Review Article

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Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: a review

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Abstract: Paddy-wheat crop rotation is responsible for declining soil health, underground water table, arising new micronutrient deficiencies, new weed flora, and resistance to herbicides, declining both land and water productivity and is claimed to be capital and energy-intensive, more particularly in emerging countries. This is further aggravated when micronutrients are deficient, particularly zinc (Zn), which plays an important role in human health, especially in developing countries. Zn biofortification is a technique in which the inherent Zn status of the edible portion of plants is improved by simply spraying a Zn solution onto the crop or through a soil application at a predetermined stage and a proper dose. The concentration of Zn within a wheat grain is genotype-dependent and interacts with the environment, inducing variation in a grain's concentration of micronutrients. Grain quality parameters are positively correlated with a higher dose of nitrogen in the late reproductive stage. Broadcasting of $ZnSO_4{\cdot}7H_2O$ at 62.5 kg ha^{-1} and foliar application of Zn chelates such as Zn–HEDP (C) at 2 g L^{-1}, Zn–HEDP (L) at 3 g L^{-1}, or a 0.4–0.5% $ZnSO_4$ solution during grain development stage enhanced the growth, productivity, and micronutrients concentration in the edible portion of the plant which further improves the quality of wheat grains and ultimately improves human health in the region. Given the central importance to Zn in cereal-based nutrition, zinc biofortification appears as an innovative technology to alleviate the zinc deficiency in human health, especially on the Indian subcontinent, by applying Zn either as a foliar or soil application.

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1 Introduction

The rising micronutrient deficiencies more particularly of zinc (Zn) is claimed to be the major reason for the declining land and water productivity of both rice and wheat yield in South Asia (Kataki et al. 2001a; Nayyar et al. 2001; Bhatt et al. 2016; Das et al. 2019, 2020; Hossain et al. 2019). The intensive use of minerals by crops have caused rapid depletion of micronutrient reserves, including zinc (Zn), from the soil causing deficiencies of micronutrients (Manojlović et al. 2019). Human dependence upon cereals with a poor Zn status, especially in developing countries, deepens the gap between the available amount, and the amount required for good health, which is 40–50 ppm (Cakmak 2010). In India, Zn availability in wheat cultivars varies from 20 to 30 ppm (Shukla et al. 2014). Second, the inherent Zn capacity of the soils decides Zn in wheat grains (Cakmak and Kutman 2018; Figure 1). Hence, if Zn-deficient soils are used for cultivating cereals, then their availability in the grain is decreased to many folds. Therefore, it is essential to sustain a satisfactory level of Zn and water in the soil during the reproductive stage of wheat to improve the Zn status in wheat grains (Cakmak and Kutman 2018). On the other hand, Zn is generally found in excess in the aleurone and embryo (100 ppm of Zn) of a wheat grain whereas white flour, which is derived from the endosperm, contains about 5–10 ppm. When wheat flour is milled, Zn-rich parts (i.e., the aleurone and embryo) are mostly removed and only the endosperm (Zn-poor; about 5–10 mg Zn kg^{-1}) remains, making wheat flour Zn-poor (Ozturk et al. 2006; Cakmak and Kutman 2018; Figure 2).

In addition to these factors, the "dilution effect" is responsible for decreased Zn content in the edible portion of cereals with significantly increased

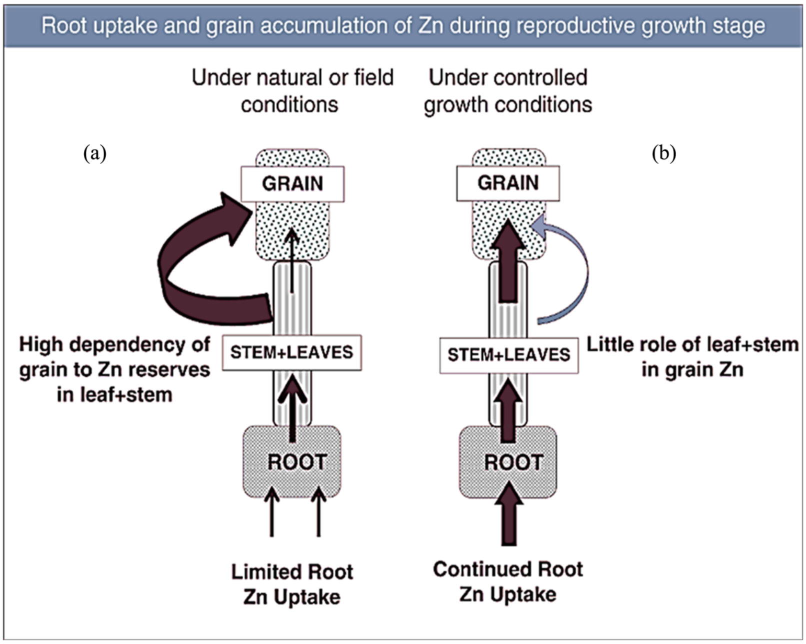

Figure 1: Zn uptake and re-translocation from root to vegetative organs then to grains of wheat: (a) plants under limited Zn and available water or both during seed-filling; (b) plants under adequate water and Zn supply. (Adapted from Cakmak and Kutman 2018.)

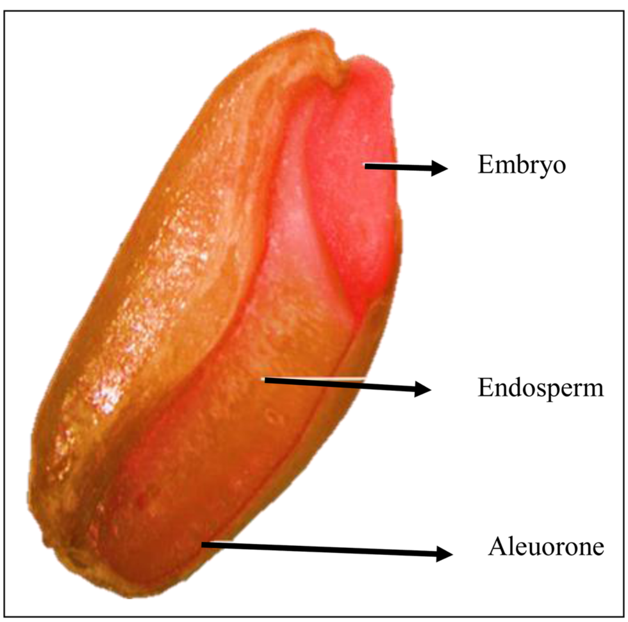

Figure 2: Localization of Zn in wheat grain: the intensity of the red color is linked with dithizone as a Zn-sensing dye that develops a red complex. (Adapted from Ozturk et al. 2006.)

production (Shewry et al. 2016). Further, when soil pH exceeds 7.8, Zn particles adhere to clay sites more strongly and hence making it difficult to fill soil solution, causing Zn deficiency (Dotaniya and Meena 2015; Goulding 2016). Diffusion is mainly responsible for the inward movement of the Zn particles in roots, which is severely restricted in the soils equipped with lower organic matter and lower moisture regimes (Rengel 2015).

More than three billion people worldwide are affected by zinc deficiency (Cakmak et al. 2010). Human health complications such as stunting, infections, impaired brain function, poor mental development, weakness in babies and anemia are because of zinc deficiency (Fraga 2005; Cakmak et al. 2010). Further around 200 enzymes responsible for growth, development, immune function, and resistance to infections are regulated by zinc (Fischer and Black 2004). For fulfilling the daily calorie intake, wheat is an important cereal crop and enhancing its nutritional quality will certainly improve the consumer's health (Cakmak 2008).

Institute of Medicine, Food and Nutrition Board (IMFN) reviews and finalizes the daily limits of Zn and other micronutrients that must be consumed or dietary reference intake (DRI) for a healthy life (Table 1; IMFN 2001) which changes as per variation in age and gender (Table 1; IMFN 2001). However, in poor or developing countries, hunger can arise from the lack of vitamins and/or mineral elements (Müller and Krawinkel 2005). One way to address the latter is by frequently eating fish and animal products, although poverty and religious backgrounds would be a financial limiting factor. Further, Zn-deficient rhizosphere complicates the situations as products are Zn deficient too (Welch and Graham 2005). In such a case, biofortification could be

Table 1: Recommended dietary allowances (RDAs) for zinc. Source: (IMFN 2001)

Age	Male (mg)	Female (mg)	Pregnancy (mg)	Lactation (mg)
0–6 months	2*	2*		
7–12 months	3	3		
1–3 years	3	3		
4–8 years	5	5		
9–13 years	8	8		
14–18 years	11	9	12	13
19+ years	11	8	11	12

*Adequate intake (AI).

a suitable solution, although strategies to increase mineral intake through a diet depend upon many factors and, therefore, might not be successful. Crop biofortification is therefore recommended for directly satisfying the plants' needs to produce the healthy edible portion. The wheat crop is estimated to remove 66–209 g of Zn for every 2 tons of wheat grains. Micronutrient analysis of soil samples across Indo-Gangetic plains revealed that 45.4% of soil was deficient in Zn (Singh and Yadav 2006), as much as 48% of soil in India is deficient in Zn (Narwal et al. 2010), while in the Punjab, India, 22% of soil is deficient in Zn (Benbi et al. 2011). Zn insufficiency led to increased hunger as a direct result of lower yield, crop failure, and poor accumulation of Zn into edible plant parts (i.e., grains) and thus poor-human nutrition (De Valença et al. 2017; Liu et al. 2017). Despite the latest breeding advances in improving nutrient uptake efficiency to cereal grains, these newly bred varieties are unable to fortify the Zn content of cereals in zinc-deficient soils (Ortiz-Monasterio et al. 2011). The higher concentration of minerals may be restricted as their supply is governed by different physicochemical properties of soils, which if adversely effected then certainly restrict the micronutrient supply (Frossard et al. 2000). Problematic soils and arid and semi-arid environments mainly magnify zinc deficiency. Zinc deficiency significantly appeared in soils of India (50%), Turkey (50%), China (0.33%), and Western Australia (Ismail et al. 2007), since diffusion and the rate of translocation of Zn are the major limiting factors for the lesser Zn availability in soils for absorption by plants. Therefore, spraying zinc solution or biofortification is a suitable and reliable answer to improve zinc status and, hence, the quality of the produced grains. Zinc is consumed across the plasma, which covers root cells as Zn^{2+} (Ismail et al. 2007) while zinc is also permeable to plasma membrane Ca^{2+} channels (White et al. 2002a). As the cell proteins are generally bonded by available Zn^{2+}, which ultimately reflects on the cytoplasmic Zn^{2+} concentrations (Broadley et al. 2007). In such cases, Zn has to be applied from the outside, either as a foliar spray or soil application, to attempt to enhance Zn availability in grains. Given the central importance of Zn in cereal-based nutrition, especially on the Indian subcontinent, this review aims to assess Zn biofortification as a way to alleviate Zn deficiency in human health.

2 Physiological basis of agronomic biofortification

Agronomic biofortification allows for mineral density to be increased in grains or fruits through fertilization strategies at responsive growth stages of crop plants (Welch 2005; Farhad et al. 2018; Das et al. 2019, 2020; Hossain et al. 2019). Mineral supply to a developing cereal grain takes place either by direct uptake from the soil or by remobilization of stored minerals in leaves. Nutrient density per unit of grain dry weight is more important for estimating grains' quality (Marles 2017). At critical growth stages of a crop, proper supply of micronutrients improves not only the quality of grains but also the health status of ultimate consumers – the human (Marschner 1995). However, oversupply or limiting micronutrients can have negative consequences. Mineral enrichment occurs when a nutrient exceeds the level of sufficiency within a crop plant (Figure 3). Through leaves, plants have the capability to absorb the different nutrients; therefore, foliar spray of micronutrients would theoretically imply that an applied nutrient will be absorbed from leaves to the point of utilization, *viz.*, growing tissue (Khoshgoftarmanesh et al. 2010). Nutrients are exported from leaves and transported within the stem via phloem or xylem (Rengel et al. 1999). The biofortification of micronutrients at specific and critical growth stages of wheat may contribute toward grain mineral enrichment and

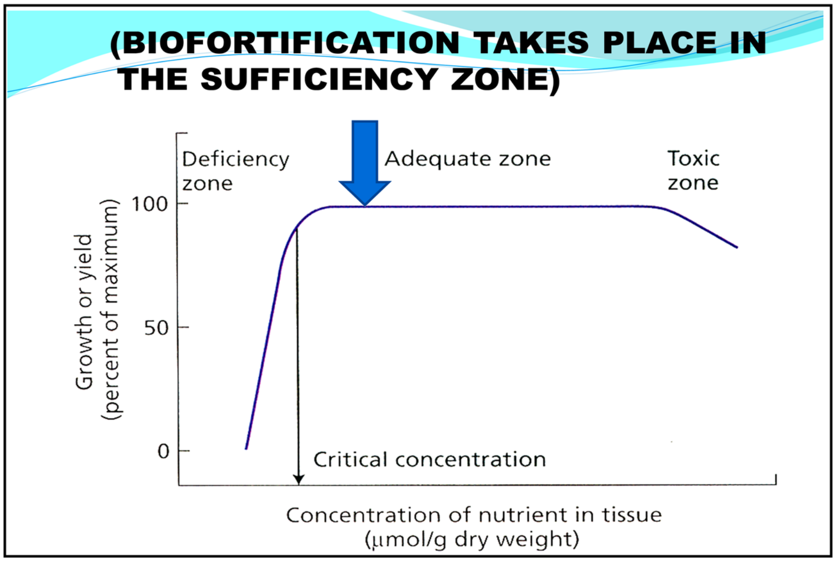

Figure 3: Fertilizer dose–response curve for biofortification of Zn.

enhanced yield by improving their availability (Marschner 1995). Application of the zinc either through soil or through foliar application is reported as an effective method for improving grain Zn concentration (Cakmak 2010). The timing of a micronutrient foliar spray is an important parameter that delineates its effectiveness in increasing its content in grain. For instance, significant land productivity increase is most likely with foliar application of Zn fertilizer (Li et al. 2013; Abdoli et al. 2014). During the milking stage to grain filling stage, grain Zn concentration increased in wheat, and therefore, this is a quite effective period for biofortification operation (Ozturk et al. 2006). Starchy endosperm adsorption is up to three-fold if fortification is done in the grain ripening stage near harvesting (McKevith 2004; Aisbitt et al. 2008). Since in the starchy endosperm (i.e., white flour), phytate quantity is at sub-optimal level (Pomeranz 1988; Velu et al. 2014); therefore, an increase in Zn implies a positive effect on the seed grain overall quality (Das et al. 2019, 2020; Hossain et al. 2019).

3 Zn transport mechanism in plants

Nutrients need to move through simplistic cells of the plant before reaching to the grains. Generally, two methods are used by the plant roots for making metal ions more available for uptake (Figure 4). Foremost, soil inherent fertility decides the roots acidification of the rhizosphere through plasma membrane H^+–ATPase. The cation exchange capacity improves, which further releases the tightly held divalent metal cations (Gaxiola et al. 2007). Second,

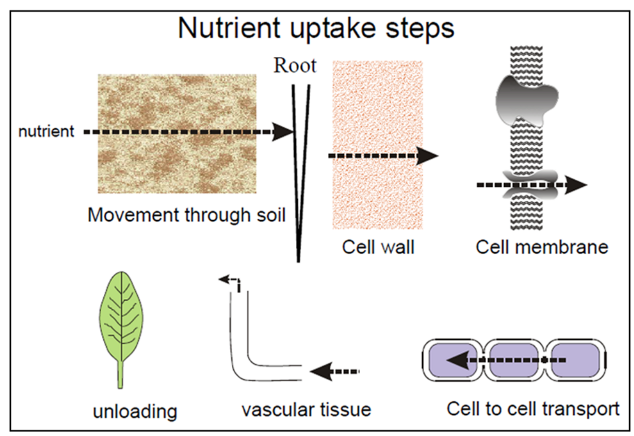

Figure 4: Mechanism of the transport pathway of a metal ion from soil to the grain.

metal chelators (organic acids and phytosiderophores) are actively produced by plant roots in the soils, which facilitate the uptake of Zn^{2+} in graminaceous plants (Oburger et al. 2014). Metal ions are moved to xylem parenchyma cells (through diffusion) to dead xylem, then to vessel-associated cells and via the phloem to the grain by specialized metal transport proteins during the period of grain filling (Palmgren et al. 2008).

4 Significance of biofortification

The biofortification technique for grain quality improvement is practiced for popular wheat cultivars. This approach is beneficial to the farmers because they get nutrient-fortified seed which is in high demand in the local market for overall improving their livelihood. The farmer is protected from buying new seeds and investment in micronutrient fertilizers is saved. Improvements in micronutrient concentration are associated with an increase in land productivity. The application rates of micronutrient fertilizers applied as a foliar spray are much smaller. Hence, this technique is a win-win approach for all. The current global challenge of mineral malnutrition is avoidable with biofortification techniques if properly and timely adopted (Das et al. 2019, 2020; Hossain et al. 2019). Biofortification approaches, therefore, harmonize the existing agricultural techniques as Zn biofortification could improve grain Zn (Velu et al. 2014). In such intervention, Punjab Agricultural University, Ludhiana, Punjab, India introduces Unnat "PBW 343" which is a modified form of earlier "PBW 343" wherein Zn content is being improved and farmers showed a keen interest in it and expected to get a good market for improved livelihoods along with good health status.

5 Human health *vis-à-vis* Zn contents

Zn is important for different metabolic processes of the human body and controls different enzymatic processes, which are responsible for good human health. Nearly 3,000 proteins in the human body depend upon the availability of correct amounts of Zn; otherwise, the deficiency leads to different physiological and mental disorders and deprived birth outcomes in pregnant women (Terrin et al. 2015; Krezel and Maret 2016). Zn deficiency is mostly reported in the kids up to the age of 5 years because of their higher Zn demand for their proper growth and today's changing food habits as kids mostly like the fast food (Wessells and Brown 2012). Higher economic losses along with overall poor human health would be reported if deficiencies of Zn and other micronutrients in developing countries of South Asia, *viz.*, India does not take necessary measures well in time through biofortification approaches.

6 Review of previous research to assess research gaps

The nutrient density of seed is dependent on inherent fertility status, soil type, crop species, season, and cultivars (Ascher 1994). Different genotypes may differ in phonological behavior and interaction with diverse management practices due to genetic variation. Varieties with heights have lesser both land and water productivity than the dwarf ones as lesser responsive to applied fertilizers. Dry matter accumulation and yield attributing characters mostly differ when different cultivars were selected based on their genotypic sequencing. Being greater in the efficient use of nitrogen fertilizer and resistant to lodging, dwarf cultivars were responsible for the green revolution in the region. In research trials at PAU Ludhiana, wheat genotypes, *viz.*, HD 2329 and WH 542 showed significantly higher plant production parameters than durum wheat (Singh et al. 1996). Hence, the varietal performance may change according to the genetic constitution and the agroclimatic conditions in the ambiance.

7 Performance of varieties in terms of micronutrients accumulation in grain

It was reported that Zn efficiency in the region varied from 0.33% to 0.77% (Torun et al. 2000). Further, tolerance of Zn deficiency is totally independent of the Zn shoot concentration. A significant variation among wheat cultivars in relation to land productivity and Zn-use efficiency is already reported (Khoshgoftarmanesh et al. 2004). Zn-use efficiency varied under texturally divergent soils, under different agroclimatic conditions and the cultivar selected for cultivation. Zn concentration and uptake were significantly higher in the Zn-efficient cultivars. Zn concentration improved from 15 to 35 ppm in some genotypes, due to the effect of the genotype and high genotype and environment interactions whereas Fe concentration ranged from 20 to 60 ppm (Oury et al. 2006). Genotype x environment interaction effect in the eastern Gangetic plains of India observed for variation in Zn concentrations of wheat grains, and it was found that micronutrient concentration in the grain is highly variable for different genotypes sown at different locations. Zn amounts of seeds ranged from 16.85 to 60.77 ppm (Joshi et al. 2010). In an accession from Spain "ANDALUCIA 344," highest levels of Zn concentrations were reported. Significant and positive correlation was reported between Zn and Fe concentrations (r = 0.81; p < 0.01), in "HONG DUAN MANG" (Chinese spring bread wheat). Therefore, cultivar selection is a must step to consider not only to maintain but also to improve the Zn-use efficiency, which further needs to consider the climate, soil type, organic matter content, and inherent fertility of the soil.

8 Nitrogen *viz-a-viz* Zn bioavailability

Both nitrogen and Zn are important to the different metabolic and enzymatic activity of the plants; therefore, plants properly nourished with N is able to use Zn with higher efficiency. Furthermore, the plant's N nutritional status greatly influences the uptake, transport, re-translocation, and grain deposition of Zn as it depends on various proteins, along with peptides. Zn-regulated transporter protein is involved in the root uptake of Zn, phloem to xylem exchange, phloem loading, and Zn deposition in grains (Palmer and Guerinot 2009). Sink for Zn is grain protein, where it finds the place (Kutman et al. 2010). Root uptake and transport of Zn via chelation with nitrogenous compounds improved proportionately with N-levels and improves seed deposition of Zn (Cakmak et al. 2010). Co-segregating genes are generally controlling the concentration of Zn in the plants. Accumulation of grains' Zn, Fe, and protein have a positive correlation and the close link with the relevant loci on the same chromosome and physiological mechanisms of plants. Elevated N supply can magnify Zn concentration in grains through improving the grain protein amounts and thereby escalating the sink strength in grains for Zn. Seed protein and Zn concentrations are reported to have positive co-relations that were also documented in various studies (Zhao et al. 2009). The effect of long-term 0, 130, and 300 N kg ha^{-1} on micronutrient density in winter wheat grain delineates that N fertilization increased the concentration of Zn, Fe, Cu, and protein in wheat grains (Shi et al. 2010). Hence, N is related to the translocation of micronutrients within plants. The grain protein is basically a genetic trait, but it can be manipulated through nitrogen application at elevated levels at critical stages. It has been seen that the addition of nitrogen at an adequate time could boost both grain yield and protein amounts (Ottoman et al. 2000). It has been observed further that the application of nitrogen fertilizer during the vegetative phase affected the grain yield but application during the reproductive phase affected protein content (Strong 1982). Increased nitrogen supply increased gliadin and glutenin contents, but not of albumins and globulins (Johansson et al. 2001). On loam soils, the protein content of grains increased with increasing nitrogen level up to 1.67% when N fertilization rates almost doubled from 100 kg N ha^{-1} which further increased but with a significant decreasing trend (Llovares et al. 2001). Maximum protein percentage and content were found in seeds plots applied with fertilized at 180 kg N ha^{-1}, while control plot's seeds receiving no fertilizer reported to had lowest protein contents (Singh et al. 2002; Warraich et al. 2002). In silt loam soils, with the application of 33.3 kg N ha^{-1}, around 70% increase in grain protein concentration was observed (Anthony and Howard 2003) and maximum grain weight was observed on the application of 150 kg N ha^{-1} because of highest protein percentage. The protein synthesis is controlled by the mobilization of N to the sink (grains) (Ali et al. 2003). Sustained supply of N from the anthesis phase onwards produces more proteins in the grains, which in turn results in the more recovery of semolina (Sardana 2003). Nitrate reductase and glutamate dehydrogenase activities observed to be a positive correlation in wheat. New structural protein involvement needs to be identified in response to a level of nitrogen fertilization (Bahrman et al. 2005). The application of 150 kg N ha^{-1} resulted in an increase in protein content and yield (Jakhar et al. 2005). Wheat cultivars responded differently to organic, *viz.*, vermicompost and inorganic fertilizers (Malik et al. 2005). Plant biomass of wheat was over control increased to 41% with 120 kg N ha^{-1} (Kalita and Nair 2005). The β-carotene pigment content was 6.6, 6.4, and 6.7 ppm at 120, 150, and 180 N kg ha^{-1}, respectively (Sardana et al. 2005). Favorable photosynthesis process might be responsible for the hike in land productivity, as well as grain zinc content when a higher dose of N-fertilizers was broadcasted. Similarly, Seiling et al. (2005) delineated from Kashmir with 120 kg N ha^{-1} (maximum dose) to wheat responsible for maximum nitrogen uptake with respect to 40 kg N ha^{-1} or 80 kg N ha^{-1} as compared to the controlled plots. The augmented N content in wheat with a higher dose of urea is responsible for the higher nitrogen-use efficiency (Kachroo and Rajdan 2006). Maximum protein content (12.0%) was found at 180 kg N ha^{-1} on loamy sand soils (Mehta et al. 2006) which agree with the results of Kumar and Ahlawat (2006) showing significantly higher N uptake in wheat-maize cropping system with 120 kg N ha^{-1} than 0 and 60 kg N ha^{-1}. Sedimentation value determines the functional quality of gluten proteins present in the grain. However, nitrogen has a non-significant effect on the sedimentation value of wheat grains in sandy loam soils (Kaur et al. 2006).

The grain appearance score depends upon grain-size, shape, luster and color. Grains are uniform in shape, bold in size, glossy in luster, and amber in color due to significantly their higher protein and beta-carotene content. Hardness and virtuousness are mechanical and optical properties, respectively. Vitreous endosperms have high gliadin content that causes higher adhesion of the protein matrix on starch granules during kernel desiccation causing compact endosperm shape (Samson et al. 2005). The application of 150 kg N ha^{-1} recorded maximum nitrogen uptake over control in wheat (Singh and Yadav 2006). The maximum protein content (12.3%) was found at 180 kg N ha^{-1} on loamy sand soils (Kaur et al. 2006). An attempt to improve the functional quality of indigenous wheat was made by Anureet et al. (2010) and Mehta et al. (2006) through the management of N, and the authors reported the improvement in the physicochemical characteristics and baking performance of bread wheat variety "PBW 343" at higher levels and late application of nitrogen. N has no significant effect on grain hardness in sandy loam soil (Kaur et al. 2006). The protein content of bread wheat was significantly affected by the variation in nitrogen dose (Otteson et al. 2007). For yielding high protein wheat, 180 kg N ha^{-1} was applied in three split doses, *viz.*, 60 kg before sowing + 60 kg in the shooting phase + 60 kg in the heading phase (Suek and Podolska 2008). Increasing N dose from 90 to 150 kg ha^{-1} increased protein content significantly (Stankowski et al. 2008; Brennan and Bolland 2009; Singh et al. 2009). Significantly higher grain as well as straw N was observed under fertilized plots [68 kg N at sowing + 75 kg N at irrigation + 7 kg N (3% urea spray) at anthesis] as compared to the control plots (Kaur et al. 2010). N uptake was significantly higher at the application of 150 kg N ha^{-1} over 120 kg N ha^{-1} (Arora et al. 2010). Split-application of N (40:40:40) kg ha^{-1} always improved the N-use efficiency than the 2 splits (0:80:40) kg ha^{-1} particularly on free-draining light-textured soils (Kharub and Chander 2010; Meena 2010). Further, protein content in the flour of wheat improved significantly in response to higher doses of N rate (Mattas et al. 2011; Ooro et al. 2011). Therefore, during various phases of growth and development of wheat, the proper dose of N fertilizers is an important factor influencing both the quantity and quality of the produced wheat grain. Further, applied N through urea at a higher dose and in the late reproductive stage is expected to have a higher positive influence on the grain quality parameters.

9 Effect of Zn nutrition on growth and land productivity

The normal concentration range of Zn is 25 to 150 mg kg^{-1} in plants. Zn toxicity occurs when the Zn leaf concentration exceeds 400 mg kg^{-1}. Plant roots absorb Zn as Zn^{+2} ions as the component of synthetic and natural complexes. Zn complexes can also enter the plant system through leaves. Zn is transported to the xylem although a substantial fraction may traverse the root and reach the xylem via the apoplast (White et al. 2002b). Generally, a decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, decreased cytochrome and nucleotide synthesis, decreased auxin synthesis, decreased chlorophyll synthesis, and inhibition of Zn enzymes are observed in plants grown on zinc-deficient soils (Marschner 1995). The average amounts of Zn vary between 20 and 35 ppm in wheat (Cakmak 2010). Reported Zn concentrations are too low to meet the daily human requirement; therefore, populations on a high-cereal (wheat) diet may suffer from its deficiency. For a measurable biological impact on human health, the concentration of Zn in whole wheat grains need to be increased at least by 10 ppm (Pfiffer and Mcclafferty 2007). Further, Zn-detailed effect are discussed under the subsections.

9.1 Growth parameters and grain yield

Applying Zn fertilizers to wheat resulted in improved grain quality and higher land and water productivity (Yilmaz et al. 1997). Seeds sown in Zn-deficient soils resulted in week plants, which ultimately produced inferior quality sprinkled grains which further had lower zinc concentrations (Yilmaz et al. 1998). The application of Zn either through soil and leaves lead to a direct effect on all the yield parameters (Ranjbar and Bahrmaniar 2007). Zn fertilization improved the overall land productivity along with the reported Zn content in the grains and straw samples. Furthermore, it is reported that $ZnSO_4$ and ZnO were equally effective in improving wheat land productivity. The recovery efficiency of applied Zn was improved at 0.5 to 1% of Zn enrichment. Zn enrichment of urea with $ZnSO_4$ provided significantly higher agronomic efficiency than ZnO (Shivay et al. 2008). The maximum increase in grain yield was achieved when the recommended dose of $ZnSO_4$ at 25 kg ha^{-1} was applied as soil application and 0.5% solution of $ZnSO_4$ as a foliar spray (Narwal et al. 2010). The application of Zn in soils increased the grain yield of wheat by 29% (Hussain et al. 2012). Similar results of Zn nutrition in wheat were reported by Habib (2009). Various plant parameters of wheat (variety "PBW 550") such as plant height, tillers m^{-2} significantly increased with soil application of $ZnSO_4{\cdot}7H_2O$ at 62.5 kg ha^{-1} and foliar spray of Zn chelates, more particularly in zinc-deficient soils (Dhaliwal et al. 2012). The application of

N at 150 kg ha^{-1} and Zn at 15 kg ha^{-1} resulted in improved growth, highest yield components, and highest land productivity (Jan et al. 2013). Therefore, Zn alone or in combination with N not only improves growth parameters but also improves land productivity. Applying Zn fertilizers to wheat grown in fields in central Anatolia, Turkey, improved grain Zn concentration (Yilmaz et al. 1997). An increased Zn transport from leaves into seeds can be achieved by spraying Zn solution of 0.5%, particularly under environmental stress conditions (e.g., drought) and on potentially deficient Zn soils (Yilmaz et al. 1997). The higher increase in the percentage of protein could easily be achieved through the soil and foliar application of Zn fertilizers (Ranjbar and Bahrmaniar 2007). Therefore, the role of the agricultural scientists cannot be ignored as far as biofortification or enriching edible portion of plants with micronutrients, *viz.*, Zn is concerned. Furthermore, the foliar Zn application doubled grain Zn concentration (Peck et al. 2008). It was also reported by Cakmak (2008) that a 3.5-fold hike in the grain Zn concentration could be achieved by zinc biofortification.

The boost in Zn percentage through the foliar application over non-foliar application was more in Zn-deficient soils as compared to Zn-sufficient soils (Ram et al. 2011). Foliar Zn application was much more effective than soil Zn application in the enrichment of wheat grains. The foliar 0.4% $ZnSO_4{\cdot}7H_2O$ application resulted in the best effect on grain Zn with 58% increase in grain Zn concentration, 76% increase in wheat flour Zn, and up to 50% decrease in the molar ratio of phytic acid to Zn in flour (Zhang et al. 2011). At the early grain development stage, Zn solution (0.5%) spray considerably improved Zn concentration in edible seeds and decreased concentration of phytic acid and ratio of phytic acid to Zn molar (Xi-wen et al. 2011). Zn application combined with foliar spray during the grain development stage increased grain Zn concentration by 95% and whole-grain estimated bioavailability by 74% (Hussain et al. 2012). Foliar Zn application alone or in combination with soil Zn application resulted in significant improvement in grain Zn concentrations as it increased from 27.4 mg kg^{-1} to 48.0 ppm by foliar Zn application (Zhang et al. 2012). Foliar application of {1-hydroxyethane 1,1-diphosphonic acid concentrated and liquid formulations of chelates} Zn–HEDP (C) at 2 g $liter^{-1}$ and Zn–HEDP (L) at 3 g $liter^{-1}$ increased the grain concentration by 29.2% over control. Zn–HEDP (L) at 3 g $liter^{-1}$ was more effective for Zn enrichment but Zn–HEDP (C) at 2 g $liter^{-1}$ showed significantly a higher uptake of Zn (Dhaliwal et al. 2012). Finally, it could be concluded that the soil application of $ZnSO_4{\cdot}7H_2O$ at 62.5 kg ha^{-1} and spray of Zn–HEDP (C) at 2 g $liter^{-1}$ and Zn–HEDP (L) at 3 g $liter^{-1}$, and 0.4–0.5% solution of $ZnSO_4$ can improve land productivity along with improving the Zn concentration in seed grains.

9.2 The cumulative effect of Zn along with Fe, Mn, and Cu on grain quality

The impact of mineral nutrients stored in seed increases crop growth rate and productivity through the direct involvement of mineral nutrients in synthesis, partitioning, and utilization of photosynthesis (McDonald et al. 1996). In wheat, an improvement in land productivity was demonstrated by Bedi and Kataki (1999); Zn solution (0.5%) was sprayed at various crop stages. The biomass increased its maximum when sprayed at ear emergence and anthesis stage. This increase was maximum and significant in wheat varieties "PBW 154" and "WH 542." Zeidan et al. (2010) showed that the application of Zn, Fe, and Mn significantly increased grain yield and yield components of wheat in comparison to control. Foliar application of Zn surpassed other treatments followed by Mn and Fe. The effects of micronutrients showed an increase of 4–11% in wheat grain yield by the addition of each micronutrient (Fe, Cu, Zn, and B) (Malakouti 2000). The foliar application of Mn at 0.5% and Cu at 0.2% improved the wheat productivity significantly in sandy loam soil (Dhaliwal et al. 2011). Crop sprayed with a micronutrient solution containing Zn improved seed micronutrient content by 63% of Zn (Kataki et al. 2001b). Foliar application of 0.5% $ZnSO_4$ increased Zn concentration in wheat grains by 99%. The highest number of grains were produced in soil application of $ZnSO_4$ at 80 kg ha^{-1} (Mohammad and Mohammad 2009). The improvement in grain quality parameters may be attributed to the role of microelements in enhanced accumulation of assimilates in the grain during grain filling stage and thus the resulting seeds had greater individual masses (Fenner 1992). Depending on the application methods, application of Zn could significantly increase the Zn concentration in the grains up to three- or fourfold (Yilmaz et al. 1997). Foliar applications of Zn enhanced plant absorption of Zn thereby increasing the concentrations in the flag leaves of the wheat crop which further enhances Zn contents in grains than in control plots (Zeidan et al. 2010). For ameliorating the Zn deficiency and prevent yield losses in cereals, Zn needs to be applied to deficient soil, typically in the form of $ZnSO_4$, at rates that range typically from 50 to 62.5 kg Zn ha^{-1}. Several factors, *viz*., inherent zinc concentration, soil texture and method of zinc application, and alkaline or calcareous soils, affect zinc biofortification (Alloway 2009). Zn fertilization has residual effects for up to 10 years and is not needed every year as only a small amount is being taken up by crops every year (Shivay et al. 2008). Therefore, zinc biofortification does not need to be carried out every year but even then farmers have to keep a watch on the crop stand, and it should be applied based on the appearance of its deficiency symptoms or on the basis of soil test reports.

10 Conclusion

Zinc-biofortification of crops either by soil or by the foliar method is required in the present era of intensive agriculture. Mineral fertilizers both macro and micro combined with proper soil fertilization approaches with an increased ability to improve the zinc density of grains, are advocated. Humans in both poor/developing countries, *viz*., India will accept biofortified grains if not more expensive than nonfortified grains as they are almost similar in appearance, taste, texture, or cooking quality of foods (Bouis 2003). Biofortified crops will have a great demand if their beneficial aspects to human health are demonstrated to consumers. Certainly, biofortified crops along with targeted genetic manipulation show great potential to address hidden hunger in humans across the world. However, there is a need to check the extent of increasing mineral density throughout the world in texturally divergent soils under different climatic conditions and different eating food habits of the inhabitants. Furthermore, there is a need to revise our old formulated fertilizer recommendations keeping in view the present trends of micronutrient deficiencies more particularly of Zn for the overall improvement of the health status of poor/developing nations and to get rid of the "Hidden Hunger" for the inclusive betterment of human beings.

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