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Zinc mediated agronomic bio-fortification of wheat and rice for sustaining food and health security: A review

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Abstract

Zinc (Zn) is essential for growth and development of plants, animals and human beings. Its deficiency poses a serious challenge for crop production, especially for wheat and rice, which are considered as staple food crops in most of the developing countries. Beside plants, health related issues in human beings also reported due to deficiency of Zn, which calls for suitable research strategies that can boost the yield and quality of crops, Zn concentration in the grains of cereals such as rice and wheat, increase Zn bio-availability in diets. Across the globe several strategies viz., supplementation, pharmaceutical preparation, food fortification and dietary diversification have been attempted to solve the problem of Zn deficiency induced malnutrition but the agronomic biofortification seems more feasible option particularly in developing countries, where other alternative could prove costly. It could easily be achieved through improved approaches such as integrated nutrient management (INM) and integrated soil fertility management (ISFM) because these helps in enhancing the uptake of micronutrients like Zn, particularly in deficient soils. Though, agronomic biofortification helps to reduce the health hazards still more research efforts are needed to solve the problem of malnutrition and food insecurity for a better and healthy future.

Keywords: Agronomic bio-fortification, Zinc, wheat, rice, food and health security

Introduction

The Zinc (Zn) is recognized as an essential nutrient element for plants and animals during 1920s and 1930s [59, 62]. It is also essential component of more than 300 enzymes [17] involved in synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients. It plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity [56] and acts as a catalytic, structural and regulatory cofactor [40].

Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally [8, 52] affecting both crops and human beings [66, 30, 65]. According to World Health Organization (WHO) report [67], zinc deficiency ranks fifth amongst the most important health risk factors in developing countries and eleventh worldwide. About 230 million undernourished people belong to India (> 27% of the world's population) [15]. Zn deficiency in human beings causes impairment in the immune system, learning ability and physical growth and increase in mortality and infections [18], such disorders affects 27% of the total population in India [68], particularly the children's of less than 5 years of age with nearly 4, 50,000 deaths [3].

Globally, rice and wheat is considered as major staple food crops for majority of the population and contributes to two-thirds of the energy needs of humans [48]. In India, rice-wheat cropping system occupies about 10 million ha area mainly in the states of Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal [47]. Adoption of such cropping system led to micronutrient malnutrition [65]. Most prevalent micronutrients deficiencies are iron, iodine, zinc and vitamin A induced malnutrition affecting more than two billion peoples in developing countries [36]. Among cereals, wheat and rice are inherently low in Zn and growing them on potentially Zn deficient soils (around 50% cereal cultivated soils) further reduces the Zn content in grains and diets, crop yields and nutritional quality of crops [11, 25]. Micronutrient deficiencies, particularly Zn, in staple food crops are really a cause for concern to feed the large population with adequate quantity of healthy food.

Thus, efforts have been made to address this malady through various interventions like pharmaceutical preparation, food fortification and dietary diversification but proved unrealistic and unacceptable due to many reasons^[23] viz., beyond the reach of large masses in the developing nations due to their high cost^[5, 60]. As a consequence bio fortification of staple food crops with higher micronutrients contents through genetic bio-fortification and agronomic bio-fortification gaining prominence now a day. The former involves selection of varieties genetically rich in micronutrients such as zinc while, the later relies on application of appropriate fertilizers to enrich the micronutrients status in the grains. Agronomic bio-fortification has so far been most effective with zinc and selenium^[14]. Research results from Turkey showed increased yield and grain zinc concentrations with zinc fertilization of various cereals (maize, sorghum, barley, wheat) and dicotyledonous (soybean, safflower, pea, common bean, canola, common vetch) crops^[13, 71] and upto three folds increase in yield and grain Zn concentrations of rice with the application of Zn-enriched urea were reported from the field studies in India^[12]. Zn mediated agronomic biofortification of cereals such as wheat and rice proved useful for enriching the micronutrient content in the edible parts and thus overcome hidden hunger caused due Zn micronutrient deficiency to some extent besides rectifying the deficiency in dietary intake. However, it is treated as complementary to breeding (genetic biofortification) approach and considered as a short-term panacea to increase micronutrient availability^[24, 64] rather than long term sustainability.

It is high time to join the hands across the globe-developed and developing nations- to address the issue of micronutrient deficiencies occurred in staple food crops such as rice and wheat. Along with food security due attention must be paid to nutritional and healthy food enriched with micronutrients like Zn in adequate amount for the masses whose staple food being the rice and wheat, most of them belongs to developing countries.

Agronomic biofortification

It is an agronomic strategy to increase micronutrient content and bioavailability for human nutrition in the edible parts of crops by adding micronutrient fertilizers to the soil or plant leaves^[19, 20]. However, successfulness to enhance micronutrient availability for crop uptake is largely depends on better soil health. It could be achieved through improved strategies like integrated soil fertility management (ISFM) which is defined as a set of soil fertility management practices that necessarily include the use of fertilizer, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic use efficiency of the applied nutrients and improving crop productivity^[63].

Agronomic bio fortification for improvement in wheat and rice yield

Ananda and Patil² observed highest grain yield with combined application Zn and Fe micronutrients (42.23 q ha⁻¹) followed by sole application of Fe @ 25 kg ha⁻¹ (40.94 q ha⁻¹) and sole application of Zn @ 25 kg ha (40.74 q ha⁻¹). Significantly higher grain and straw yield were obtained at 20 kg Zn ha⁻¹ along with recommended doses of fertilizers^[37]. Foliar application of Zn @ 216 g ha⁻¹ to wheat crop produced more number of spikes per unit area than no zinc treatment^[73]. Wheat grain yield is also significantly influenced under N, Zn and B treatment^[34]. Soil and foliar spray of Zn to wheat crop

under alkaline soil conditions significantly increase the grain yield as well as 1000 grain weight^[38]. High wheat grain yield was also observed under soil application of Zn as well as foliar Zn + propiconazole treatment than no Zn application^[49].

Agronomic bio-fortification of rice with zinc is effective in improving the grain as well as straw yield of rice under several studies. Application of ZnSO₄ as soil application @ 10 Kg ha⁻¹ increase grain as well as straw yield of rice in the tune of 31.9 q ha⁻¹ and 68.24 q ha⁻¹, respectively^[42]. Zinc application has significant effect on grain as well as straw yield increase^[60] as compared to control. Mustafa *et al.*^[43] reported that yield and yield attributes of rice was significantly superior due to foliar application of 0.5% Zn solution 15 days after transplanting (21.0 g) over control treatment (18.52 g). Organic matter application increases total Zn content, proportion of readily available form (labile Zn) for plant uptake^[53]. Green manure crops (as mulch or soil amendment) are also proved effective to enhance nutrient bioavailability, yields and grain Zn nutritional quality^[46]. Combined application of mineral fertilizers and organic matter increase the agronomic efficiency of mineral fertilizers in case of Zn biofortification in rice^[21].

Agronomic bio fortification for improvement in wheat and rice quality

Various researchers made significant efforts for quality improvement of wheat grains through bio-fortification with different mineral nutrients, of which application of Zn as a bio-fortification material has significant affects on total carbohydrate and wet gluten content in wheat grain^[37]. Zn application was quite effective even under adverse soil conditions (soil alkalinity) in improving the protein content of wheat grains^[38]. Protein content in wheat grain was significantly increased under micronutrient treatments⁵⁷. Foliar application of micro elements is also effective in improving the protein content in wheat grains^[72]. Application of N, Zn and B significantly affects the Zn concentration in grain as well as straw of wheat crop^[34]. Zinc mediated bio-fortification significantly improved quality parameters of rice grain viz., crude protein (5-19%) in rice genotypes⁶¹. Studies from various countries indicated average Zn concentration in the range of 20 to 35 mg kg⁻¹ in whole grain of wheat^[51, 10, 54].

Effect on concentration and uptake of Zn and other nutrients in wheat and rice grains

Application of Zn to wheat crop at different doses significantly influenced the total uptake of macro nutrients like N, P, K and micronutrients like Zn, Fe and Mn when applied in soil^[1]. Application of Zn along with RDF at higher doses increases the total N, K and Zn uptake³⁷. Application of N, Zn and B significantly affected the total Zn uptake by grain and straw of wheat^[34]. Soil Zn application increase the Zn concentration in wheat grains by 19% and by 63% through foliar application under various Zn-enriched fertilizers experiments in several African countries^[33]. Mc Donald *et al.*^[41] found that application of zinc @ 7.6 kg ZnSO₄ ha⁻¹ significantly increased the concentration of zinc (20 ppm) in wheat grain as compared to control treatment. Similarly, application of zinc increased the concentration of zinc in wheat grains from 45.45 to 67.27 microgram gram⁻¹ under pot experiment^[50].

Highest Zn concentration in paddy rice with foliar Zn spray @ 0.5% ZnSO₄ applied at panicle initiation, booting, 1 and 2 weeks after flowering^[4], while foliar application of Zn at

early growth stage (panicle initiation and booting) had no effect on seed Zn concentration. Site (soil and foliar) of zinc nutrient application has also significant effect on Zn content in whole rice grain ^[61]. Soil and foliar zinc application increased Zn concentration in rice by 7% and 25%, respectively ^[33].

Improvement in bioavailability of Zn for nutritional and human health security

Zn deficiency is related to growth failure, decreased immunity, increased susceptibility to infection, morbidity and mortality²². Therefore, Zn bioavailability plays a crucial role in rectifying these odds arose as a consequence of zinc deficiency occurred due to low bioavailability of Zn²³. It is reported that food with high amounts of phytic acid has low Zn bioavailability ^[9, 16]. Thus, phytate level is most reliable approach used to measure the Zn bioavailability in human being. Hotz and Gibson²⁹ suggested to use phytic acid to zinc molar ratios of < 5, 5-15 and >15 for a corresponding Zn bioavailability factor of 55, 35 and 15%, respectively. Gibson²⁶ in another study further reported the revised estimates of WHO's categorization of the diets into low, moderate, and high bioavailability based on the Zn bioavailability in the following order i.e. ~15%, ~30% or 35%, and ~50% or 55%. Efforts have been made in India through Zn-enriched urea to improve the Zn bioavailability, rice yields and grain Zn concentrations up to three times¹². Research indicated Zn fertilization increase Zn bioavailability in humans ^[31] as well as increase concentration in crops and reduction in phytate concentration level ^[32].

Constraints and potentials

In low-income countries accessibility and affordability of the fertilizers are two major concerns for resource poor farmers, which favours breeding intervention to improve the crop nutrition status ^[13, 39]. The other challenges faced by the marginal and small farmers in the effective implementation of agronomic biofortification include limited availability of organic manures and mineral fertilizers, extra labour requirements and costs and environmental stress ^[27]. Besides, it requires sophisticated infrastructure and hence high costs to improve the Zn concentration in grain cereals like rice and wheat ^[55, 16]. However, these shortcomings could be addressed through a whole supply chain approach ^[58]. Agronomic biofortification holds great promise and offers a potential solution for alleviating the Zn induced deficiency *per se* and malnutrition in general. It has greater spatial extent and requires less behavioral change in comparison to other nutrition improving interventions ^[28]. Therefore, it is more accepted strategy as compare to supplementation and diet diversification ^[6].

Conclusion

Today, malnutrition and food insecurity are the biggest challenges before the humanity with their vicious presence in the developing countries. These are mostly dominated by the micronutrients led deficiencies occurred in the cereal crops such as rice and wheat, which used to be the staple food grains for satisfying the food needs of majority of the population in these nations. Rice and wheat grains generally lacks in micronutrients particularly zinc causing staple food induced Zn deficiency malnutrition. Such micronutrient deficiencies could be addressed through transgenic or genetic engineering bio-fortification but its success is limited due to genetic variation. This kind of limitations calls for suitable

alternatives to overcome the zinc induced malnutrition and undernourished situation. In this context, attempts have been made through several approaches viz., supplementation, pharmaceutical preparation, food fortification and dietary diversification but due to their cost and technological requirements restricts their spatial extent. In such situations, agronomic biofortification strategy seems more feasible option particularly in developing countries, where other alternative could prove costly. It could be a boon in mitigating the malnutrition problem on one hand and supply of healthy food on the other hand besides yield increase and health security in the years to come in many developing as well as developed countries. However, the success of agronomic biofortification largely depends on bioavailability of micronutrients like Zn to human food via soil-plant-human chain, which could effectively be strengthened with the adoption of improved nutrient management approaches such as integrated nutrient management (INM) and integrated soil fertility management (ISFM) to ensure the enhanced uptake of micronutrients particularly in micronutrient deficient soils. Agronomic biofortification of rice and wheat with Zn not only improves the yield and quality of grains but also its bioavailability in diet. Thus, it may prove to be a suitable and sustainable strategy in reducing the health hazards but with more research efforts to solve the problem of malnutrition and food insecurity for a better and healthy future.

References

1. Abbas G, Khan MQ, Muhammad J, Muhammad T, Hussain F. Nutrient uptake, growth and yield of wheat (*Triticum aestivum*) as affected by zinc application rates. *Int. J. Agric. Biol.* 2009; 11(4):389-396.
2. Ananda N, Patil BN. Effect of micronutrients (Zn and Fe) and time of nitrogen application on growth and yield of durum wheat. *Karnataka J Agric Sci.* 2005; 18(3):604-608.
3. Black RE *et al.*, Maternal and child under nutrition: global and regional exposures and health consequences. *Lancet.* 2008; 371:243-260.
4. Boonchuay P, Cakmak I, Rrerkasem B, Chanakan PT. Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. *Soil Sci Plant Nutr.* 2013; 59:180-188.
5. Bouis HE. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proc. Nutr. Soc.* 2003; 62:403-411.
6. Bouis HE, Welch RM. Biofortification –A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science.* 2010; 50:20-32.
7. Bouis HE, Hotz C, Mc Clafferty B, Meenakshi JV, Pfeiffer WH. Biofortification: A new tool to reduce micronutrient malnutrition. *Food Nutr Bull.* 2011; 32(1):531-540.
8. Cakmak I, Torun B, Erenoglu B, Ozturk L, Marscher H, Kalayci M *et al.* Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica.* 1998; 100:349-357.
9. Cakmak I, Kalayci M, Ekiz H, Braun HJ, Kilin Y, Yilmaz A. Zinc deficiency as a practical problem in plant and human nutrition in Turkey: A NATO-science for stability project. *Field Crops Research.* 1999; 60(1-2).
10. Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A *et al.* *Triticum dicoccoides*: an important genetic

- resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci. Plant Nutr.* 2004; 50:1047-1054.
11. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* 2008; 302:1-17.
 12. Cakmak I. Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. *Journal of Trace Elements in Medicine and Biology.* 2009; 23(4):281-289.
 13. Cakmak I, Pfeiffer WH, Mc Clafferty B. Biofortification of durum wheat with zinc and iron. *Cereal Chem.* 2010; 87(1):10-20.
 14. Cakmak I. Agronomic biofortification. Conference brief #8, The 2nd Global Conference on Biofortification: Getting Nutritious Foods to People, Rwanda, 2014.
 15. Chakraborti M, Prasanna BM, Hossain F, Singh AM. Evaluation of single cross quality protein maize (QPM) hybrids for kernel iron and zinc concentrations. *Indian J Genet Pl Br.* 2011; 71(4): 312-319.
 16. Clemens S. Zn and Fe biofortification: The right chemical environment for human bioavailability. *Plant Science.* 2014; 225:52-57.
 17. Cousins RJ, McMahan RJ. Integrative aspects of zinc transporters. *J Nutr.* 2000; 130:1384S-7S.
 18. Cunningham-Rundles S, Mc Neeley DF, Moon A. Mechanisms of nutrient modulation of the immune response. *J Allergy Clin. Immunol.* 2005; 115:1119-1128.
 19. De Valençaa AW, Bake A. Micronutrient Management for Improving Harvests, Human Nutrition, and the Environment. Background Document for Stakeholder Workshop on Micronutrients, Wageningen University, Food and Business Knowledge Platform, IFDC, 2016.
 20. De Valençaa AW, Bakeb A, Brouwerb ID, Gillera KE. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global Food Security.* 2017; 12:8-14.
 21. Duffner A, Hoffland E, Stomph TJ, Melse-Boonstra A, Bindraban PS. Eliminating zinc deficiency in rice-based systems. VFRC Report 2014/2. Virtual Fertilizer Research Center, Washington, D.C. 2014.
 22. Etcheverry P, Griffin IJ, Abrams SA. Micronutrient Deficiencies: New solutions to a seemingly irresolvable problem. 2005; 6(1):77-86.
 23. Frossard E, Bucher M, Machler F, Mozafar A, Hurrell R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agr.* 2000; 80(7):861-879.
 24. Garcia-Banuelos ML, Sida-Arreola JP, Sanches E. Biofortification – promising approach to increasing the content of iron and zinc in staple food crops. *Journal of Elementology.* 2014; 19(3):865-888.
 25. Gibbson RS. Zinc: the missing link in combating micronutrient malnutrition in developing countries. In *Proceedings of the Nutrition Society, University of East Anglia, Norwich, 2005.*
 26. Gibbson RS. A historical review of progress in the assessment of dietary zinc intake as an indicator of population zinc status American Society for Nutrition. *Adv. Nutr.* 2012; 3:772-782. doi:10.3945/an.112.002287.
 27. Giller KE. Targeting management of organic resources and mineral fertilizers: Can we match scientists' fantasies with farmers' realities? *Integrated Plant Nutrient Management in sub-Saharan Africa: From Concept to Practice* (eds B. Vanlauwe, J. Diels, N. Sanginga & R. Merckx), CAB International, Wallingford. 2002, 155-171.
 28. Groote H De, Gunaratna NS, Gameda S, Tessema M. Addressing Human Zinc Deficiency through Agriculture Innovations in Ethiopia. In *Organized Symposium proposed for the Conference of the African Association of Agricultural Economics.* 2016.
 29. Hotz C, Gibson RS. Complementary feeding practices and dietary intakes from complementary foods amongst weanlings in rural Malawi. *European Journal of Clinical Nutrition.* 2001; 55:841-849.
 30. Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* 2004; 25:94-204.
 31. Hussain S, Maqsood MA, Rengel Z, Aziz T, Abid M. Estimated zinc bioavailability in milling fractions of biofortified wheat grains and in flours of different extraction rates. *International Journal of Agriculture and Biology.* 2013a; 15(5):921-926.
 32. Hussain S, Maqsood MA, Aziz T, Basra SMA. Zinc bioavailability response curvature in wheat grains under incremental zinc applications. *Archives of Agronomy and Soil Science.* 2013b; 59(7):1001-1016.
 33. Joy EJM, Stein AJ, Young SD, Ander EL, Watts MJ, Broadley MR. Zinc-enriched fertilisers as a potential public health intervention in Africa. *Plant Soil.* 2015; 389(1-2):1-24.
 34. Kapoor S, Sharma SK, Rana SS, Shankhyan N. Effect of the Application of Nitrogen, Zinc and Boron on micronutrients concentration and uptake in grain and straw of wheat in a silty clay loam soil of mid hills. *Int J Adv Agric Sci Technol.* 2016; 3(6):25-39.
 35. Kaya Y, Arisoy RZ, Göcmen A. Variation in grain yield and quality traits of bread wheat genotypes by Zn fertilization. *Pak J Agron.* 2002; 1:142-144.
 36. Kennedy G, Nantel G, Shetty P. The scourge of "hidden hunger": Global dimensions of micronutrient deficiencies. *Food Nutr Agric.* 2003; 32:8-16.
 37. Keram KS, Sharma BL, Sawarkar SD. Impact of Zn application on yield, quality, nutrients uptake and soil fertility in a medium deep black soil (vertisol). *Int J Sci Environ Tech.* 2012; 1(5):563-571.
 38. Khattak SG, Dominy PJ, Ahmad W. Effect of Zn as soil addition and foliar application on yield, and protein content of wheat in alkaline soil. *J Natn Sci Foundation Sri Lanka.* 2015; 43(4):303-312.
 39. Ma G, Jin Y, Li Y, Zhai F, Kok FJ, Jacobson E, Yang X. Iron and zinc deficiencies in China: What is feasible and cost-effective strategy? *Public Health Nutrition.* 2008; 11(6):632-638.
 40. Maret W, Krezel A. Cellular zinc and redox buffering capacity of metallothionein/thionein in health and disease. *Mol Med.* 2007; 13(7-8):371-5.
 41. McDonald GK, Genc Y, Lewis J, Graham RD. Relationships between zinc and other nutrients in wheat grain. 2007. www.zinc.crop.org/2007
 42. Md AA, Kumar M. Effect of Zinc on growth and yield of rice var. Pusa Basmati-1 in Saran district of Bihar. *Asian J Plant Sci Res.* 2015; 5(2):82-85.
 43. Mustafa G, Ehsanullah NA, Saeed AQ, Asif I, Khan H, Jabran ZK *et al.* Effect of Zinc application on growth and yield of rice (*Oryza sativa* L.). *IJAVMS* 2011; 5(6):530-535.

44. Nubé M, Voortman RL. Human Micronutrient Deficiencies: Linkages with Micronutrient Deficiencies in Soils, Crops and Animals. Combating Micronutrient Deficiencies: Food-Based Approaches, 2011, 289-311.
45. Phattarakul N *et al.* Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant Soil*. 2012; 361:131-141.
46. Pooniya V, Shivay YS. Enrichment of basmati rice grain and straw with zinc and nitrogen through ferti-fortification and summer green manuring under indo-gangetic plains of India. *Journal of Plant Nutrition*. 2013; 36(1):91-117.
47. Prasad R. Rice–wheat cropping systems. *Adv Agron* 2005; 86:255-339.
48. Prasad R. Zinc biofortification of food grains in relation to food security and alleviation of zinc malnutrition. *Curr Sci* 2010; 98:1300-1304.
49. Ram H, Sohu VS, Cakmak I, Singh K, Buttar GS, Sodhi GPS *et al.* Agronomic fortification of rice and wheat grains with zinc for nutritional security *Curr Sci*. 2015; 109(6):1171-1176.
50. Ranjbar GA, Bahmaniar MA. Foliar application of Zn fertilizer on yield and growth of bread wheat (*Triticum aestivum* L.) cultivars. *Asian J Plant Sci*. 2007; 6(6):1000-1005.
51. Rengel Z, Batten GD, Crowley DE. Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research*. 1999; 60(1-2):27-40.
52. Salunke R, Rawat N, Tiwari VK, Kumari N, Randhawa GS, Dhaliwal HS *et al.* Determination bioavailable-zinc from biofortified wheat using a coupled in vitro digestion/Caco-2 reporter-gene based assay. *J Food Comp Anal*. 2012; 25(2):149-159.
53. Santos S, Costa CAE, Duarte AC, Scherer HW, Schneider RJ, Esteves VI *et al.* Influence of different organic amendments on the potential availability of metals from soil: A study on metal fractionation and extraction kinetics by EDTA. *Chemosphere*. 2010; 78(4):389-396.
54. Seilsepour M. The study of Fe and Zn effects on quantitative and qualitative parameters of winter wheat and determination of critical levels of these elements in Varamin plain soils. *Pajouhesh & Sazandegi*. 2007; 76:123-133.
55. Shahzad Z, Rouached H, Rakha A. Combating mineral malnutrition through iron and zinc biofortification of cereals. *Comprehensive Reviews in Food Science and Food Safety*. 2014; 13(3):329-346.
56. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr*. 1998; 68(2 Suppl):447S-463S.
57. Sharma R, Agarwal A, Kumar S. Effect of micronutrients on protein content and productivity of wheat (*Triticum aestivum* L.). *Vegetos*. 2008; 21(1):51-53.
58. Slingerland MA. Biofortification in a food chain approach in West Africa. Case study #3-6 of the program “Food policy for developing countries: The role of government in the global food system”, 2007 (Eds. Pinstrup-Andersen, P. and Cheng, F.). Ithaca, New York: Cornell University Press, 2007, 69-80
59. Sommer AL, Lipman CB. Evidence of indispensable nature of zinc and boron for higher green plants. *Plant Physiol*. 1926; 1:231-49.
60. Stein AJ, Nestel P, Meenakshi JV, Qaim M, Sachdev HPS, Bhutta ZA. Plant breeding to control zinc deficiency in India: how cost effective is biofortification? *Pub. Health Nutr*. 2007; 10:492-501.
61. Sudha S, Stalin P. Effect of zinc on yield, quality and grain zinc content of rice genotypes. *Intern J Farm Sci*. 2015; 5(3):17-27.
62. Todd WR, Elvehjem CA, Hart E. Zinc in the nutrition of the rat. *Am J Physiol*. 1934; 107:146-56.
63. Vanlauwe B, Bationo A, Chianu J, Giller KE, Merckx R, Mokwunye U *et al.* Integrated soil fertility management – Operational definition and consequences for implementation and dissemination. *Outlook on Agriculture*. 2010; 39:17-24.
64. Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP. Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of Cereal Science*. 2014; 59(3):365-372.
65. Welch RM, Graham RD. A new paradigm for world agriculture: Meeting human needs—Productive, sustainable, nutritious. *Field Crops Res*. 1999; 60:1-10.
66. White JG, Zasoski RJ. Mapping soil micronutrients. *Field Crop Res*. 1999; 60:11-26.
67. WHO. World Health Rep. 2002 <http://www.who.int/whr/2002/>
68. WHO. UNICEF India, Children Issues. Global Database on Child Growth and Malnutrition in United Nations Administrative Committee on Coordination/Sub-Committee on Nutrition, Low Birth Weight, Nutrition Policy, 2007, 18. <http://www.who.int/nutgrowthdb/en/>
69. Yang XEE, WenRong C, Ying F, Chen WR, Feng Y. Improving human micronutrient nutrition through biofortification in the soil-plant system: China as a case study. *Environmental Geochemistry and Health*. 2007; 29(5):413-428.
70. Yassen AA, Hellal FA, Abo-Basha DM. Influence of organic materials and foliar application of zinc on yield and nutrient uptake by wheat plants. *J Appl Sci Res*. 2011; 7(12):2056-2062.
71. Yilmaz A, Ekiz H, Torun B, Gultekin I, Karanlik S, Bagci SA *et al.* Effect of different zinc application methods on grain yield and zinc concentration in wheat cultivars grown on zinc-deficient calcareous soils. *J Plant Nutr*. 1997; 20(4-5):461-471.
72. Zeidan MS, Mohamed MF, Hamouda HA. Effect of foliar fertilization of Fe, Mn and Zn on wheat yield and quality in low sandy soils fertility. *World J Agric Sci*. 2010; 6(6):696-699.
73. Zoz T, Steiner F, Fey R, Castagnara DD, Seidel EP. Response of wheat to foliar application of zinc. *Cienc Rural*. 2012; 42 (5). Santa Maria. <http://dx.doi.org/10.1590/S0103-84782012005000015>