are crucial for maximum effects and phytotoxicity prevention. $ZnSO_4$ has 23% elemental Zn, making it a possible food fortifier to counteract Zn deficiency [23]. Zinc citrate (ZnCi) is rarely added to meals, but recent clinical research shown that young adults taking ZnCi absorbed more Zn than those taking ZnO [24]. Thus, agronomic and genetic biofortification and functional food additives can reduce micronutrient deficit. Another way to raise the zinc content of crops is to use fertilizers that contain zinc but to prevent soil zinc accumulation and environmental contamination, this method must be properly controlled. In fortifications, the zinc compounds (sulphate, gluconate, oxide, chloride, and stearate combinations) are most frequently utilized. Zinc fortificants are chemicals that are white or colourless, and they have a lower likelihood of generating physical alterations. However, their insoluble nature can lend unacceptable tastes to food [25]. In spite of the fact that it is less soluble than zinc fortificants, zinc oxide is the most extensively utilized of the zinc fortificants since it is the least expensive. Furthermore, besides being added directly to food, zinc oxide nanoparticles have shown promising results when added to soil samples that are used to grow sorghum [26]. In recent years, biofortification through microbes is expanding in prominence as a method of increasing Zn and other micronutrient levels in grain crops [27]. In this context, [28] identified species from the genera *Pseudomonas, Brevibacterium, Bacillus, Enterobacter* and *Acinetobacter* as viable candidates for plant biofortification and biocontrol aligns with current research trends. Each of these bacterial genera has been recognized for its potential benefits in promoting plant growth, enhancing nutrient uptake, and providing protection against pathogen. The aforementioned microbes can also incorporate micronutrients into eatable plant tissues by solubilizing their indigenous insoluble sources found in the soil [29]. These microorganisms assist plants in absorbing more Zn from the soil by solubilizing Zn via organic acid secretions [30] and increasing root surface area through auxin synthesis in the rhizosphere.

2.1.1 Ferti-fortification

Foliar spraying: Plants are able to circumvent the restrictions of the soil and more quickly absorb the micronutrient zinc when it is applied directly to the surfaces of the leaves and readily absorbed and translocated to other parts of the plant, which facilitates its incorporation into essential enzymes and metabolic pathways (Fig. 2).

Foliar spray of zinc influences the zinc concentration in finger millet grains [31]. Sai Divya [32] also revealed that zinc spray effective for enhancing concentration in grain upto 40 percent. Study conducted by [33] discovered that zinc concentration was higher in foliar applied zinc rice crop. Research conducted by [34] found that the application of zinc nanoparticles improved the bioavailability of zinc in plants. The study showed that foliar application of ZnO nanoparticles led to higher zinc concentrations in plant tissues compared to larger conventional zinc sources, indicating enhanced nutrient efficiency. According to [35], the application of zinc nanoparticles not only improved zinc uptake but also stimulated physiological responses in plants, such as increased chlorophyll content and enhanced photosynthetic activity. This, in turn, can lead to improved plant growth and productivity. Further, study conducted by [36] highlighted that foliar application of zinc nanoparticles provides a rapid delivery system for micronutrients, allowing plants to access essential nutrients quickly, especially during critical growth stages when nutrient demand is high. Additionally, it helps to avoid interactions with soil components that may inhibit zinc uptake, such as high pH or high levels of certain soil minerals. Foliar spraying zinc can fix zinc shortages in crops, but it should be used as part of an integrated nutrient management approach that addresses soil fertility and other nutrient availability variables.

Soil fertilizer treatment: According to [37], the use of dextran-coated zinc oxide nanoparticles on developing wheat seeds resulted in a similar rise in the amount of zinc found in the seeds. The findings of these research collectively point to a less expensive technique of biofortifying zinc, which may be more advantageous to the process of food fortification in sub-Saharan Africa and other places that are struggling with micronutrient deficiency. Zinc oxide seems to be popular in food fortification since it absorbs similarly to zinc sulphate, the most soluble zinc fortifier [38]. Earlier research by [39] showed that fortified wheat bread and porridge absorbed zinc oxide and zinc sulphate similarly; however, the authors found that bread absorbed zinc at a far faster rate than porridge. This proves that different kinds of food and different matrices have different effects on micronutrient absorption. Additionally, the authors concluded that the reduced zinc absorption from porridge was due to the increased phytate level in comparison to bread, even though they did not measure the phytate concentrations. It is possible that the phytate-zinc complexes were broken down by the high temperatures that occurred during baking [39].

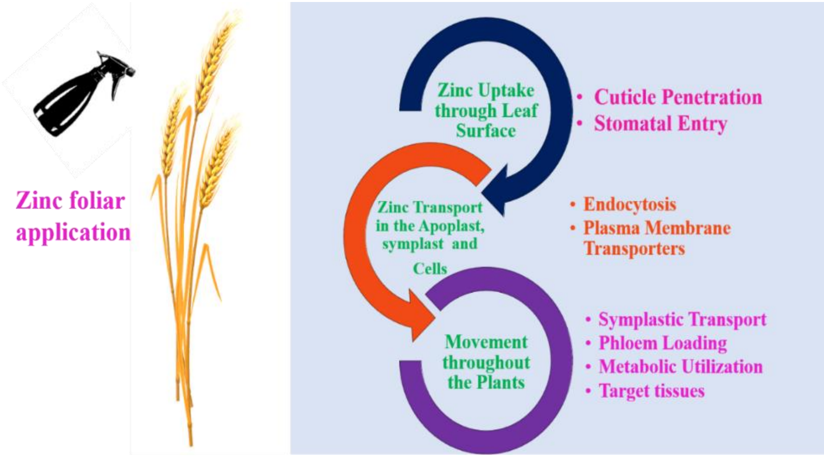


Fig. 2. Pathways of zinc delivery through foliar spraying

Organic amendments: A number of researches on impact of zinc concentration by the application of organic amendments have been conducted as depicted in Table 2. For Instance, Poultry Manure-amended soils induced higher zinc accumulation compared to sewage sludge applied in the wheat crop [40]. Another research showed that cow dung amended plots showing higher concentration [41]. Study at Madhya Pradesh on Organic farming revealed that organic wheat contained more Zn despite same yield level due to higher nutrient efficiency [42]. Field study conducted at two diverse locations [10] and indicated that grain Zn content of maize and succeeding wheat significantly affected by Zn application and organic amendments and it was observed highest values when applied poultry manure at Peshawar calcareous soil. Organic manure plays a vital role in improving zinc availability in soils while also enhancing soil microbial activity. [43] studied different sources of organic fertilizers in maize and found out that highest zinc (%) in maize was observed in the crops produced in biochar treated plots.

Rhizobacteria-based biofortification: Synthetic and natural sources fertilizers, as well as microorganisms, augment micronutrient acquisition, resulting of higher levels of micronutrients, particularly Fe and Zn in plants [44]. A balanced nitrogen application, in conjunction with other macro/micronutrients, proves crucial to ensuring optimum grain production and superior quality [45]. White and Broadley [46] studied that the interaction between PGPR and plants can be complex and multifaceted. While PGPR can enhance nutrient availability and uptake for plants, the specific effects on biofortified supplements may depend on various factors such as the type of supplement, the specific strain of PGPR used, soil conditions, and the plant species involved [47]. The selection of specific strains within these genera is crucial, as different strains can exhibit varying levels of effectiveness in promoting plant growth and providing biocontrol. Moreover, considerations such as environmental conditions and the target plant species should be taken into account for successful application in agriculture. The findings underscore the importance of harnessing the potential of beneficial bacteria for promoting both plant nutrition and health in agricultural systems. Further research into the mechanisms of action and optimization of microbial inoculation strategies can help unlock the full potential of biofortification and biocontrol approaches for sustainable crop production. In agriculture, zinc (Zn) availability to plants is a common concern, especially in places with low zinc soils or where soil pH, organic matter concentration, or specific soil features can limit its availability [48,47]. However, Zn deficiency in Indian soils is primarily triggered by their calcareous and alkaline nature. The use of zinc-solubilizing Plant Growth-Promoting Rhizobacteria (PGPR) can be a valuable strategy for enhancing zinc availability to plants and facilitating its absorption [49, 46]. Zinc deficiency causes growth retardation and immune system dysfunction; these symptoms can be alleviated by increasing the zinc content of certain crops. include supplementing plants with zinc in chelated or nanoparticle forms, which plants absorb more effectively. Zinc deficiency causes growth retardation and immune system dysfunction; these symptoms can be alleviated by increasing the zinc content of certain crops include supplementing plants with zinc in chelated or nanoparticle forms, which plants absorb more effectively.

Table 2. Organic amendments affect the Zinc availability in different crops

Citations	Organic source	Crop tested	Effects
[50]	Farmyard Manure	Wheat	Increased soil Zn availability
[51]	FYM, Press mud and Green Manure	Rice, Chickpea	Improved zinc bioavailability in soil
[52]	Vermicompost	Various	Positive correlation between organic matter and Zn levels
[53]	Poultry Manure	Maize	Increased concentration of zinc in plant tissues
[54]	Cow Manure	Soybean	Higher soil zinc levels observed
[55]	Compost	Mustard	Enhanced Zn uptake in plant tissues
[48]	Organic Fertilizer	Tomato	Elevated soil and fruit Zn levels
[56]	Green Manure	Cowpea	Increased Zn levels in soil and plants
[57]	Biochar	Spinach	Elevated soil Zn availability
[58]	Integrated Organic Inputs	Barley	Improved soil Zn and Fe levels
[59]	Green Manure	Peas	Increased soil Zn levels and improved nutrient uptake
[60]	Biochar	Lettuce	Enhanced soil properties and increased Zn bioavailability
[61]	Farmyard Manure	Potato	Increase in soil Zn content and plant growth parameters
[62]	Compost	Groundnut	Improved Zn concentration in pods and overall yield
[63]	Poultry Manure	Sorghum	Enhanced soil Zn availability and increased biomass

2.2 Plant Breeding-Based Biofortification

Plant breeders strive to create new crop types with improved soil-uptake and zinc-transport efficiency so that people can eat more zinc-rich foods like grains and fruits. Zinc bio-fortification is an effort to improving staple food nutrition, particularly those eaten by susceptible communities, like beans, rice, wheat, and maize. If these difficulties are addressed, biofortification becomes cost-effective because fortified crops can be grown seasonally without additional adjustments. Insufficient zinc intake by humans in their standard diets is caused by a zinc deficiency in the soil, which reduces the amount of zinc that is bioavailable [64]. The treatment of this ailment can be approached from a number of various angles, including the consumption of a diversified diet, the use of food supplements, and the formulation of a diet that is fortified. However, these are expensive and unaffordable for those who are impoverished. Within this paradigm, agronomic biofortification using zinc fertilizers is a sustainable and effective way to alleviate micronutrient deficiencies, especially in low-income countries worldwide [65]. Mineral fertilizers or the selection of plant varieties or combination of these strategies may be used synergistically to achieve optimal zinc biofortification outcomes and address zinc deficiency in populations that rely heavily on staple crops for their dietary zinc intake [65]. Plant breeding-based biofortification has been previously considered as a potential strategy to address global hunger [66]. Biofortification aims to create safe, sustainable, ample, and nutritious food sources. Staple foods are now the focus for maximizing the benefits [67]. Effective biofortification in plant breeding depends on a varied gene pool to regulate nutrient levels in the selected crop variety. If there is a paucity of diversity, transgenic approaches may be a more appropriate option [68]. Biofortification is considered a potential method to address micronutrient deficiencies, particularly in underdeveloped nations where it is prevalent. To achieve success in biofortification, a high level of expertise in plant genetics is required for effective transgenic improvements. It is possible to boost the zinc content of plants in a straightforward and inexpensive manner by employing this method. Although it is a time-consuming process, genetic biofortification is an excellent strategy for reducing the likelihood of micronutrient shortages [69]. However, technological processing of food raw materials reduces nutrition, resulting in inadequate mineral and vitamin intake. Several food fortification methods remedy micronutrient deficits. the selection of mineral-containing functional food additives for zinc biofortification relies on several critical factors, including solubility, bioavailability, digestibility, and cost-effectivenes [27]. As a result, selecting an appropriate dietary matrix for the purpose of fortification is of the utmost importance. For products that are designed for mass consumption or specific consumption, it is recommended to fortify them.

2.3 Biological Chelating

Chelating agents are compounds that can form complexes with metal ions, effectively sequestering them and preventing their involvement in undesirable reactions that can lead to food spoilage or degradation and also remove unwanted metal ions from food matrices, such as heavy metals or metal contaminants. By forming stable complexes with these ions, chelators help reduce their presence in food products, thereby enhancing food safety and compliance with regulatory standards.

2.3.1 Chelated zinc biofortification

Food technology is increasingly exploring the use of biological chelating agents to protect food from chemical, enzymatic, and oxidative processes. Food fortification with them is still rare. Biological chelating agents, derived from natural sources such as plants, microorganisms, or animal tissues, offer several advantages over synthetic chelators, including safety, sustainability, and consumer acceptance. Some researchers have investigated the biofortification of cereals and cereal-based products by chelating Zn with amino acids, peptides, and solubilizing bacteria. Xu *et al*. [70] investigated that chelated Zn biofortification of staple foods is less phytotoxic than conventional biofortification. It boosts plant-based product growth, productivity, and nutrition by increasing Zn content [69]. The synthesis of metal chelate complexes with biological structures allows for highly stable trace element forms. Plant polysaccharides and peptides have also emerged as promising biological materials with various applications, including their potential as zinc chelators to enhance zinc uptake, bioavailability, and bioactivity in both plants and humans. There are several benefits to taking a biological zinc supplement that includes the natural polysaccharide-Zn complex [70]. Unlike some synthetic chelating agents, plant-derived polysaccharides and peptides are generally regarded as safe (GRAS) and exhibit low toxicity, making them suitable for use in food and agriculture without adverse health effects. It has been found that polysaccharide-Zn complexes have powerful antioxidant, anti-proliferative, and anti-diabetic properties. They may be able to alleviate zinc deficiency symptoms by regulating the immune system [60]. Furthermore, the use of plant polysaccharides and peptides as zinc chelators aligns with the principles of sustainability and environmental responsibility, as they are derived from renewable plant sources and contribute to the development of eco-friendly solutions for enhancing zinc nutrition in both plants and humans. Natural polysaccharide-chelating Zn complexes outperformed inorganic and organic Zn supplements in in vitro and in vivo studies measuring bioavailability and efficiency of Zn absorption. Ongoing research in this area aims to optimize the properties and applications of these novel biological materials for improved zinc biofortification and nutritional supplementation strategies.

2.3.2 Zinc microencapsulation

Enclosing zinc particles or compounds into small protective capsules or spheres is called zinc microencapsulation. Improving stability, regulating release, and boosting zinc's functionality in varied applications are all goals of this approach. There are several methods for microencapsulation, including spray drying, fluid bed coating, coacervation, and emulsion techniques. Each method has its advantages and is chosen based on factors like the desired particle size, release profile, and the nature of the encapsulated material. It helps to protect zinc from environmental factors such as oxidation, moisture, and light, which can degrade its efficacy. This enhanced stability prolongs the shelf life of zinc-containing products and ensures their effectiveness over time. By encapsulating zinc, it's possible to control its release kinetics, allowing for sustained or delayed release profiles. This feature is particularly useful in applications where a steady supply of zinc is required over time, such as in nutritional supplements or agricultural fertilizers. Microencapsulation enables targeted delivery of zinc to specific sites within the body or to particular areas in agricultural settings. For example, in agriculture, zinc microencapsulated fertilizers can be designed to release zinc directly to the root zone of plants, maximizing nutrient uptake and minimizing waste. Zinc can be microencapsulated and released at a controlled rate, facilitating slow or targeted dosing [71]. Mineral encapsulation, including that of zinc, has been the subject of much research and practice [72]. The most popular method for mineral microencapsulation is spray drying because of its high production rate and inexpensive cost. When it comes to encapsulating a wide variety of materials, spray drying offers a number of benefits. Thanks to these benefits, spray drying has become one of the few unit procedures that can be easily scaled up and used on an industrial scale [72]. Zinc microencapsulation finds applications in various industries, including pharmaceuticals, food and beverages, agriculture, cosmetics, and textiles. In pharmaceuticals, it can be used to improve the bioavailability of zinc supplements or to deliver zinc-based drugs to specific tissues or cells. In the food industry, microencapsulated zinc can be added to fortify foods and beverages without affecting their taste or texture. [73] reported a recent application of spray-dried Zn compound microencapsulation for food fortification. Overall, zinc microencapsulation offers a versatile approach to enhancing the stability, delivery, and efficacy of zinc-based products across a range of industries and applications.

2.3.3 Zinc nanoencapsulation

The study of substances centered on the atomic and molecular dimensions is the primary emphasis of nanotechnology, which has become an emerging area of research. (sizes 1-100 nm). There have been recent applications of nanoscale zinc in the food business, particularly in processing and packaging [74]. A number of physical and chemical methods have been devised for the synthesis of zinc nanoparticles (Zn NPs), such as hydrothermal procedures, laser ablation, vapour deposition, and precipitations [75]. However, there is a danger to human and environmental health from these procedures. A green manufacturing approach has been devised to decrease the toxicity of Zn NPs [76]. In order to produce nanoparticles, the environmentally friendly approach makes use of natural components that are derived from bacteria and plants [77]. This method is not only less hazardous to the environment, but it also has a cheaper cost than the traditional chemical and physical procedures compared to the alternatives [78]. The process of encapsulation involves the creation of a protective barrier around the zinc, which helps to prevent interactions with other components of the meal that could potentially impair the zinc's bioavailability [79]. Udechukwu et al. [80] define chelation is the building of stable zinc-molecule complexes. The formation of chelate complexes of metals with biological structures extracted from plants (peptides and polysaccharides), highly stable and encapsulating mineral elements in microcapsules that protect against food system interaction and prevent digestive tract destruction, and using nanoparticles as food additives are promising trends in food fortification/biofortification with Zn. Encapsulation and chelation could differ depending on the food matrix and fortification method, so further investigation needs to be conducted to determine the best ways to increase zinc bioavailability in fortified foods and zinc nutrition.

2.4 Biotechnical/Transgenic Approaches for Zn Fortification

Forward and reverse genetics approaches are two major biotechnical approaches utilized for crop improvement programs and the development of zinc-fortified crops [81]. Forward genetics approaches; Molecular mapping, marker-trait association studies, Eco-TILLING, Subtractive Hybridization, Gene trapping, RNA seq approaches, Fine mapping, *etc.* are the biotechnological tools and techniques that search for the genetic basis of a particular trait and phenotypes. Reverse genetics approaches; TILLING, Knock-out approaches, Homologous recombination, insertional mutagenesis, gene silencing, Knock-down approaches, Knock-On and Knock-In approaches are used to generate variation in the target gene to ascertain the function of the gene for the particular mechanism and phenotype. Various genes have been mapped for zinc enhancement in crop plants [82]. The zinc fortification through the grain depends upon; the uptake of Zn from soil to the root, transportation of Zn to the grain, and the availability of Zn in the grain. The uptake of minerals from the soil depends upon the pH of the soil and the interaction of Zn with other minerals present in the soil such as the presence of Cd in the soil affects the uptake of Mn, Zn, and Ca [83]. Biotechnical approaches are looking for the transporter promoting the accumulation of dispensable minerals in soil and resisting co-transportation of heavy metals.The transporter genes are widely being mapped for Zn uptake and transportation to the grain for the enrichment of Zn (Table 3). While the genes responsible for the accumulation of anti-nutritional factors are also being identified. Their expressions are being modulated using CRISPR/Cas approaches to enhance the availability of Zn in the grain [84]. While GmIPK1 gene has been edited for the resistance of the accumulation of phytic acid in soybean seed that interferes with the bioavailability of Zn [85]. The expression of genes responsible for Zn enhancement in seed grain is regulated by the signalling components, transcription factors, and regulatory proteins which convey the message of Zn-deficiency and Zn–availabilities in the soil. The seed quality attributes are regulated by the plant growth-promoting regulators. Exogenous application of growth regulators enhances the seed quality attributes in peas enhancing the efficient signalling module for better nutrition uptake and transportation [86].

2.5 Nutri-Priming

In recent years, imbalanced and inappropriate macronutrient application, organic fertilizer constraints and manures, and reduced plant residue retention have resulted in micronutrient deficiencies in India's soil. Nano or microcapsules can be effective for bio-fortification of crops. These are small particles made of a shell material that contain active components like vitamins, minerals, bioactive chemicals, and even helpful microbes. These encapsulated components can be released gradually or in response to specified triggers, ensuring regulated delivery and tailored functionality. Utilizing nano or microcapsules presents a multifaceted and efficacious strategy for attaining food fortification, biofortification, and enhancements in food product packaging and coating. This presents an abundance of prospects for innovation and sustainability within the domains of agriculture and the food sector. According to [94], much of the research on nutri-priming has concentrated on cereals and leguminous grain crops, with the primary goals being to enhance germination, seed vigor, and growth under stress conditions, as well as to meet biofortification requirements. Biofortification aims to improve the nutritional quality of grains produced from nutrient-primed seeds, making them richer in essential nutrients. Additionally, seed nutri-priming has been suggested as an effective method to enhance the bioaccessibility and bioavailability of essential minerals, such as zinc and iron, particularly in soybean sprouts. Research by [95], highlighted that during the nutrient priming process, soaking seeds can lead to the leaching of antinutrient compounds, such as phytic acid. This reduction in phytic acid is noteworthy because phytic acid can inhibit the absorption of essential minerals, thus improving their bioavailability. However, [95, 86], also noted that there is a lack of research on the biofortification of vegetables via nutrient priming. This gap highlights an area for future investigation, as improving the nutritional quality of vegetables through nutri-priming could significantly contribute to addressing micronutrient deficiencies in human diets.

Table 3. Transporter genes and their role in Zinc transportation

Sl. No.	Target Gene	Role	References
01	Zinc-regulated transporter (ZRT)/ or ZRT and iron-regulated transporter (IRT)-related protein (ZIP)	Uptake from soil and translocation	[87]
02	Heavy metal ATPase transporter (HMA)	Involved in active transportation of Zn across membrane and vacuolar sequestration	[88]
03	Metal tolerance proteins (MTP) and Cation diffusion facilitator (CDF)	Sequestration of Zn into subcellular compartmentalization such as vacuole, endoplasmic reticulum and Golgi body	[89]
04	Vacuolar Iron Transporter (VIT)	Involved in uptake and transportation to seed grain	[90]
05	Natural Resistance-Associated Macrophage Protein (NRAMP)	Intracellular transportation and enhanced uptake by root	[91,92]
06	Yellow Stripe-Like proteins (YSLs) / Oligo peptide transporter (OPT)	Involved in zinc uptake and transportation to the grain	[93]

3. ZINC FORTIFIED CROPS

Bio-fortified crops are agricultural crops that have been conventionally bred or genetically modified to contain higher levels of essential nutrients. Bio-fortified crops are often developed to address specific nutrient deficiencies prevalent in certain populations. Bio-fortification can be applied to a wide range of staple crops, including rice, wheat, maize, cassava, beans, and sweet potatoes, among others. The choice of crops depends on their significance in local diets, agricultural suitability, and the feasibility of introducing and promoting bio-fortified varieties. For instance, Bio-fortified crops, such as orange-fleshed sweet potatoes or golden rice, which is a genetically engineered rice type that contains beta-carotene, have been produced to provide a source of provitamin A. This is because vitamin A insufficiency is a significant public health issue in numerous underdeveloped countries. The estimated daily intake of zinc by the consumption of zinc-biofortified rice was 2.9 g per person. This estimation was calculated based on a daily rice consumption of 220 g per person [96]. Wang *et al*. [97] reported an average Zn content of 3.34 mg/100 g, which varied between 0.79 to 5.89 mg/100 g in 57 rice genotypes. Based on a comprehensive analysis of multiple studies, it has been noticed that brown rice exhibited the greatest variability in zinc content, ranging from 6.2 to 71.6 mg/kg [98]. According to the study conducted by [1], percent distribution of zinc (Zn) in different parts of rice grain (hull, bran and starch endosperm together with embryo) are approximately 19.6, 23.8 and 56.6 % respectively. Akram *et al*. [99] noted in their experiments in Pakistan that small core hybrid rice exhibited a 1.08, 2.48 and 2.47-fold increase in grain yield, Zn concentration in grain, and Zn uptake after soil Zn application ($ZnSO_4$ @ 10 kg Zn/ha). Further, a 2.06-fold increase (4.27 mg/300 g/day) in average Zn bioavailability was observed during field trials by employing foliar applications of zinc oxide nanoparticles (ZnO NPs) at concentrations ranging from 20 to 60 mg/L. When compared to $ZnSO_4$ alone, the combination treatment with ZnO NP40 (40 ppm ZnO NPs) increased yield, NPK absorption, and grain Zn content [100]. High-zinc wheat cultivars development is crucial towards combating malnutrition under Indian situations. Due to its better agronomic features, high-zinc wheat is predicted to become more popular. Breeding programs has focused to increase the zinc content in wheat for which multilocation trials are underway in India. Zinc content was increased in their study [101] in the *Amaranthus cruentus* through soil application of biosynthesized nanoparticles Study on zinc spraying in wheat leaves increases grain zinc concentration more than soil treatment, and its usage early in seed development is beneficial as suggested by [102] and also claimed that repeating procedure can boost grain zinc concentration. In addition, [103] also investigated that biofortification of zinc in wheat increases grain and flour zinc (Zn) concentration by 50 and 76% respectively. Agronomic biofortification should increase grain zinc content from 35 to 45 mg kg^{-1} to have a significant influence on people's health [104]. A 10-year study on Zn fortification of maize in Zimbabwe found that using NPK fertilizers and local organic nutrient resources, such as woodland leaf litter and dung from cattle, may raise the grain Zn concentration by 19.3 mg/kg, which results in a 55% reduction in dietary Zn deficit. The Uttarakhand government released two early-maturing high zinc cowpea cultivars, Pant Lobia-1 and Pant Lobia-2, in 2008 and 2010, respectively. Quality of potato and productivity is significantly affected by zinc. Daily requirement per day zinc is up to 15 mg, as recommended by the World Health Organization. Hazra *et al*. [105] revealed that potato is a strong Zn accumulator compared to rice, which can accumulate up to 18.6 to 28.1 mg Zn kg^{-1} of dry matter. According to the results of the IZA-MOA joint study, applying $ZnSO_4$, H_2O @ 15 kg ha^{-1} to potatoes resulted in the greatest percentage of increase in yield (up to 25%) when compared to other fruit and vegetable crops. Biofortification enhances the micronutrient content of rice grains through the application of molecular and contemporary plant breeding techniques [106]. Recent studies have yielded evidence regarding the potential outcomes of rice biofortification, such as the identification of genetic resources, transgenic rice, and released varieties that exhibit high cereal zinc content in conjunction with increased grain yield [107]. In 2014, [108] effectively interbred IR 68144 rice to produce milled rice grain containing 1.54 times more zinc. IRGC 81848 and IRGC 81832, two distinct accessions of *Oryza nivara*, were discovered to have zinc contents that were two to three times greater than those of the recipient progenitor Swarna [109]. A number of studies identified about 29 zinc fortified varieties across the world. Among them 13 were reported so far from India viz. Chhattisgarh Zinc Rice 1, CR Dhan 315, Zinco Rice-MS, Chhattisgarh Zinc Rice-2 (RRHZ-LI-23) having 22-24, 24.9, 27.4 and 23 mg/kg Zn) by [98]; DRR Dhan 45, DRR Dhan 48, DRR

Dhan 49 and Surabhi (22.3, 20.9, 26.1 and 22.8 mg/kg) by [96]; CR Dhan 311 (Zn: 20.1mg/kg) by [112] and DRR Dhan 63, DRR Dhan 67 (BRRI Dhan 84) and DRR Dhan 69 (BRRI Dhan 100) (24.2, 27.6, 25.7 mg/kg (https://www.icar-iirr.org); GR-15: 21.6 mg/kg Zn) (https://nau.in/ nauvariety). Highest Zn concentration (18.46 mg kg^{-1} dry weight) was in potato tubers with application of recommended dose of fertilizer along with Zn (6.0 kg ha^{-1}) accounting for 28.0% greater than control [113-115] reported. Zn ferti-fortification experiments carried out across the world showed that Zn loading in potato (foliar as well as soil-applied Zn) elevated tuber Zn concentration 3-4 times (35 mg Zn kg^{-1} of dry matter), which is significantly higher than most commonly known crops [116-115]. Zn-fortified potato can be a promising approach to alleviate Zn-induced malnutrition among underdeveloped countries [116-118].

4. CONCLUSION

In conclusion, zinc (Zn) is a critical physiological metal necessary for the metabolism of most living organisms, playing vital biological roles in various physiological processes. Addressing zinc deficiencies through dietary technologies, particularly bio-fortification, represents a sustainable and effective strategy for combating malnutrition. By enhancing the nutrient content of staple crops, bio-fortification integrates nutrition directly into the food supply, making it more accessible to communities without relying on external interventions such as supplementation programs. The successful implementation of zinc bio-fortified crops hinges on several key factors, including consumer acceptance, market availability, and agronomic performance. To achieve widespread adoption, it is essential to promote awareness among consumers about the benefits of zinc bio-fortified foods, establish strong partnerships with farmers and stakeholders, and ensure that these crops remain affordable for all segments of the population.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The assistance and direction provided by each and every electronic database that has been subjected to peer review is greatly appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/124948