

Review Paper

Agronomic innovations for enhancement of zinc content in pulses: A review

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ABSTRACT

The rapid pace of climate change is adversely affecting crop yields, both quantitatively and qualitatively, with staple cereals requiring high inputs, posing ecological, economic, and nutritional risks. This shift contributes to a global concern known as 'hidden hunger', where micronutrient malnutrition has severe health repercussions, particularly in developing nations. The important nutrient deficiencies like zinc (Zn) in major food crops, posing a significant public health threat. In this context, pulse crops emerge as a promising solution within low-input farming systems, known for their suitability and nutritional richness. These crops can play a pivotal role in climate-resilient food systems. This review deals with the untapped potential of pulses as a crucial component in low-input food-production systems, emphasizing the need for zinc biofortification in pulses to sustain human nutrition.

Key words: Biofortification, Pulses, Zinc, Foliar application, Soil application

INTRODUCTION

Zinc (Zn) stands as an indispensable micronutrient for the human population, and its insufficiency poses a substantial challenge to public healthcare systems in developing nations, resulting in significant disease burden and mortality. A considerable segment of the population is affected by zinc deficiency (Hussain *et al.* 2022), giving rise to manifestations such as stunted growth (Sangeetha *et al.* 2022), aberrant or diminished infant birth weight, diminished energy levels, compromised immune functionality, heightened child mortality, and other deleterious consequences (Krebs *et al.* 2014). Zinc plays a pivotal role in catalyzing a multitude of enzymes, including Ribonucleic acid (RNA) polymerase and superoxide dismutase, thereby augmenting the efficacy of cellular proteins. It constitutes an integral component of nearly 3000 proteins, comprising 10% of the total protein content within the human body, and actively engages in neurobiological and physiological processes (Krężel and Maret 2016).

As per the World Health Organization (WHO), over 800,000 individuals succumb to zinc deficiency annually, with half of the mortality burden being borne by children under the age of 5. Additionally, 38.9% of adults from Africa to North America are identified as overweight or obese. Consequently, zinc deficiency has become a major reason for

sickness and death in many developing countries (Gupta *et al.* 2020).

The response to zinc deficiency in the body depends on factors like how much zinc is already in the body and the age of person. Nonetheless, a gamut of issues directly influenced by inadequate zinc intake encompasses diarrhea, dermatological maladies, heightened vulnerability to infectious pathogens, growth faltering in pediatric populations, unfavourable pregnancy outcomes, and ulcerative conditions (Prasad *et al.* 2014). Although the precise mechanisms underpinning zinc's actions in afflictions such as diarrhea and pregnancy-related complications remain incompletely elucidated, the supplementation of zinc is fervently recommended (Udechukwu *et al.* 2016). Scientists worldwide are also keen on investigating different aspects of how our bodies get zinc. They are working on various ways to improve how our bodies absorb and use zinc (Saha *et al.* 2017).

ZINC CONTENT IN SOILS

Zinc deficiency in soil has detrimental repercussions on crop yield and compromises the nutritional quality of agricultural produce. It is estimated that approximately half of the world's arable land is afflicted by zinc deficiency, with this deficiency being particularly widespread in major cereal-producing nations, including India. In India,

cereals are cultivated in soils deficient in zinc, exacerbating the issue in the human population. Soil sample analyses conducted in the northern plains of India have revealed that over 45% of soils exhibit zinc deficiency, affecting nearly half of India's total soil area (Narwal *et al.* 2010). Inadequate zinc content in soils results in decreased crop yields and, in severe cases, complete crop failure (De Valença *et al.* 2017). Furthermore, crops grown in zinc-deficient soils exhibit reduced nutritional value (Liu *et al.* 2017). Despite advancements in technologies such as mutation breeding and improved breeding approaches that have introduced various crop varieties capable of enhanced nutrient uptake, these have not effectively addressed the challenge of zinc fortification in soils with low zinc content (Ortiz-Monasterio *et al.* 2011).

The concentration of micronutrients in soils is influenced by various physicochemical soil properties, and arid and problematic soils contribute to zinc deficiency in different regions worldwide. The elevated incidence of zinc deficiency in Indian soils can be attributed to factors such as intensive cropping practices, limited availability of organic manure, excessive use of chemical fertilizers, and the widespread application of phosphorus-based fertilizers in agricultural fields. Moreover, there is a looming possibility of increased zinc deficiency in the future. Several reports suggest that zinc deficiency could escalate from the current rate of 50% to nearly 60% within the next 5-10 years, primarily due to the conversion of non-cropped areas into intensive cultivation zones.

POSSIBLE SOLUTION: ZINC BIOFORTIFICATION IN PULSES

The various options exist for fortifying the food we consume, encompassing industrial fortification, supplementation, and biofortification. Industrial fortification necessitates robust infrastructure, a consumer base with higher purchasing power, and easy access to fortified products. Supplementation demands initial high expenditures and better accessibility for urban populations as opposed to non-urban areas, along with the availability of zinc in forms conducive to efficient absorption by the human body. Genetic and agronomic biofortification represent pivotal avenues for biofortification.

Genetic biofortification entails the development of novel crop varieties endowed with genetic traits enabling them to accumulate higher zinc levels compared to conventional varieties.

However, this approach incurs substantial costs and necessitates significant infrastructure, which hampers its widespread implementation. The genetic approach provides a long-term strategy to combat micronutrient deficiencies by developing new crop varieties enriched with higher levels of zinc in their edible parts (Zou *et al.* 2012). Although the initial investment and development process can be costly and time-consuming, once a new variety is created, farmers do not have to incur any additional costs. The primary expenses are borne by breeding agencies and scientists involved in identifying suitable germplasm and conducting plant selection. This strategy encompasses several steps, including crossing, backcrossing, trait stabilization in different environments, and testing improved agronomic practices under diverse conditions (Cakmak 2008).

The concept of enriching food through breeding strategies was introduced by Welch and Graham (2004). They emphasized the importance of maintaining nutrient content and yield stability across different environments while developing new genotypes for enhanced nutritional value. It is crucial that end consumers can easily incorporate these new varieties into their traditional diets. In the context of staple food systems, particularly rice and wheat, genetic and agronomic biofortification approaches have been commonly employed (Cakmak and Kutman 2018).

During the Green Revolution era, the primary focus was on developing high-yielding varieties, with little attention given to enriching the nutritional content of the new genotypes (*i.e.*, biofortification) alongside increased production. As a result, there are currently very few, globally released varieties that have been biofortified with zinc for general cultivation. A conspicuous correlation has also been discerned between the heightened prevalence of zinc deficiency in populations that predominantly consume cereals in developing countries. Cereals are known to possess low nutrient content and high levels of phytate, thereby diminishing the accessibility of zinc to end-users (Sharma *et al.* 2013). Recent shifts in dietary patterns resulting from lifestyle changes, urbanization, and globalization have led to an increased consumption of nutrient-poor processed and ready-to-eat foods, characterized by elevated levels of sugar, fat, and salt, coupled with diminished protein, fiber, vitamin, and mineral content (Farzana *et al.* 2017). Although fortified foods have been developed to mitigate these concerns, their prohibitive cost renders them inaccessible to low-income populations.

Consequently, the most viable solution appears to be the promotion of naturally balanced diets, with pulses serving as vital components.

The incorporation of pulses into ready-to-eat/cook foods such as biscuits, noodles, soups, and snacks is imperative to augment their nutritional and phytochemical profiles. The consumption of pulses, while not independently adequate for ensuring comprehensive nutritional security, serves as an economical and readily available protein source, particularly catering to populations adhering to vegetarian diets or facing financial constraints that limit access to meat or other animal products. Traditionally, legumes have been synergistically consumed with cereals in numerous Asian and African nations. Notably, they offer a valuable supply of dietary fibre and the essential amino acid lysine, thereby complementing cereal-based diets often characterized by low lysine content (Leinonen *et al.* 2019). Furthermore, certain pulses, such as chickpeas and lentils, are recognized for containing β -carotene, lutein, and zeaxanthin, precursors to vitamin A which are directly related to the zinc content in plant (Margier *et al.* 2018).

A diverse array of methods is employed in the application of zinc fertilizers to crops to augment the plant's zinc content. Among the techniques within agronomic biofortification, foliar application of fertilizers is deemed the most cost-effective and convenient means of elevating nutrient levels in the reproductive parts of plants. Constraints on zinc concentration in plant cells are imposed by limitations in translocation from roots to shoots and diffusion, which constitute critical factors that must be addressed when seeking to enhance zinc levels. This review discusses various ways which can be used for improving the zinc content in pulses.

AGRONOMIC BIOFORTIFICATION METHODS/MECHANISMS

Zinc can be applied to crops through a variety of methods, which include soil application, foliar application, seed priming, or a combination of these strategies. Each of these methods operates through distinct mechanisms to enhance the zinc content in the reproductive structures of plants.

Soil application

The application of zinc to soil through fertilization is the primary and direct method for enhancing the nutritional content of plants. However, the effectiveness of applied fertilizers

is greatly influenced by the diverse physical and chemical properties of the soil. Zinc availability is relatively higher in acidic soils when compared to other soil types. The solubility of zinc decreases significantly with each unit increase in pH, resulting in a 100-fold reduction (Lindsay and Mortvedt, 2018). Conversely, alkaline soils also exhibit low zinc availability to crops. Calcareous soils may experience zinc deficiency due to the formation of insoluble calcium-zinc compounds (Prasad 2007). Soil properties such as low moisture content and organic matter also hinder the diffusion of zinc in the rhizosphere, thereby diminishing its availability to crops (Cakmak and Kutman 2018). Consequently, soils with arid and semi-arid conditions are more susceptible to limited zinc availability compared to well-irrigated conditions.

Zinc availability is also influenced by the presence of other nutrients (Erenoglu *et al.* 2011). It exhibits positive interactions with nitrogen (Kutman *et al.* 2010) but negative interactions with phosphorus (Prasad *et al.* 2016), iron, and copper (Prasad *et al.* 2014). These interactions can vary depending on the soil type and nutrient concentrations. Moreover, soil biological characteristics play a role in increasing or decreasing zinc content in plants. Plant growth-promoting rhizobacteria and arbuscular mycorrhiza have been found to enhance zinc availability in the soil (Vejan *et al.* 2016).

The optimal dosage of zinc fertilizer and the specific crop type determine the appropriate amount of zinc fertilizer to be applied. The application of zinc fertilizer is highly contingent on various factors, including the specific crop, soil composition, climate conditions, as well as the crop's age and type. Problematic soils, such as alkaline and calcareous soils, generally require higher application rates (Alloway 2008). In the Indian context, a recommended practice for mitigating zinc deficiency in soils involves the uniform application of 5 to 25 kg of $ZnSO_4 \cdot 7H_2O$ per hectare, regardless of soil type. Alternatively, if zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) is not available, an alternative recommendation is to apply approximately 5 kg of zinc per hectare annually or biennially as a soil application. For foliar application, a 0.5% spray solution of $ZnSO_4$ is generally advised, applicable to all soil types. The residual impact of zinc in specific soil types can persist for up to 10 years, contingent upon the particular crop type and the intensity of cultivation (Singh 2008). The data regarding soil application of zinc on zinc content in grain of pulses are presented

in Table 1. There is substantial improvement in zinc content in the grain of various pulse crops with the soil application of zinc.

Foliar application

Foliar application of nutrients offers several advantages over other methods. It requires a lower quantity of fertilizer compared to soil application. When zinc is applied through foliar tissues, it can readily penetrate the outer cell wall and enter the phloem tissue, facilitating efficient transfer to both vegetative and developing grain tissues (Cakmak and Kutman 2018). Plants require zinc in small quantities, it plays a crucial role in the formation of enzymes and proteins within plants. Regardless of the concentration and method of application, the introduction of zinc results in increased growth parameters. Additionally, the application of zinc, whether through soil or foliar methods, effectively increases the zinc content in plant shoots (Soni and Kushwaha 2020). While foliar sprays provide a solution for the plant, they do not address soil-related issues.

The choice of the application method also depends on the specific zinc fertilizer used. Foliar application of zinc sulphate as fertilizer has proven highly effective in enhancing nutrient content in various legumes such as lentil and chickpea. Additionally, it stands as the most cost-effective approach for augmenting crop nutrient levels. Applying nutrients during the later stages of crop growth is more likely to increase grain nutrient content compared to early-stage foliar application (Malesh *et al.* 2016). This may be attributed to the presence of more active photosynthates during the reproductive stage and the increased mobilization of these photosynthates towards the reproductive organs, which serve as the final sink for nutrients. The reproductive stage also exhibits higher phloem tissue mobility (Haslett *et al.* 2001), facilitating the re-translocation of zinc from vegetative tissues to reproductive organs. For example, in chickpea, foliar application of zinc at the pod formation stage resulted in the highest zinc accumulation in sink tissues (Pal *et al.* 2019a, Pal *et al.* 2021, Pal *et al.* 2023).

The efficacy of applied fertilizer is also dependent on the timing of application (Cakmak 2008). Foliar application during the evening hours of the day has been shown to exhibit the highest tissue permeability (Alshaal and El-Ramady 2017). The foliar application of zinc on lentil in the evening hours of the day at pre flowering and pod formation

stages resulted in the accumulation of higher zinc content in lentil grains (Dhaliwal *et al.* 2021).

The interaction of Zn with other plant nutrients can be synergistic, antagonistic and/or zero-interactive which clarify that the supply of a nutrient can affect the function of another nutrient that ultimately influences the various physiological processes in the plant (Rietra *et al.* 2017). The positive correlations of Zn with Fe in biofortification of edible parts in pulses depict the close linkage of the relevant loci on the same chromosome number which ultimately affects the various physiological mechanisms in the plant and escalated the sink strength for the nutrients (Diaz *et al.* 2022). However, the positive relationship between zinc and nitrogen illustrate the hypothesis that protein represent an important sink for zinc and iron in the grain tissue and all the nutrients have positive effect on the biofortification of pulses (Bhatt *et al.* 2020). Therefore, the application of micronutrients through foliar sprays has been found to have a beneficial impact on promoting growth and enhancing seed yield. The data regarding foliar application of zinc on zinc content in grain of pulses are presented in Table 2, which clearly shows tremendous potential of zinc enhancement with foliar application of zinc. The combined use of zinc and urea (nitrogen) further improves zinc content in the grain over the sole use of zinc (Kaur and Singh 2022).

Seed priming

Another way to enhance the nutritional content of pulses is by using zinc-enriched nutrient solutions on the seeds before planting. There are different types of zinc available, like inorganic salts, nano-particles, and chelated forms. Zinc sulphate ($ZnSO_4$) is a popular choice because it is cost-effective, dissolves easily, and is widely accessible (Veena and Puthur 2022). Chelated synthetic fertilizers, such as Zn-EDTA, are also commonly used. Zn-EDTA gradually releases in the soil, making it efficient for plant absorption, especially in alkaline conditions, and helps prevent the formation of insoluble complexes (Zhao *et al.* 2018). However, it's important to note that chelated fertilizers are usually more expensive than inorganic ones. The literature shows that plants absorb zinc differently depending on the type applied, but the results in the literature are not consistent. For example, Montanha *et al.* (2020) found that soybean plants absorbed more zinc from zinc sulphate than Zn-EDTA.

Another method for agronomic biofortification

is seed nutri-priming, a simple technique where seeds are soaked in a solution containing nutrients before planting. While most research on nutri-priming focuses on improving germination, seed vigor, and growth in cereals and leguminous grain crops (Farooq *et al.* 2019), it is also suggested for enhancing the availability of minerals like Zn in soybean (Zou *et al.* 2014). An interesting point is that during nutri-priming, soaking the seeds may decrease the concentration of compounds like phytic acid, which can be harmful, through leakage (Egli *et al.* 2002). Microgreens are a great option for nutrient biofortification through nutri-priming, as these are consumed at an early seedling stage, allowing for quick nutrient absorption from the seed to the greens (Di Gioia *et al.* 2021). Additionally, microgreens have high nutrient content and low harmful compounds like phytate.

Tillage

Conservation Agriculture (CA) plays a crucial role in regulating soil thermal dynamics, thereby sustaining optimal soil moisture levels. This, in turn, enhances water and nutrient-use efficiency, leading to improved crop productivity. Incorporating agronomic biofortification, CA practices offer a promising avenue for enhancing nutrient content in crops. The zero tilled cropping systems under CA not only contribute to nutrient efficiency but also positively impact soil physico-chemical and biological properties. In chickpea, the influence of tillage practices and system intensification on micronutrient (Zn) bio-fortification in chickpea grains and straw was found to be significant. Among the various tillage treatments, Conservation Agriculture with crop residues (Cac) and Conservation Agriculture with residues partially removed (Cap) demonstrated the highest micronutrient content in both chickpea grains and straw, followed by Conventional Tillage (Conv Till). The Zn content exhibited a notable increase of 1.56% and 10.1% in chickpea grains and 3.8% and 6.2% in straw during the cropping seasons of 2019–2020 and 2020–2021, respectively, compared to Conv Till (Bana *et al.* 2023).

The observed enhancement in micronutrient content under Cac is attributed to increased microbial activity and the synchronous release of nutrients during the decomposition process of crop residues in the soil organic matter. Minimum tillage or no-till has been widely adopted as a sustainable solution of conservation agriculture that could help to overcome problems of soil fertility

and micronutrient availability and improving the biofortification also. Combining pulses and cereals rotations with minimum tillage practices has been widely promoted as an efficient solution of integrated crop management to sustain agricultural cropping systems and address nutritional deficiencies (Conti *et al.* 2021). In lentil, under no-till, substantial genetic variation for zinc (9–48 mg kg⁻¹) was observed, indicating the possibility of lentil biofortification under conservation agriculture (Aboutayeb *et al.* 2023).

Biofertilizers

Environments within the rhizosphere that are enriched with nutrients play a pivotal role in stimulating various ecological processes, including the decomposition of organic matter, homeostasis, and nutrient cycling. Such enriched environments contribute significantly to reducing crop dependency on synthetic fertilizers, thereby fostering sustainable and stable ecosystems (Hakim *et al.* 2021). Plant growth-promoting bacteria (PGPBs) play an important role in improving the plant growth as well improving the nutritional quality (Khoshru *et al.* 2020a). Zinc solubilizing bacteria (ZSB), a subset of PGPBs, can contribute to these mechanisms and elevate Zn solubility by generating organic and inorganic acids (Khoshru *et al.* 2020b). Numerous bacterial species, including *Rhizobium*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Acinetobacter*, among others, demonstrate the ability to solubilize or tolerate zinc, thus functioning as agents of both Zn solubility enhancement and plant growth promotion (Mumtaz *et al.* 2017).

In a pot experiment, a study was carried out to evaluate the effect of two potential ZSB (BT3 and CT8) on growth promotion and Zn biofortification in chickpea. Through atomic absorption spectroscopy Zn content in the plants was determined. BT3 and CT8 strains of bacteria recorded 59 and 60 mg kg⁻¹ of zinc as compared to 56.5 mg kg⁻¹ in control plot (Kushwaha *et al.* 2021). In another field study, application of PGPB (*Enterobacter* sp. MN17) recorded higher zinc concentration in chickpea grain along with foliar application of zinc, followed by seed coating, osmopriming, hydropriming and soil application of zinc fertilizer (Ullah *et al.* 2019).

The application of Zn-solubilizing plant growth-promoting bacteria (PGPB) emerges as a strategic approach to enhance Zn availability for plants. Diverse PGPB strains have been documented to exert positive effects on plant growth through

Table 1. Effect of soil application of zinc on zinc content of different pulses

Crop	Treatment	Zinc content in grain (mg kg ⁻¹)	Reference
Chickpea	No application of zinc (Control)	38.7	Hidoto <i>et al.</i> (2017)
	Soil application of 25 kg ZnSO ₄ ·7H ₂ O ha ⁻¹	43.7	
Chickpea	Recommended dose of fertilizer (Control)	39.05	Nandan <i>et al.</i> (2018)
	Recommended dose of fertilizer + Seed treatment 1g Zn/kg + Soil application of ZnSO ₄ @ 25 kg/ha	43.62	
Chickpea	No seed priming	30.3	Ullah <i>et al.</i> (2019)
	Seed priming @ 0.001 M Zn	47.2	
Cowpea	Recommended dose of fertilizer (Control)	23.9	Manzeke <i>et al.</i> (2021)
	Recommended dose of fertilizer + Soil application of organic fertilizer	30.1	
Cowpea	Urea @ 25 ml to each pot at the concentration equivalent to 0.10 g pot ⁻¹ + No application of ZnSO ₄ ·7H ₂ O	47.59	Silva <i>et al.</i> (2021)
	Urea @ 25 ml to each pot at the concentration equivalent to 0.10 g pot ⁻¹ + Soil application of 25 mg kg ⁻¹ of Zn applied as ZnSO ₄ ·7H ₂ O ha ⁻¹ as Zn equivalent to 25 mg dm ⁻³	57.11	
Mungbean	No application of zinc (Control)	35.8	Dev <i>et al.</i> (2023)
	Soil application @ 6 kg Zn ha ⁻¹	39.6	
Mungbean	No application of zinc (Control)	45.60	Haider <i>et al.</i> (2021)
	Soil application of 10 kg ha ⁻¹	64	
Mungbean	No application of zinc (Control)	29	Hussain <i>et al.</i> (2021)
	Soil application of 10 kg Zn ha ⁻¹	50	

Table 2. Effect of foliar application of zinc on zinc content of different pulses

Crop	Treatment	Zinc content in grain (mg kg ⁻¹)	Reference
Chickpea	No application of zinc (Control)	37.73	Purushottam <i>et al.</i> (2018)
	Foliar application of 0.5% zinc sulphate at branching + pre flowering + pod development	48.11	
Chickpea	Recommended dose of fertilizer as basal application+ No application of zinc (Control)	26.12	Rathod <i>et al.</i> (2021)
	Recommended dose of fertilizer as basal application + Foliar application @ 0.5% Zn at flowering stage	29.98	
Chickpea	No application of zinc (Control)	42	Shivay <i>et al.</i> (2015)
	Foliar application of 0.5% Zn-EDTA application at vegetative, flowering and grain-filling stages	67.9	
Cowpea	No application of zinc (Control)	43.24	Açık and Sümer (2023)
	Foliar zinc application @ 60 kg ha ⁻¹ at flowering period	88.57	
Lentil	Recommended dose of fertilizer as basal application + No application of zinc (Control)	55.9	Dhaliwal <i>et al.</i> (2021)
	Recommended dose of fertilizer as basal application + Foliar spray of ZnSO ₄ ·7H ₂ O (0.5%) at Pre-flowering + Pod formation	63.0	
Lentil	No seed coating + No application of zinc (Control)	34.9	Karmakar <i>et al.</i> (2021)
	Seed coating @ 1.2% ZnSO ₄ ·7H ₂ O + two foliar spray @ 0.5% ZnSO ₄ ·7H ₂ O	42.1	
Lentil	Recommended dose of fertilizer as basal application + foliar spray of 0.5% ZnSO ₄ + 0.5% FeSO ₄ + 2% urea at Flowering stage + Pod formation stage	36.38	Kaur <i>et al.</i> (2024)
	Foliar spray of 0.5% ZnSO ₄ + 0.5% FeSO ₄ + 2% urea at Flowering stage + Pod formation stage	61.97	
Mungbean	Recommended dose of fertilizer as basal application+ No application of zinc (Control)	103.1	Dhaliwal <i>et al.</i> (2023)
	Recommended dose of fertilizer as basal application + Foliar spray of 0.5% ZnSO ₄ ·7H ₂ O at 40 days after sowing	127.1	

various mechanisms, including the enhancement of nutrient uptake, augmentation of biological nitrogen fixation, synthesis of enzymes, improvement in grain zinc concentration and the production of phytohormones and siderophores (Mitter *et al.* 2013). Microbial application can indirectly facilitate plant growth by elevating the uptake of Zn and other essential nutrients which can be achieved by carboxylation and the solubilization of nutrients via the release of organic acids, chelation, and acidification in the rhizosphere (Rehman *et al.* 2018).

LIMITATIONS AND BENEFITS OF AGRONOMIC BIOFORTIFICATION

One of the primary challenges in biofortification lies in the additional costs associated with zinc fertilization. The return on investment may not be optimal unless premium prices are obtained for the biofortified produce. However, studies have demonstrated that application of zinc through soil or foliar sprays can have a significantly positive impact on crop yield and quality, with only marginal increases in production costs compared to the yield benefits achieved. In-depth analysis has indicated that foliar application of zinc fertilizers represents a cost-efficient strategy for increasing grain zinc levels in cereals, as the cost is comparable to that achieved through zinc fortification in flour (Joy *et al.* 2016).

The primary cost associated with zinc fertilization is the application cost, but this can be mitigated by timing the application to coincide with pesticide treatments for the crops (Ram *et al.* 2016). There are no published reports suggesting compatibility issues between pesticides and zinc fertilization. Zinc can also be mixed with other nutrients in the same tank mixture to avoid additional costs associated with separate spraying treatments (Pal *et al.* 2023). Farmer awareness programmes should be conducted to educate them about the positive impacts of tank mix application of zinc with other nutrients and pesticides.

However, it is important to note that excessive amounts of zinc can also pose health hazards in humans, although zinc toxicity is rare and typically limited to areas affected by mining and smelting activities or contaminated water, industrial waste, and sewage sludge accumulation. Plant tissues exhibit signs of toxicity at levels above 300 micrograms per gram of dry weight (Marschner 2012). Nevertheless, zinc toxicity in plant tissues is generally less severe compared to copper or cadmium toxicity (Alloway 2008).

The inorganic phosphorus in grains transforms into phytic acid, which is considered an anti-nutritional factor, diminishing zinc bioavailability. When zinc is applied to soil, it has been observed to decrease the uptake and accumulation of phosphorus. The reduced uptake of phosphorus may lead to a decline in phytate content in grains (Cakmak 2008), contributing to the zinc enriched grains. Grains with low phytic acid content can improve zinc bioavailability, with the phytate-zinc molar ratio serving as an indicator. Seeds with low zinc concentration exhibit poor resilience to environmental stresses. Planting such seeds in zinc-deficient soil leads to lower crop establishment and seedling vigor (Donia and Carbone 2023). Maintaining adequate zinc levels through seed coating with zinc fertilization in seeds also acts as a defence against soil-borne pathogens (Cabot *et al.* 2019).

One significant health benefit of zinc fertilization is its competitive effect on cadmium, a highly toxic heavy metal that is not essential for human health (Cakmak 2009). Crops grown in cadmium-rich soils tend to have higher cadmium uptake and accumulation in grain (Harris and Taylor 2013). Zinc and cadmium share similar chemical properties and follow the same transport mechanisms. Transporters involved in zinc and iron regulation, such as zinc-regulated transporter (ZRT), iron-regulated transporter (IRT), and heavy metal transporters, also facilitate cadmium transport from roots to the upper parts of plants (Cun *et al.* 2014). Genetic modifications that affect the genes responsible for heavy metal transport, including cadmium uptake, also impact zinc accumulation in grain. Agronomic interventions with zinc can help reduce cadmium uptake, root-to-shoot translocation, re-translocation, and deposition in grain tissues (Jiao *et al.* 2004).

Application of zinc not only improves zinc content in the grain but also increases the productivity as well as profitability of pulses (Pal *et al.* 2019b, Pal *et al.* 2020, Kaur *et al.* 2024). Therefore, agronomic biofortification is a win-win situation for the growers as well as the consumers.

CONCLUSION

Addressing future agricultural challenges is crucial to ensure global access to affordable and nutritious food amidst growing population. Zinc deficiency poses a significant health threat in present scenario. This review identified pulse

crops as a promising strategy to mitigate Zn deficiencies, emphasizing their potential for nutrient accumulation. Despite being overlooked in many economies, pulses warrant increased research attention for combating micronutrient malnutrition. Their higher capacity for nutrient accumulation and adaptability to low-input systems makes them a cost-effective and environmentally friendly option for resource-poor farmers. For future purposes, there is need to overlook the multiple factors affecting the nutritional quality of pulses in different climatic conditions. This will help us understand how climate affects the level of zinc in pulses

REFERENCES

- Aboutayeb R, Baidani A, Zeroual A, Benbrahim N, Aissaoui AE, Ouhemi H, Houasli C, Mazzucotelli E, Gadaleta A and Idrissi O. 2023. Genetic variability for iron, zinc, and protein content in a Mediterranean lentil collection grown under no-till conditions: towards biofortification under conservation agriculture. *Sustainability* **15**(6): 5200. DOI:10.3390/SU15065200
- Acik A and Sümer OF. 2023. Foliar application of zinc improves agronomical and quality parameters and biofortification of cowpea (*Vigna sinensis*) under deficit irrigation. *Agronomy* **13**(4): 1021.
- Alloway BJ. 2008. Zinc in Soils and Crop Nutrition, 2nd edn. International Zinc Association and International Fertilizer Industry Association, Brussels.
- Alshaal T and El-Ramady H. 2017. Foliar application: from plant nutrition to biofortification. *Environment Biodiversity and Soil Security* **1**: 71-83.
- Bana RS, Faiz MA, Sangwan S. 2023. Triple-zero tillage and system intensification lead to enhanced productivity, micronutrient biofortification and moisture-stress tolerance ability in chickpea in a pearl millet-chickpea cropping system of semi-arid climate. *Scientific Reports* **13**(1): 10226. DOI: 10.1038/S41598-023-36044
- Bhatt R, Hossain A and Sharma P. 2020. Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: a review. *Open Agriculture* **5**: 176-187. DOI: 10.1515/opag-2020-0018
- Cabot C, Martos S, Llugany M, Gallego B, Tolrà R and Poschenrieder C. 2019. A role for zinc in plant defense against pathogens and herbivores. *Frontiers in Plant Science* **10** DOI:10.3389/FPLS.2019.011171
- Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant Soil* **302**: 1-7. DOI: 10.1007/s11104-007-9466-3
- Cakmak I. 2009. Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. *Journal of Trace Elements in Medicine and Biology* **23**(4): 281-289.
- Cakmak I and Kutman UB. 2018. Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science* **69**: 172-180.
- Conti MV, Guzzetti L, Panzeri D, De Giuseppe R, Cocchetti P, Labra M and Cena H. 2021. Bioactive compounds in legumes: implications for sustainable nutrition and health in the elderly population. *Trends in Food Science & Technology* **117**: 139-147.
- Cun P, Sarrobert C, Richaud P, Chevalier A, Soreau P and Auroy P. 2014. Modulation of Zn/Cd P_{1B2} -ATPase activities in Arabidopsis impacts differently on Zn and Cd contents in shoots and seeds. *Metallomics* **6**: 2109-2116.
- De Valença A, Bake A, Brouwer I and Giller K. 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global Food Security* **12**: 8-14.
- Dev P, Singh U, Singh LN, Shivay YS, Kumar M and Raiger PR. 2023. Zinc biofortification of mungbean (*Vigna radiata* L.) as influenced by varieties and zinc fertilization. *Journal of Environmental and Agricultural Sciences* **25** (1&2): 1-17.
- Dhaliwal SS, Sharma V, Shukla AK, Kaur J, Verma V, Singh P, Singh H, Abdel-Hafez SH, Sayed S, Gaber A, Ali R and Hossain A. 2021. Enrichment of zinc and iron micronutrients in lentil (*Lens culinaris* Medik.) through biofortification. *Molecules* **26**(24): 7671.
- Dhaliwal SS, Sharma V, Shukla AK, Kaur J, Verma V, Singh P, Singh H, Abdel-Hafez SH, Sayed S, Gaber A, Ali R and Hossain A. 2023. Biofortification of mungbean (*Vigna radiata* L. (Wilczek)) with boron, zinc and iron alters its grain yield and nutrition. *Scientific Reports* **13**(1): 3506.
- Di Gioia F, Petropoulos SA, Ferreira ICFR and Roskopf EN. 2021. Microgreens: from trendy vegetables to functional food and potential nutrition security resource. *Acta Horticulturae* **1321**: 235-242.
- Diaz S, Polania J, Ariza-Suarez D, Cajiao C, Grajales M, Raatz B and Beebe SE. 2022. Genetic correlation between Fe and Zn biofortification and yield components in a common bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science* **12**. DOI: 10.3389/fpls.2021.739033
- Donia DT and Carbone M. 2023. Seed priming with zinc oxide nanoparticles to enhance crop tolerance to environmental stresses. *International Journal of Molecular Sciences* **24** (24): 17612. DOI:10.3390/ijms242417612
- Egli I, Davidsson L, Juillerat M, Barclay D and Hurrell R. 2002. The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *Journal of Food Science* **67**: 3484-3488.
- Erenoglu EB, Kutman UB, Ceylan Y, Yildiz B and Cakmak I. 2011. Improved nitrogen nutrition enhances root uptake, root-to-shoot translocation and remobilization

- of zinc (^{65}Zn) in wheat. *New Phytologist* **189**(2): 438-448.
- Farooq M, Usman M, Nadeem F, Rehman H, Wahid A, Basra SMA and Siddique KHM. 2019. Seed priming in field crops: potential benefits, adoption and challenges. *Crop and Pasture Science* **70**(9): 731-771.
- Farzana T, Mohajan S, Saha T, Hossain M and Haque M. 2017. Formulation and nutritional evaluation of a healthy vegetable soup powder supplemented with soy flour, mushroom, and moringa leaf. *Food Science and Nutrition* **5**(4): 911-920.
- Gupta S, Brazier AKM and Lowe NM. 2020. Zinc deficiency in low- and middle-income countries: prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics* **33**(5): 624-643.
- Haider MU, Hussain M, Farooq M, Ul-Allah S, Ansari MJ, Alwahibi MS and Farooq S. 2021. Zinc biofortification potential of diverse mungbean [*Vigna radiata* (L.) Wilczek] genotypes under field conditions. *PLoS One* **17**(11): e027759. DOI: 10.1371/journal.pone.0253085.
- Hakim S, Naqqash T, Nawaz MS, Laraib I, Siddique MJ, Zia R, Mirza MS and Imran A. 2021. Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems* **5**: 617157.
- Harris NS and Taylor GJ. 2013. Cadmium uptake and partitioning in durum wheat during grain filling. *BMC Plant Biology* **13**: 103-119.
- Haslett BS, Reid RJ and Rengel Z. 2001. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. *Annals of Botany* **87** (3): 379-386.
- Hidoto L, Taran B, Worku W and Mohammed H. 2017. Towards zinc biofortification in chickpea: performance of chickpea cultivars in response to soil zinc application. *Agronomy* **7**: 11.
- Hussain A, Jiang W, Wang X, Shahid S, Saba N, Ahmad M, Dar A, Masood SU, Imran M and Mustafa A. 2022. Mechanistic impact of zinc deficiency in human development. *Frontiers in Nutrition* **9**: 717064.
- Hussain M, Shahid MZ, Mehboob N, Minhas WA and Akram M. 2021. Zinc application improves growth, yield and grain zinc concentration of mung bean (*Vigna radiata* L.). *Semina Ciencias Agrarias* **42**(2): 487-500.
- Jiao Y, Grant CA and Bailey LD. 2004. Effects of phosphorus and zinc fertilizer on cadmium uptake and distribution in flax and durum wheat. *Journal of the Science of Food and Agriculture* **84**: 777-785.
- Joy EJM, Ahmad W, Zia MH, KumssaDB, Young SD and Ander EL. 2016. Valuing increased zinc (Zn) fertilizer-use in Pakistan. *Plant and Soil* **411**: 139-150.
- Karmakar M, Sarkar NC and Shivay YS. 2021. Agronomic biofortification of zinc in lentil. *International Journal of Bio-resource and Stress Management* **12**(2): 95-107.
- Kaur A and Singh G. 2022. Zinc and iron application in conjunction with nitrogen for agronomic biofortification of field crops – a review. *Crop & Pasture Science* **73** (7-8): 769-780.
- Kaur A, Singh G, Singh K and Dhaliwal SS. 2024. Foliar application of combined zinc sulphate, ferrous sulphate and urea has synergistic effect on grain yield enhancement and biofortification in lentil (*Lens culinaris* Medik.). *Journal of Soil Science and Plant Nutrition*. DOI:10.1007/s42729-024-01612-4
- Khoshru B, Mitra D, Khoshmanzar E, Myo EM, Uniyal N, Mahakur B, Mohapatra PK, Panneerselvam P, Boutaj H and Alizadeh M. 2020a. Current scenario and future prospects of plant growth-promoting rhizobacteria: an economic valuable resource for the agriculture revival under stressful conditions. *Journal of Plant Nutrition* **43**(20): 3062-3092.
- Khoshru B, Mitra D, Mahakur B, Sarikhani MR, Mondal R, Verma D and Pant K. 2020b. Role of soil rhizobacteria in utilization of an indispensable micronutrient zinc for plant growth promotion. *Journal of Critical Reviews* **7**(12): 4644-4654.
- Krebs NF, Miller LV and Hambridge KM. 2014. Zinc deficiency in infants and children: a review of its complex and synergistic interactions. *Paediatrics and Child Health* **34**(4): 279-288.
- Krezel A and Maret W. 2016. The biological inorganic chemistry of zinc ions. *Archives of Biochemistry and Biophysics* **6**(11): 3-19.
- Kushwaha P, Srivastava R, Pandiyan K, Singh A, Chakdar H, Kashyap PL, Bhardwaj AK, Murugan K, Karthikeyan N and Bagul SY. 2021. Enhancement in plant growth and zinc biofortification of chickpea (*Cicer arietinum* L.) by *Bacillus altitudinis*. *Journal of Soil Science and Plant Nutrition* **21**: 922-935.
- Kutman UB, Yildiz B, Ozturk L and Cakmak I. 2010. Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chemistry* **87**: 1-9.
- Leinonen I, Iannetta PPM, Rees RM, Russell W, Watson C and Barnes AP. 2019. Lysine supply is a critical factor in achieving sustainable global protein economy. *Frontiers in Sustainable Food Systems* **3**(27): 1-11.
- Lindsay WL and Mortvedt JJ. 2018. Inorganic equilibria affecting micronutrients in soils. *Micronutrients in Agriculture* **4**: 89-112.
- Liu J, Yang M, Li H, Li D, Shi X and Zhang Y. 2017. Genetic processes of iron and zinc accumulation in edible portion of crops and their agro-biofortification: a review. *American Journal of Agriculture and Forestry* **5**(3): 65-72.
- Malesh AA, Mengistu DK and Aberra DA. 2016. Linking agriculture with health through genetic and agronomic biofortification. *Agricultural Sciences* **7**(5): 295-307.
- Manzeke KMG, Mtambanengwe F, Watts MJ,

- Broadley MR, Lark RM and Mapfumo P. 2021. Can nitrogen fertilizer management improve grain iron concentration of agro-biofortified crops in Zimbabwe? *Agronomy* **11**(1): 124.
- Margier M, Georgé S, Hafnaoui N, Remond D, Nowicki M, Du Chaffaut L, Amiot MJ and Reboul E. 2018. Nutritional composition and bioactive content of legumes: characterization of pulses frequently consumed in France and effect of the cooking method. *Nutrients* **10**(11): 1668.
- Marschner P. 2012. Marschner's mineral nutrition of higher plants, 3rdedn. academic press, Elsevier, San Diego, CA.
- Mitter B, Brader G, Afzal M, Company S, Naveed M, Trognitz F and Sessitsch A. 2013. Advances in elucidating beneficial interactions between plants, soil and bacteria. *Advances in Agronomy* **121**: 381-445.
- Montanha GS, Rodrigues ES, Romeu SLZ, Almeida E, Reis AR and Lavres J. 2020. Zinc uptake from ZnSO₄ (aq) and Zn-EDTA (aq) and its root-to-shoot transport in soybean plants (*Glycine max*) probed by time-resolved in vivo X-ray spectroscopy. *Plant Science* **292**: 110370.
- Mumtaz MZ, Ahmad M, Jamil M and Hussain T. 2017. Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. *Microbiological Research* **202**: 51-60.
- Nandan B, Sharma BC, Chand G, Bazgalia K, Kumar R and Banotra M. 2018. Agronomic fortification of Zn and Fe in chickpea an emerging tool for nutritional security—a global perspective. *Acta Scientific Nutritional Health* **2**: 12-19.
- Narwal RP, Malik RS and Dahiya RR. 2010. Addressing variations in a few nutritionally important micro-nutrients in wheat crop. *Proceedings of 19th World Congress of Soil Science*. Published on DVD.1-6 A Brisbane, Australia.
- Ortiz-Monasterio I, Trethowan R, Holm PB, Cakmak I, Borg S, Erenoglu B, Tauris B and Brinch-Pedersen H. 2011. Breeding, transformation, and physiological strategies for the development of wheat with high zinc and iron grain concentration. In: Bonjean AP, Angus WJ, Van Ginkel M, editors. *The world wheat book, a history of wheat breeding*. Aarhus, Kolding, Copenhagen: Lavoisier. pp. 951-977.
- Pal V, Singh G and Dhaliwal SS. 2019a. Agronomic biofortification of chickpea with zinc and iron through application of zinc and urea. *Communications in Soil Science and Plant Analysis* **50**: 1864-1877.
- Pal V, Singh G and Dhaliwal SS. 2019b. Yield enhancement and biofortification of chickpea (*Cicer arietinum* L.) grain with iron and zinc through foliar application of ferrous sulfate and urea. *Journal of Plant Nutrition* **42**(15): 1789-1802.
- Pal V, Singh G and Dhaliwal SS. 2020. Symbiotic parameters, growth, productivity and profitability of chickpea as influenced by zinc sulphate and urea application. *Journal of Soil Science and Plant Nutrition* **20**: 738-750.
- Pal V, Singh G and Dhaliwal SS. 2021. A new approach in agronomic biofortification for improving zinc and iron content in chickpea (*Cicer arietinum* L.) grain with simultaneous foliar application of zinc sulphate, ferrous sulphate and urea. *Journal of Soil Science and Plant Nutrition* **21**: 883-896.
- Pal V, Singh G and Dhaliwal SS. 2023. Effects of sequential and tank mix applications of zinc, iron and nitrogen on symbiotic parameters, productivity and economics of chickpea (*Cicer arietinum* L.) under field conditions. *Journal of Soil Science and Plant Nutrition* **23**: 2673-2686.
- Prasad R, Shivay YS and Kumar D. 2014. Agronomic biofortification of cereal grains with iron and zinc. *Advances in Agronomy* **125**: 55-91.
- Prasad R, Shivay YS and Kumar D. 2016. Interactions of zinc with other nutrients in soils and plants - A Review. *Indian Journal of Fertilizers* **12**: 16-26.
- Prasad R. 2007. *Crop nutrition-principles and practices*. New Vishal Publications: Delhi, India, pp. 272.
- Purushottam B, Puhup K, Kumar C and Sodi B. 2018. Effect of irrigation scheduling and zinc application on chlorophyll content, zinc content, uptake and yield of chickpea (*Cicer arietinum* L.). *Journal of Pharmacognosy and Phytochemistry* **7**: 1834-1837.
- Ram H, Rashid A, Zhang W, Duarte AP, Phattarakul N and Simunji S. 2016. Biofortification of wheat, rice and common bean by applying foliar zinc fertilizer along with pesticides in seven countries. *Plant and Soil* **403**: 389-401.
- Rathod PS, Patil DH, Bellad SB and Haveri RV. 2021. Biofortification of Zn and Fe in chickpea through agronomic intervention in medium black soils of Karnataka. *Legume Research* **10**: 18805.
- Rehman A, Farooq M, Naveed M, Nawaz A and Shahzad B. 2018. Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat. *European Journal of Agronomy* **94**: 98-107.
- Rietra RP, Heinen M, Dimkpa CO and Bindraban PS. 2017. Effects of nutrient antagonism and synergism on yield and fertilizer use efficiency. *Communications in Soil Science and Plant Analysis* **48**: 1895-1920. DOI: 10.1080/00103624.2017.1407429
- Saha S, Chakraborty M, Padhan D, Saha B, Murmu S, Batabyal K, Seth A, Hazra GC, Mandal B and Bell RW. 2017. Agronomic biofortification of zinc in rice: influence of cultivars and zinc application methods on grain yield and zinc bioavailability. *Field Crops Research* **210**: 52-60.
- Sangeetha VJ, Dutta S, Moses JA and Anandharamkrishnan C. 2022. Zinc nutrition and human health: overview

- and implications. eFood doi:10.1002/efd2.17.
- Sharma A, Patni B, Shankhdhar D and Shankhdhar SC. 2013. Zinc-An indispensable micronutrient. *Physiology and Molecular Biology of Plants* **19**: 11-20.
- Shivay YS, Rajendra P and Madan P. 2015. Effects of source and method of zinc application on yield, zinc biofortification of grain, and Zn uptake and use efficiency in chickpea (*Cicer arietinum* L.). *Communications in Soil Science and Plant Analysis* **46**: 2191-2200.
- Silva VM, Nardeli AJ, Mendes NAC, Rocha MM, Wilson L, Young SD, Martin RB, Philip JW and Reis AR. 2021. Agronomic biofortification of cowpea with zinc: variation in primary metabolism responses and grain nutritional quality among 29 diverse genotypes. *International Journal of Plant Physiology and Biochemistry* **162**: 378-387.
- Singh MV. 2008. Micronutrient deficiencies in soils and crops of India. In: Alloway BJ (ed) *Micronutrient deficiencies in global crop production*. Springer Sciences Business Media, Dordrecht, pp 93-125.
- Soni J and Kushwaha HS. 2020. Effect of foliar spray of zinc and iron on productivity of mungbean [*Vigna radiata* (L.) Wilczek]. *Journal of Pharmacognosy and Phytochemistry* **9**(1): 108-111.
- Udechukwu MC, Collins SA and Udenigwe CC. 2016. Prospects of enhancing dietary zinc bioavailability with food-derived zinc chelating peptides. *Food Function Journal* **7**: 4137-4144.
- Ullah A, Farooq M, Hussain M, Ahmad R and Wakeel A. 2019. Zinc seed priming improves stand establishment, tissue zinc concentration and early seedling growth of chickpea. *Journal of Animal and Plant Science* **29**: 1046-1053.
- Veena M and Puthur JT. 2022. Seed nutri-priming with zinc is an apt tool to alleviate malnutrition. *Environmental Geochemistry and Health* **44**: 2355-2373.
- Vejan P, Abdullah R, Khadiran T, Ismail S and Boyce AN. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-A review. *Molecules* **21**: 573.
- Welch RM and Graham RD. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* **55**: 353-364.
- Zhao A, Yang S, Wang B, Tian X and Zhang Y. 2018. Effects of ZnSO₄ and Zn-EDTA broadcast or banded soil on Zn bioavailability in wheat (*Triticum aestivum* L.) and Zn fractions in soil. *Chemosphere* **205**: 350-360.
- Zou CQ, Zhang YQ, Rashid A, Ram H, Savasli E, Arisoy RZ, Ortiz-Monasterio I, Simunji S, Wang ZH and Sohu VS. 2012. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant and Soil* **361**: 119-130.
- Zou T, Xu N, Hu G, Pang J and Xu H. 2014. Biofortification of soybean sprouts with zinc and bio-accessibility of zinc in the sprouts. *Journal of the Science of Food Agriculture* **94**: 3053-3060.