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Biofortification Approaches to Enhance Grain Mineral Nutrient Concentration in Relation to Zinc - A Review

Clinical Research

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ABSTRACT

Globally, micronutrient malnutrition alone afflicts more than two billion people, mostly among resource-poor families in developing countries, with Zn, Fe, I and vitamin A deficiencies most prevalent. More than five million childhood deaths occur from micronutrient malnutrition every year. Currently, mineral malnutrition is considered to be among the most serious global challenges to human kind and is avoidable. Among different micronutrients, zinc deficiency is a well-documented problem in food crops, causing decreased crop yields and nutritional quality. Generally, the regions in the world with Zn-deficient soils are also characterized by widespread Zn deficiency in humans. Recent estimates indicate that nearly half of world population suffers from Zn deficiency. Cereal crops play an important role in satisfying daily calorie intake in developing world, but they are inherently very low in Zn concentrations in grain, particularly when grown on Zn-deficient soils. The reliance on cereal-based diets may induce Zn deficiency-related health problems in humans, such as impairments in physical development, immune system and brain function. Among the strategies being discussed as major solution to Zn deficiency, biofortification appears to be a most sustainable and cost-effective approach useful in improving Zn concentrations in grain. Scientific evidence shows this is technically feasible without compromising agronomic productivity.

INTRODUCTION:

Zinc is the twenty fourth most abundant element on earth's crust with a content of 75 mg kg^{-1}. Soil contains 5-770 mg kg^{-1} of Zn with an average of 64 mg kg^{-1}, sea water has 30 µg Zn L^{-1}, and the atmosphere contains 0.1^{-4} µg Zn m^{-3} (Emsley, 2001). However, zinc (Zn) is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases) (Auld, 2001). Zinc is an essential micronutrient for humans, animals and plants. Higher plants generally absorb Zn as a divalent cation (Zn^{2+}), which acts either as the metal component of enzymes or as a functional structural or a regulatory co-factor of a large number of enzymes.

However, zinc deficiency is one of the most common micronutrient deficiencies in human populations affecting health of over three billion people worldwide (Welch and Graham, 2004; Cakmak et al., 2010). According to a report published by the World Health Organization in 2002, deficiency of Zn rank fifth in terms of leading causes of diseases in developing high-mortality countries. Zinc deficiency causes impairments in brain development and wound healing and increases susceptibility to infectious diseases including diarrhea, pneumonia and malaria by weakening the immune system (Hotz and Brown, 2004; Black 2008).

In most cases, the reason behind Zn deficiency is inadequate dietary intake of Zn (Welch and Graham, 2004). In our country, cereal crops are the main component of the diet and responsible for more than 50 per cent of the daily caloric intake (Cakmak, 2008). However, these crops are inherently too poor in Zn to meet the recommended dietary allowances for human beings and also rich in compounds reducing the bioavailability of Zn, such as phytic acid.

According to a Zn-staining study in wheat seed, Zn concentrations were found to be around 150 mg kg–1 in the embryo and aleurone layer and only 15 mg kg^{-1} in the endosperm (Ozturk et al. 2006). The Zn-rich parts of wheat seed are removed during milling, thus resulting in a marked reduction in flour Zn concentrations. Consequently, heavy consumption of high proportion of milled wheat and other cereal products may result in reduced intake of Zn.

Processing of food grains also affects Zn concentration. For example, although unhulled rice (paddy) contains 27–42 mg kg^{-1} Zn, polished rice contains only 13 mg kg^{-1} Zn. High consumption of cereal based foods with low contents of micronutrients is causing health hazards in humans. The contents of micronutrients in food can be elevated either by supplementation, fortification or by agricultural strategies i.e., biofortification and application of micronutrients containing fertilizers.

Enrichment of cereal grains with Zn is, therefore, a high priority area of research and will contribute to minimizing Zn deficiency related health problems in humans.

Approaches to increase zinc in plants:

Biofortification provides a sustainable solution to Zn deficiency in food around the world as it is the process of enriching the nutrient contents of staple crops. Biofortification of staple food crops with micronutrients by either breeding or fertilization or transgenic can be an effective strategy to address widespread dietary deficiency in human populations (Bouis et al., 2000). Biofortification is a means to enhance the grain mineral nutrient concentration in plants and provide micronutrients to the most vulnerable people in a comparatively inexpensive and cost-effective way, using an agricultural intervention that is sustainable (Bouis, 2003 and Nestel et al., 2006).

Increasing Zn content of food grains could be achieved by breeding crop cultivars with higher Zn concentration in grains or by fertilizing crops with Zn. There are 3 biofortification strategies, viz., agronomic, genetic and transgenic biofortification strategies.

1. Agronomic approaches for biofortification:

Application of Zn fertilizers to soil and/or foliar seems to be a practical approach to improving grain Zn concentration (e.g., agronomic biofortification). Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvement of the solubilisation and mobilization of mineral elements in the soil. When crops are grown where mineral elements become immediately unavailable in the soil, targeted application of soluble inorganic fertilizers to roots or to leaves is practised. In situations where mineral elements are not readily translocated to edible tissues, foliar applications of soluble inorganic fertilizers are made (Graham et al., 2007).

Zinc fertilization of crops using fertilizers (Fertifortification):

Zinc can be directly applied to soil as both organic and inorganic compounds. Although soil application of inorganic Zn fertilizers is widely used, organic and chelated sources are better from economic and environmental perspectives. Use of other methods of Zn application (such as seed treatment, foliar application etc., in association with mycorrhizal fungi) may improve Zn-use efficiency in rice (Hafeez-ur Rehman et al., 2012).

Abundant supply of Zn nutrition and balanced NPK, increases the protoplasmic constituents, accelerates the process of cell division and elongation, photosynthesis processes, respiration, nitrogen metabolism-protein synthesis, other biochemical and physiological activates. This in turn increased the values of all growth and yield attributing parameters, which finally reflected in increased both grain and straw yields as well as harvest index.

Keram et al.2014 reported that the application of 5, 10 and 20 kg Zn ha-1 significantly increased the Zn concentration in root, stem, leaves and earhead of wheat over NPK fertilization alone at different growth stages of wheat. Further, the grain and straw yields as well as harvest index increased with the increasing levels of Zn as compared to NPK alone. The maximum pooled grain yield 4.66 t ha^{-1} was observed in the treatment 20 kg Zn ha^{-1}, while the minimum pooled grain 3.88 t ha-1 in the control plot (100 % NPK).

Zn enriched farm yard manure (FYM) with chelated (Zn-EDTA) and non chelated (zinc sulphate, $ZnSO_4$) Zn sources for optimum grain yield and higher Zn concentration in grains of rice grown in a salt-affected soil showed significantly increased grain yield and Zn concentration in rice grains. Application of ZnSO4 enriched FYM proved better over application of alone ZnSO4 or Zn-EDTA indicating the positive role of organic matter in increasing grain yield and grain Zn concentration on soils affected with salts and depleted in organic matter. (Ahmad et al.2012)

Zinc sulfate ($ZnSO_4$) is the most widely applied inorganic source of Zn due to its high solubility and low cost. Zinc can also be applied to soils in form of ZnO, ZnEDTA and Zn-oxysulfate. The agronomic effectiveness of Zn fertilizers is higher with ZnEDTA than the inorganic Zn fertilizers (Mortvedt 1991).

The application of Zn fertilizers to the soil is effective in increasing grain Zn concentrations in cereals growing on most, but not all, soils and foliar applications of either $ZnS0_4$ or Zn-chelates can increase grain Zn concentrations in plants with adequate Zn mobility in the phloem (Rengel et al., 1999 and Cakmak, 2002, 2004, 2008).

Zn-EDTA treatment showed the greatest Zn-mobilization- efficiency index and produced the highest yields. Zn applications like $ZnSO_4$, Zn-FYM and Zn $(NH_3)_4$-FYM to the marginally Zn-deficient soil increased the Zn concentration in the soil and promoted its uptake by the rice crop, and helped to increase the grain yield by ensuring the early emergence of panicles which permitted the greater storage of assimilates in grains (Srivastava et al., 1999).

Depending on the application method, Zn fertilizers can increase grain Zn concentration up to three- or fourfold . The most effective method for increasing Zn in grain was the soil+foliar application method that resulted in about 3.5-fold increase in the grain Zn concentration. The highest increase in grain yield was obtained with soil, soil+foliar and seed+foliar applications (Yilmaz et al. 1997).

Phattarakul et al. (2012) during their study on evaluation of the effect of soil and/or foliar Zn fertilizer application on grain yield and grain Zn concentration of rice reported that Zn application increased grain yield by about 5 %. Grain Zn concentrations were, however, more effectively increased by Zn fertilization, especially with foliar Zn applications. On average, Zn concentration in brown rice (whole caryopsis with husk removed) was increased by 25 % and 32 % by foliar and foliar + soil Zn applications in china and india.

Foliar Zn fertilization was an effective approach to promote grain Zn concentration and Zn bioavailability, especially, in case of Zn-AA and ZnSO4. On average, Zn-AA and ZnSO4 increased Zn concentration in polished rice up to 24.04% and 22.47%, respectively and ZnSO4 increased Zn bioavailability in polished rice up to 68.37% and 64.43%, respectively. Therefore, it's believed that foliar application of suitable Zn form is a feasible approach to improve the bioavailable Zn status in polished rice (Yanyan Wei et al., 2012). Thus adequate fertilization of food crops can partly help in Zn intake by humans.

Under agri-horticultural system in pearl millet there were progressive increases of both the grain and biological yield in response to increasing N supply with Zn treatment. Nitrogen and zinc fertilization had significant effect on pearl millet grain and biological yield with 60 kg N/ha and 10 kg Zn/ha fertilizer level. Grain yield increased up to 32.27% and 31.0 % from 0 to 60 kg N/ha and 0 to 10 kg Zn/ha, respectively(Prasad et al.2014).

As an agronomic approach recent studies indicate that intercropping systems contribute to grain Zn and Fe concentrations. Various field tests in China with peanut/maize and chickpea/wheat intercropping systems showed that gramineaceous species are highly beneficial in biofortfying dicots with micronutrients. In the case of chickpea/wheat intercropping, Zn concentration of the wheat grains was 2.8-fold higher than those of wheat under monocropping (Zuo and Zhang, 2009).

2. Genetic approaches for biofortification:

Plant breeding (e.g., genetic biofortification) approach to minimize the extent of Zn deficiency is thought to be cost-effective, easily applicable and affordable in the target populations (Cakmak, 2008). Genetic biofortification is the idea of breeding crops to increase their nutritional value. This can be done either through conventional selective breeding or through genetic engineering.

Increasing the concentrations of essential mineral elements in produce through the application of mineral fertilizers can be complemented by breeding crops with an increased ability to acquire and accumulate these minerals in their edible portions. However, it must be recognized that genotypic enhancement can only improve the acquisition, utilization or accumulation of mineral elements available to the crop. Considerable genetic variation appears to exist in the concentrations of the mineral elements most frequently lacking in human diets in the edible portions of most crop species.

There is genetic variation in the concentrations of mineral elements in the grains of most cereal species. Concentrations of Fe and Zn in cereal grain vary 1.5- to 4-fold among genotypes depending on the genetic diversity of the material tested. Some of this variation can be attributed to differences in grain yield. Although the cultivated germplasm of some cereals, such as wheat, may have a limited genetic variation in grain Fe and Zn concentrations, wild relatives often possess considerable variation (Monasterio & Graham, 2000; Cakmak et al., 2004; Welch et al., 2005) and accessions with grain Fe and Zn concentrations at least 2-fold higher than the most widely grown varieties are available for many cereal species (White & Broadley, 2005).

Differences in grain Zn and Fe concentrations between species of wild and cultivated wheat have been attributed, in part, to allelic variation at a chromosomal locus that promotes early senescence and remobilization of protein, Fe, Zn and Mn from senescing leaves to seeds, and the introgression of the high grain protein content (Gpc- B1) locus from a wild tetraploid wheat (Triticum turgidum ssp. dicoccoides) to a cultivated wheat (Triticum durum) resulted in higher concentrations of Fe and Zn in its grain (Distelfeld et al., 2007).

In addition, highly significant positive correlations between grain Fe and Zn concentrations have been observed in wheat (Graham et al., 1999) and sorghum (Reddy et al., 2004; Koti et al., 2009) which increase the possibilities of breeding for increased concentrations of Fe and Zn simultaneously. Interestingly, studies of related Triticum species showed strong positive correlations between protein and Zn concentrations (Ozturk et al., 2006; Distelfeld et al., 2007).

Suzuki et al. (2008) noticed the expression pattern of genes involved in DMA synthesis (Deoxymugineic acid) which is translocator of zinc in zinc deficient plants. Similarly, Lee et al. (2009) observed increased concentration of zinc and iron in $osNAS_3$ tagged mutants in rice over wild type plants that are grown under MS media and in zinc and iron deficient conditions. The OsNAS genes, particularly $OsNAS_2$, show enormous potential for Fe and Zn biofortification of rice endosperm. The results demonstrate that rice cultivars over expressing single rice OsNAS genes could provide a sustainable and genetically simple solution to Fe and Zn deficiency disorders affecting billions of people throughout the world (Alexander etal 2011).

3. Transgenic approaches for biofortification:
Biofortification of plants with microelements (zinc) can be achieved by transgenic modification (Bouis, 2003).Transgenic approaches could be a further option in improving food crops with Zn. Currently, impressive progress is being made in developing transgenic plant genotypes with increased concentrations of Zn and Fe and also increased phytoavailability of mineral elements in the soil, their uptake from the rhizosphere, translocation to the shoot and accumulation in edible tissues (White & Broadley, 2005 and Zhu et al., 2007). In addition, transgenic approaches may be used to reduce the concentrations of anti-nutrients and increase the concentrations of promoter substances.

Evidence is available showing a potential role of ZIP family Fe and Zn transporter proteins in improving micronutrient density in grain (Schachtman and Barker 1999; Eide 2006). These proteins are involved in uptake and transport of cationic micronutrients in cells. There was an increase in grain Zn concentration due to expression of the genes encoding a Zn transporter protein from Arabidopsis thaliana in roots of a barley genotype (Ramesh et al. 2004).

The mis-expression of genes affecting Zn and Cu uptake and movement within the plant can increase the concentration of these elements in edible portions. For example, pea bronze (brz) and degenerated leaflet (dgl) and Arabidopsis ferric reductase defective 3/manganese accumulator 1 (jrd3 = manl) mutants constitutively expressing rhizosphere Fe(III) reductase activity have greater shoot Zn and Cu concentrations than wild-type plants (Rogers etal, 2000), and transgenic barley plants expressing AtZIP 1 produce smaller seeds with higher Zn concentrations than wild-type plants (Ramesh et al., 2004).

Seed Fe and Zn concentrations can be increased in wheat by expressing RNA interference (RNAi) constructs of an NAC transcription factor (NAM-B 1) that accelerates senescence and increases remobilization of mineral elements from leaves to developing grain (Uauy et al., 2006). High expression of zinc (Zn)-regulated, iron-regulated transporter-like protein (ZIP) genes increases root Zn uptake in dicots, leading to high accumulation of Zn in shoots. Overexpression of HvZIP7 in barley plants increased Zn uptake. Significantly, there was a specific enhancement of Zn accumulation in shoot and grains (Jingwen et al., 2013).

Several reports have investigated the role of ferritin protein in seed accumulation of Fe and Zn. Ferritin is a major Fe protein existing in most living organisms (Harrison and Arosio 1996). Vasconcelos et al. (2003) reported that overexpression of soybean ferritin genes in rice was effective in increasing both Fe and Zn concentrations of seeds. Transforming rice with ferritin gene from soybean (Qu et al. 2005) or French bean (Lucca et al. 2006) increased grain Zn and Fe concentrations.

Recently, it has been shown that the Gpc-B1 locus from Triticum diccocoides encodes a NAC transcription factor (NAM-B1) that increases grain Zn and Fe concentrations, possibly by stimulating leaf senescence and thus remobilization of Zn and Fe from flag leaves into seeds (Uauy et al. 2006). Heavy metal transporters belonging to the P_1B-ATPase subfamily of P-type ATPases are key players in cellular heavy metal homeostasis. Heavy metal transporters belonging to the P1B-ATPase subfamily of P-type ATPases are key players in cellular heavy metal homeostasis. Such an activity could be important for redistribution of heavy metals within and between cells in response to changes in cellular demand, such as during Zn deficiency and during the process of grain filling (Maria Dalgaard et al. 2012).

Conclusion:
The challenge of hidden hunger requires building a multidisciplinary research team of scientists from different disciplines of agriculture. An important component of this is encouraging plant breeders in developing countries to include micronutrients in their breeding portfolios along with higher yield, disease resistance and other agronomic traits to enhance grain mineral nutrient concentration. As biofortification is a viable, cost-efficient and effective solution for combating micronutrient malnutrition it should be worked out with different approaches like agronomic, genetic and transgenic to enhance grain mineral nutrient concentration.

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