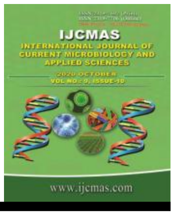
Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 2995-3008

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com



Review Article

https://doi.org/10.20546/ijcmas.2020.910.361

Agronomic Bio fortification in Rice with Zn

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ABSTRACT

Zinc (Zn) deficiency is a well documented public health issue and an important soil constraint to crop production. Also, there is a close geographical overlap between soil and human deficiency of Zn and Fe indicating a high requirement for increasing concentrations of micronutrients in food crops. Breeding new plant genotypes for high grain concentrations of Zn (genetic biofortification) is the most cost-effective strategy to address the problem; but, this strategy is a long-term process. A rapid approach is therefore required for biofortification of food crops with Zn and represents useful complementary approach to on-going breeding programs. In this regard, agronomic biofortification or fertilizer strategy (ferti-fortification) represents an effective way and rapid process for combating Zn malnutrition in developing countries. Ferti-fortification is the application of fertilisers to seeds, soil and/or foliage, at rates greater than those required for maximum yield, to increase the uptake of nutrients into the plants and its translocation into seeds. Zinc-enriched grains are of great importance for crop productivity resulting in better seedling vigour, denser stands and higher stress tolerance on potentially Zn-deficient soils. Soil plus foliar application of micronutrients is the best application method and foliar sprays should be done at proper stages of crop growth to increase the grain yield and micronutrient concentration in grain part. This review discusses bio-fortification and ferti-fortification studies of Zn, its deficiency in human populations, public health and socioeconomic concerns and future prospects/ strategies to reduce its deficiency in soil and ultimately human populations.

Keywords

Agronomic,
Bio fortification,
Rice, Zn deficiency

Article Info

Accepted:
24 September 2020
Available Online:
10 October 2020

Introduction

In the developing world Zinc is a growing public health and socioeconomic issue (Welch and Graham 2004). Micronutrient malnutrition afflicts over three billion people worldwide and the numbers are continuously increasing. It is due to strong detrimental dietary preferences, low consumption of animal products, fruits and vegetables, intake of poor quality diets like cereals as these are inherently very low in zinc contents particularly when grown on potentially deficient soils. Several strategies have been suggested as intervention programmes for the reduction of micronutrient malnutrition in human populations (Maberly *et al*., 1994). They include food fortification as done in salt, sugar, cereals, milk and tea; dietary supplementation by use of iodized oil, vitamin A capsules and iron tablets. Other strategies are dietary diversification through consumption of red meat, liver and adequacy of vitamin C and biofortification through agronomic approach and Genetic approach. Agricultural strategies that are used to improve the nutritional value of crop plants are known as biofortification strategies (Cakman and Kutman, 2018). Genetic approach is a strategy that uses plant breeding techniques to produce staple food crops with higher micronutrient levels, reducing levels of anti-nutrients and increasing the levels of substances that promote nutrient absorption (Bouis, 2003). It offers a sustainable solution to malnutrition problems by exploring natural genetic variation to develop mineral-dense crop varieties (Pfeiffer and McClafferty, 2007a) and is the most cost-effective strategy to address the problem; but, this strategy is a long-term process. A rapid and complementary approach is therefore required for bio fortification of food crops with Zn short term. In this regard, agronomic bio fortification (fertilizer strategy) seems to be a very cost-effective, fast and practical approach to improve Zn, concentration in cereal crops. Agronomic approaches to biofortification include Ferti-fortification (adequate fertilization, method of fertilization, time of application), FYM application, intercropping and Crop rotation**.** Ferti - fortification is the application of fertilisers to seeds, soil and/or foliage, at rates greater than those required for maximum yield, to increase the uptake of nutrients into the plants and its translocation into seeds (Prasad, 2007).

Zinc deficiency in human populations and crop production

Zinc deficiency is a well-documented, global micronutrient deficiency problem both in human populations and in crop production. Close geographical overlapping between soil and human deficiency of Zn, indicates a high requirement for increasing concentrations of micronutrients in food crops (Fig. 1).

It is estimated that about 50% of the cereal-cultivated soils globally are deficient in plant-available zinc, leading to reductions in crop production and also nutritional quality of the harvested grains. Zinc is an essential constituent of several enzymes like carbonic anhydrase and dehydrogenase. It also controls the synthesis of Indole acetic acid which is an important growth regulator (Graham *et al.*, 1992). Dietary zinc deficiency is associated with severe consequences in human health, including impairments in brain function and development, weakness of the immune system to deadly infectious diseases and delays in physical development. According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11th among the 20 most important factors in the world and 5th among the 10 most important factors in developing countries. Deficiencies of Zn and other micronutrients in developing countries are also reported to cause great economic losses and have a considerable effect on the gross national product by decreasing productivity and increasing the health care costs (Stein, 2014). Based on a range of reports and survey studies, the average concentration of Zn in a whole grain of wheat in various countries is between 20 to 35 mg kg^{-1} (Rengel *et al.*, 1999; Cakmak, 2004). The Zn concentrations reported are too low to meet daily human requirement, especially for those consuming a high proportion of cereal-based diets. For a measurable biological impact on human health, the concentration of Zn in whole wheat grain needs to be increased at least by approximately 10 mg kg^{-1}, assuming a 400 g per day intake for adult woman in the countries where whole grain flour is used for making food like chapatti in India (Pfeiffer and McClafferty, 2007b). Generally, recommended dietary allowance for Zn is around 15 mg per day (National Research Council 1989) (Table 1). Zinc content of some foods is shown in (Table 2).

Besides having inherently low levels of Zn, wheat grain is also rich in substances limiting utilization (bioavailability) of Zn in the human digestive tract, such as polyphenols and phytic acid (Welch and Graham 2004). Phytic acid is the major storage compound of phosphorus in grain. By binding Zn, phytic acid reduces solubility of Zn in food and restricts its utilization and retention in human body. Most of the seed-Zn is located in the embryo and aleurone layer, whereas the endosperm is very low in Zn concentration (Ozturk *et al.*, 2006). The embryo and aleurone parts are also rich in protein and phytate (Lott and Spitzer 1980; Mazzolini *et al.*, 1985), indicating that protein and phytate in seeds could be sinks for Zn. According to a Zn-staining study in wheat seed (Fig. 2), 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. 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. Ccommonly used Zinc Fertilizer materials are shown in (Table 3).

Enrichment of cereal grains with zinc through ferti-fortification

Genetic biofortification and agronomic biofortification are two important agricultural tools to improve cereal grain Zn concentration (Cakmak, 2008; Pfeiffer, 2007). However, yield factor, interactions between genotype and environment, lack of sufficient genetic diversity in current cultivars for breeding program, consumer resistance and safety of genetically modified crops are the main bottlenecks of genetic biofortification (Falk, 2002; Palmgren, 2008 and Joshi, 2010). The traditional and efficient strategy of agronomic biofortification, such as Zn fertilization (ferti-fortification) is, therefore essential and rapid solution for improving Zn concentration in rice grain to address the ongoing human Zn deficiency.

Considerable progress has been made on the impact of foliar Zn fertilization on biofortification of Zn in rice grain (Fang *et al.*, 2008, Phattarakul *et al.*, 2012, Wissuwa *et al.*, 2008) since it has the advantages of low application rates and avoiding Zn losses through soil fixation (Nasri *et al.*, 2011). Furthermore, foliar applied Zn caused greater increases in brown rice Zn concentration than soil application (Phattarakul *et al.*, 2012). There is evidence in literature demonstrating that foliar applied Zn can be absorbed by leaf epidermis, and remobilized and transferred into the rice grains through the phloem (Wu *et al.*, 2010) and several members of the Zn-regulated transporters regulate this process (Bashir *et al.*, 2012). Brown rice Zn concentration was significantly increased by foliar Zn fertilizations (Fig. 3A).

Zn concentration in brown rice was increased from 30.28 mg kg^{-1} in the control to 33.75 mg kg^{-1} by foliar Zn-EDTA application, to 35.07 mg kg^{-1} by foliar Zn-Citrate application, to 38.45 mg kg^{-1} by foliar $ZnSO_4$ application, to 39.84 mg kg^{-1} by foliar Zn-AA application, these represented increases of 11.46%, 15.81%, 27.26% and 31.58%, respectively. Zn concentration in polished rice was significantly increased by foliar Zn fertilizations. Zn concentration in polished rice was increased from 22.92 mg kg^{-1} in the control, to 25.26 mg kg^{-1} by foliar Zn-EDTA application, to 26.09 mg kg^{-1} by foliar Zn-Citrate application, to 28.08 mg kg^{-1} by foliar $ZnSO_4$ application, to 28.67 mg kg^{-1} by foliar Zn-AA application, these represented increases of 10.22%, 13.82%, 22.47% and 24.04%, respectively. Thus, foliar Zn fertilization could increase Zn concentration in brown rice and polished rice depending on Zn form (Wei *et al*., 2012). Ozturk *et al.*, (2006) studied changes in grain Zn concentration in wheat during the reproductive stage and found that the highest concentration of grain Zn occurs during the milk stage of grain development. Foliar application of Zn during reproductive growth seems to be more effective in increasing grain Zn concentration than spraying of Zn at earlier growth stage (Fig. 4).

In nutrient solution experiments with rice (*Oryza sativa* L.), post-flowering Zn uptake equalled or surpassed grain Zn content at maturity over a wide range of applied Zn levels (Jiang *et al*., 2008). Habib (2009) reported that foliar application of (Fe+Zn) at tillering and heading stage increased Zn concentration up to 20.27 from 12.17 $mg.kg^{-1}$ and also Fe concentration increased compared with control (from 84.93 to 139.6 $mg.kg^{-1}$) (Table 4). Yilmaz, *et al*., 2008 found that increase in Zn concentration with the soil-leaf application of Zn were about 7 fold for shoot and 3.5 fold for grain compared to the control (Table 5). Combined application of soil and foliar Zn fertilizers caused more than a 3 fold increase in grain Zn (Fig. 5).

Two foliar spraying of Zn (@ 0.5 % $ZnSO_4.7H_2O$) along with basal application (@ 20 kg Zn ha^{-1}) increased the grain Zn content to the level of two to three times, while only soil applied Zn increased it very slightly indicating that basal application of Zn showed a trend to increase the grain yield, whereas, soil plus foliar application was proven to enhance the grain Zn content *i.e.* to increase the quality of the final produce (Saha *et al.*, 2013). In rice, soil Zn application has been reported to increase grain yield whereas foliar-Zn application increased grain concentration of Zn (Wissuwa *et al*., 2008). Increased Zn application to soil significantly increases its concentration in the edible plant parts of staple food crops (Welch, 2002; Furlani *et al.*, 2005).

Jat *et al.*, (2008) reported that application of 2.0% ZEU with $ZnSO_4.7H_2O$ recorded significantly higher Zn concentrations in grain (23.0 mg kg^{-1}) and straw (177.7 mg kg^{-1}) of aromatic hybrid rice followed by 2.0% ZEU (ZnO). The Zn concentration in rice straw was 7-8 times higher than that in grain. So 2.0% ZEU ($ZnSO_4.7H_2O$) is an excellent source of N and Zn for improving productivity as well as for agronomic ferti-fortification of the aromatic hybrid rice (Table 6). Kanwal *et al.*, 2010 reported that incremental addition of Zn to the soil significantly affected grain Zn accumulation in maize plants which ranged from 21.82 to 30.65 mg kg^{-1} (Fig. 6). Applying Zn-coated urea fertilizers (up to 3 % Zn) increased both grain yield and grain Zn concentration in rice (Shivay *et al*., 2008; Table 7).

According to Nattinee *et al.*, (2009) Soil Zn application did not increase grain Zn concentration but foliar applied Zn significantly and consistently increased grain Zn concentration from 20.5 to 45.5 mg Zn kg^{-1} in unhusked rice and from 20.8 to 27.7 mg Zn kg^{-1} in brown rice (Fig. 7). Zeidan (2001) indicated that Zn application significantly increased grain protein and enhanced grain Zn concentration, while simultaneously reduced grain P concentration. Lungu *et al.*, (2010) reported the greatest increase in grain Zn concentration from a combination of soil Zn and foliar application (Table 8).

Table.1 Recommended dietary allowance (RDA) of Zn

Micronutrients	**Recommended dietary allowance (mg/day)**
Zinc (Zn)	Infants: 5 Children : 10 Males: 15 Females: 12

Prasad (2007)

Table.2 Zinc content of some foods

Food item	**Zn (mg/Kg)**
Brown rice	18
Polished rice	13
Wheat grain	32–59

Prasad (2007)

Table.3 Commonly used zinc fertilizer materials

Compound	**Formula**	**Zinc Content (%)**
Inorganic Compounds		
Zinc sulphate monohydrate	$ZnSO_4.H2O$	36
Zinc sulphate heptahydrate	$ZnSO_4.7H2O$	22
Zinc oxysulphate	$ZnO.ZnSO_4$	20-50
Basic zinc sulphate	$ZnSO_4.4Zn(OH)_2$	55
Zinc oxide	ZnO	50-80
Zinc carbonate	$ZnCO_3$	50-56
Zinc chloride	$ZnCl_2$	50
Zinc nitrate	$Zn(NO3)_2.3H2O$	23
Zinc phosphate	$Zn_3(PO4)2$	50
Zinc frits	Fritted glass	10-30
Ammoniated zinc sulphate solution	$Zn(NH_3)4SO4$	10
Organic Compounds		
Disodium zinc EDTA	$Na_2ZnEDTA$	8-14
Sodium zinc HEDTA	NaZnHEDTA	6-10
Sodium zinc EDTA	NaZnEDTA	9-13
Zinc polyflavonoid	-	5-10
Zinc lignosulphonate	-	5-8

Alloway (2008)

Table.4 Effect of foliar application of Zn on wheat yield (kg ha^{-1}), seed-Zn and Fe Concentration (mg kg^{-1})

Treatment\Adjective	**Yield ± SE**	**Zn ± SE**
Fe	8185 ± 93.853	22.6 ± 1.493
Zn	7919 ± 67.3	50.9 ±1.114
Fe+Zn	8954 ± 74.638	20.27 ± 1.291
Control	7665 ± 97.994	12.17 ± 1.317
LSD (=0.01)	414.7	3.85

Table.5 Effect of different Zn application methods on Zn concentration in wheat

Zn application methods	**Zn concentration (mg/kg)**	
	Whole shoot	**Grain**
Control	10	10
Seed coating	12	10
Soil	19	18
Foliar	60	27
Soil + Foliar	**69**	**35**

(1)control (no Zn application); (2) 23 kg Zn/ha as broadcast to soil; (3) seed coating (1L 30% ZnSO4 sprayed on to 10 kg seeds and then the seeds dried and sown); (4) foliar application (440 g Zn/ha as ZnSO4 in 450 l at tillering and stem elongation); (5) combination of the methods 2 and 4

Table.6 Effect of Zn fertilization on yield and Zn concentrations of aromatic hybrid rice

Treatments	**Zn applied (kg/ha)**	**Grain yield (t/ha)**	**Grain Zn conc. (mg/kg)**	**Straw Zn conc. (mg/kg)**
Absolute Control (No N & No Zn)	-	5.03	15.0	125.2
Control (Only N)	-	6.74	17.0	144.0
2.0% ZEU ($ZnSO_4.7H_2O$)	5.0	7.53	23.0	177.7
2.0% ZEU (ZnO)	5.0	7.30	20.0	164.6
5.0 kg Zn/ha ($ZnSO_4.7H_2O$)	5.0	7.17	21.1	161.3.
5.0 kg Zn/ha (ZnO)	5.0	7.04	19.2	151.8
CMCU	-	6.80	17.0	143.1
SEm±	-	0.12	0.08	0.56
CD (P=0.05)	-	0.33	0.24	1.60

ZEU*, Zn- enriched urea; **CMCU**,** Coating material coated urea

Table.7 Effect of Zn-enriched urea (ZEU) (up to 3 % Zn in urea) on grain yield and grain Zn concentrations of aromatic rice grown in India. Data show average values of 2-year field trials

Treatments	Grain Yield **(ton ha^{-1})**	Zn Concentration **(mg kg^{-1} DW)**
Prilled Urea	3,87	27
0.5% ZEU	4,23	29
1.0%	4,39	33
2.0%	4,60	39
3.0%	4,76	42

Table.8 Effect of soil and foliar application of zinc on grain zinc concentration of wheat

Treatment	**Grain Zn, mg kg^{-1}**
Control	23.5 ± 2.1
NPK + Urea	20.8 ±2.2
Soil Zn	23.8 ± 1.1
NPK + Urea + Soil Zn	24.2 ± 3.2
Soil Zn + 2X Foliar Zn	43.0 ± 5.6
NPK+ Urea + Soil Zn +2X Foliar Zn	46.4 ± 4.7

Table.9 The concentration of Zn in growth media and in plants (mg x kg^{-1}) grown on media consisting of soil control, FA1, FA2, BA or FA2+BA

Media	**Zn con.in Growth medium**	**Barley**	**Oats**	**Rye**	**Triticale**	**Winter wheat**
Soil	15.2± 0.4a	17.5± 3.3a	33.4± 6.2a	27.0± 4.1a	31.1± 3.4a	18.0± 2.4a
FA1	51.1± 7.4	92.8± 19.1b	**133.4± 22.2b**	87.8± 8.9b	78.0± 7.9b	**192.2± 29.8b**
FA2	46.9± 8.3b	89.4± 14.0b	134.2± 25.5b	93.1± 9.4b	74.2± 8.1b	167.6± 24.2b
BA	21.7±3.7c	51.4±7.7c	93.6± 17.0c	53.3± 5.2c	53.8± 6.8c	76.5± 16.0c
FA2+BA	31.1± 5.7c	50.2± 6.7c	84.8± 19.3d	60.8± 7.7c	47.9± 5.1c	73.6± 17.3c

Soil as a control
FA1 (Fly ash) from lignite coal
FA2 from semi-bituminous coal
BA (bottom ash) from semi-bituminous coal
FA/BA (1:1 weight based) from semi-bituminous coal

Table.10 Effect of zinc, iron and manganese on mineral composition of rice KS- 282

Treatment	Zn (mg/kg)
Control	27.10e
NPK	28.00e
NPK+Fe	25.23f
NPK+Mn	26.60ef
NPK+Fe+Zn_1	36.97d
NPK+Fe+Zn_2	44.47b
NPK+Mn+Zn_1	39.17c
NPK+Mn+Zn_2	46.53a

Means sharing the same letter (s) do not significantly at P=0.05 level of probability (DMR test)
NPK=100:50:50 (mg/kg soil)
Fe:Mn:Zn_1:Zn_2= 10:10:5:10 (mg/kg soil)
Design = RBD
Soil – Sandy clay loam

Fig.1

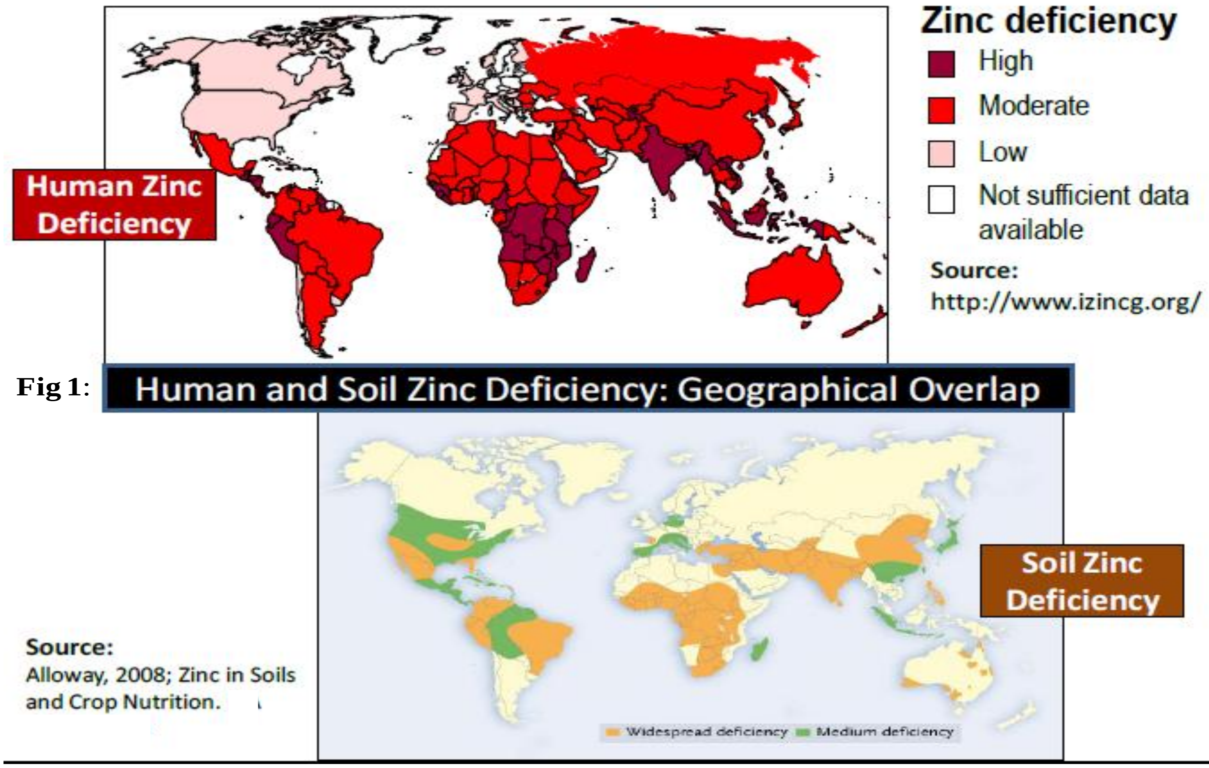


Fig.2 Diphenyl thiocarbazone (DTZ) staining a wheat seed. When reacting with Zn, DTZ forms a red DTZ-complex which indicates localization of Zn (Ozturk *et al.*, 2006)

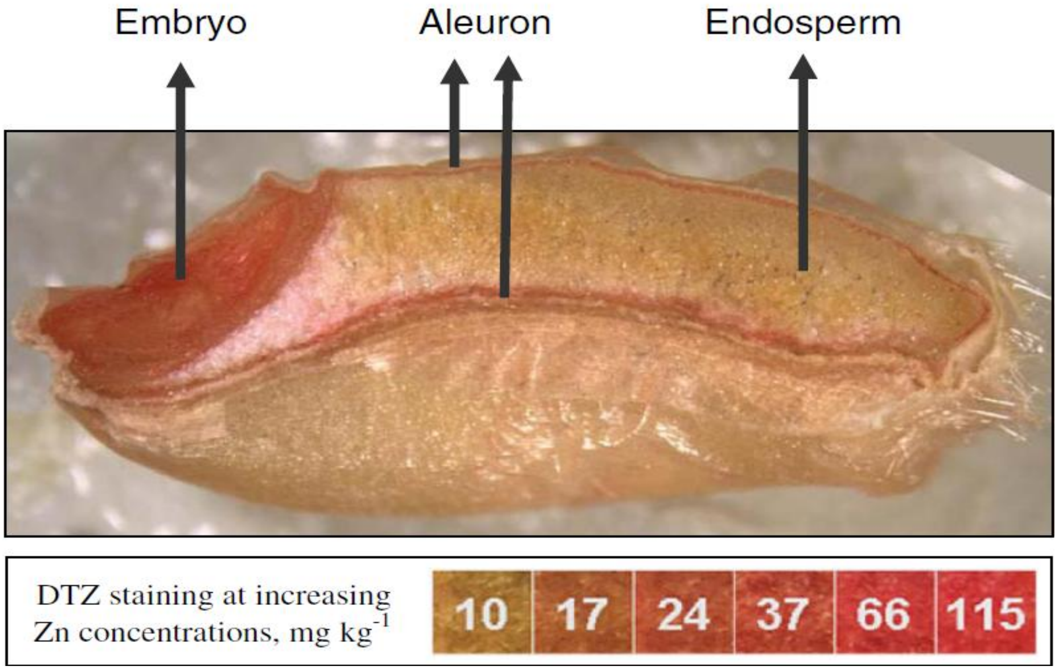


Fig.3 Effect of different forms of foliar Zn fertilization on Zn concentration in rice grain. (A) Zn concentration in brown rice. (B) Zn concentration in polished rice

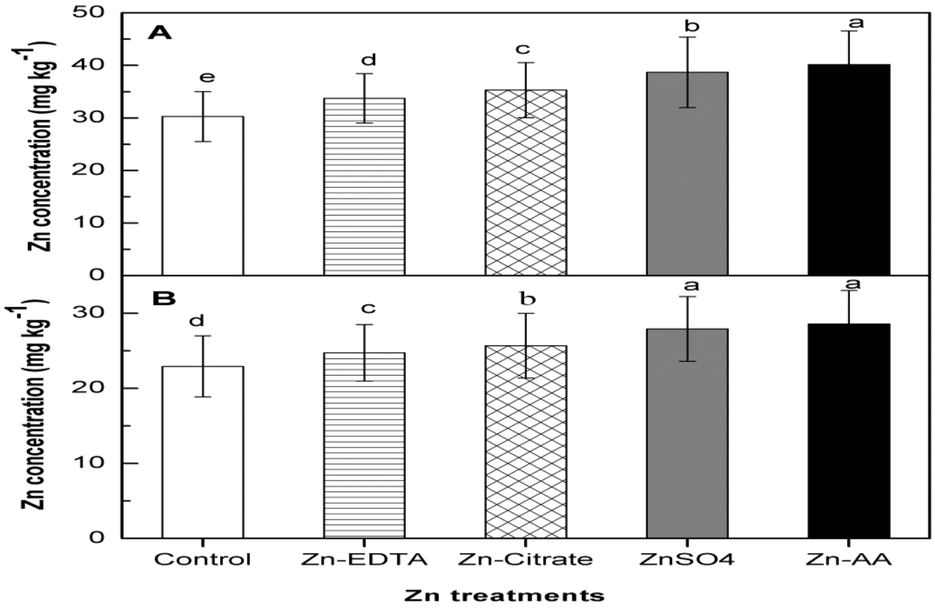


Fig.4 Effect of time of foliar application of Zn on grain Zn concentration in wheat

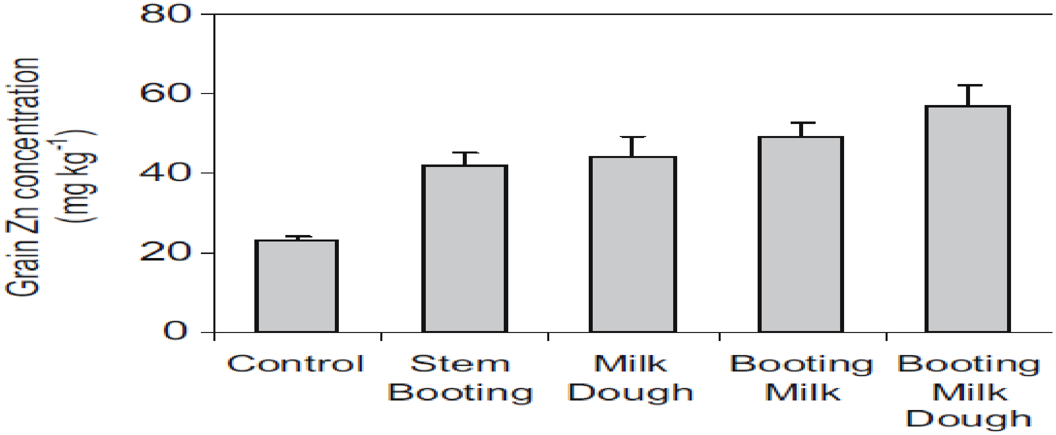


Fig.5 Enrichment of durum wheat grain with Zn applied through soil and foliar spray

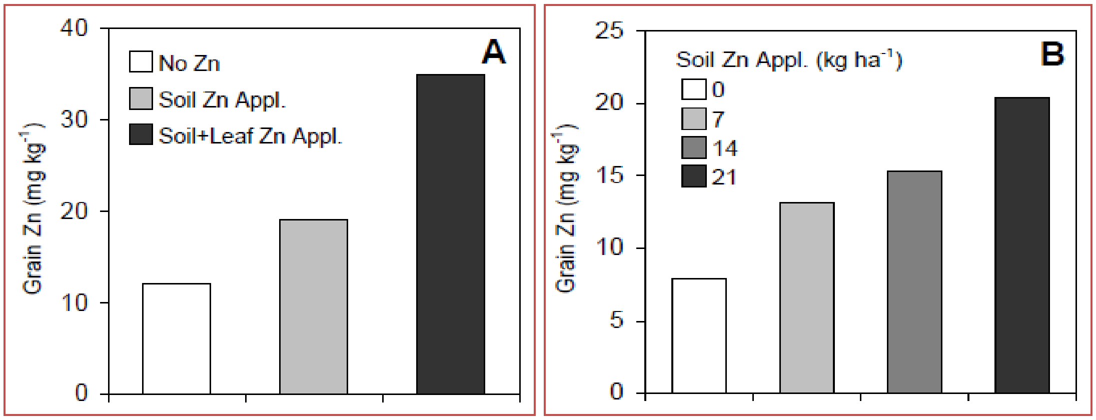


Fig.6 Grain Zn concentration of maize hybrid (FHY-421) and variety (Golden) at different rates of Zn application

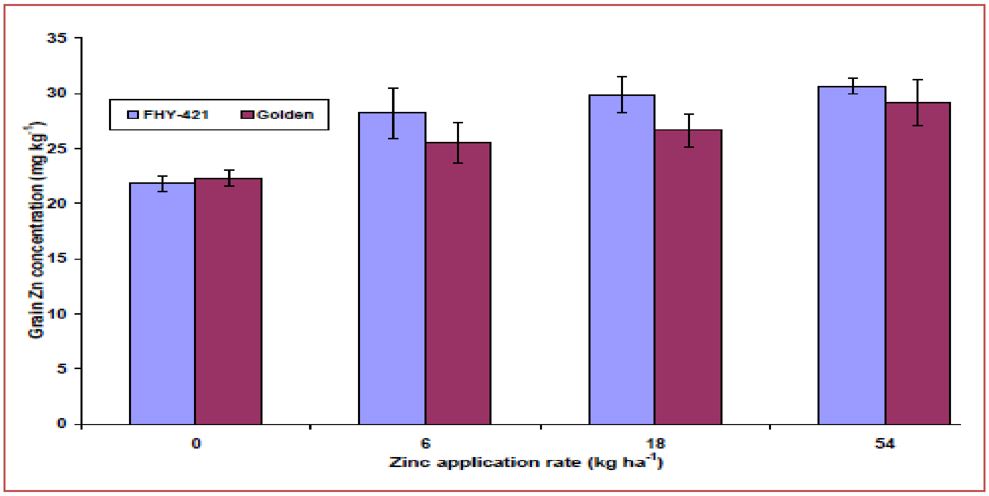


Fig.7 Concentration of Zn in rice seeds at 4 farmer's locations in Thailand

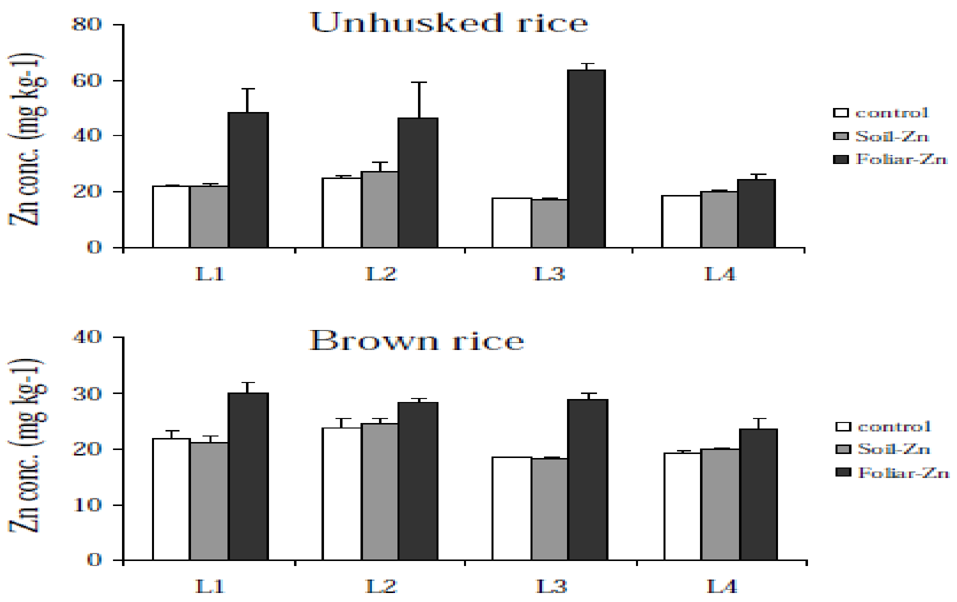
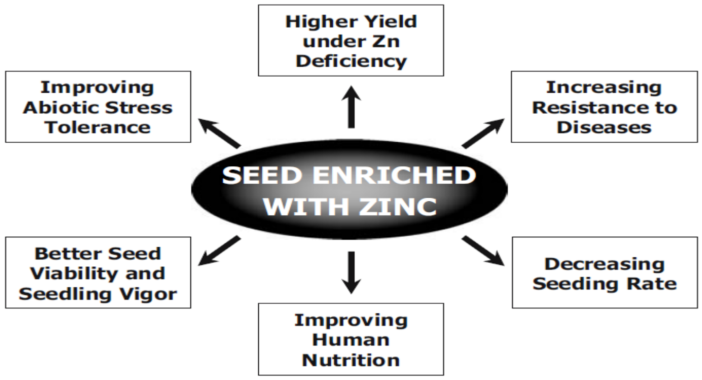


Fig.9 Agronomic and human nutritional benefits resulting from use of Zn enriched seeds



Zn fertilization significantly increased Zn density in polished rice, with a more pronounced effect of $ZnSO_4$ being observed as compared with Zn-EDTA, especially under an alternate wetting and drying **(**AWD) regime (Fig. 8). Bilski *et al.*, (2012) reported significant increase of Zn accumulation in plants grown on coal ashes and suggests that these coal combustion residues might be treated as Zn fertilizers especially desirable in various developing countries (e.g. in Africa) where the farmers cannot afford application of mineral fertilizers (Table 9).

Abid *et al.*, (2001) reported highest Zn and Mn concentration in grain with $NPK+Mn+Zn_2$ and $NPK+Mn+Zn_1$ respectively. Both these treatments differed significantly with all other treatment, was probably due to the more balanced nutrient ratio (Table 10).

Hussain *et al.*, 2014 also reported highest zinc content in brown rice with the application of EDTA-chelated zinc (53.20 mg/kg) followed by zinc FYM incubated (49.55 mg/kg), zinc sulphate (46.40 mg/kg) and control (30.04

mg/kg). Increasing grain Zn by soil and/or foliar applications also provides additional positive impacts in terms of seed vitality and seedling vigor. Priming seeds in Zn-containing solutions is an alternative way to increase seed Zn prior to sowing. High seed Zn concentrations ensure good root growth and contribute to better protection against abiotic and biotic stress like soil borne pathogens (Fig. 9) (Cakmak, 2012).

In conclusion, all of the approaches of fortification in general and biofortification in particular are complementary to each other and should go hand in hand to alleviate the major concern of under nutrition. Ferti-fortification can be a rapid solution to enrich the cereals. Seed biofortification by foliar feeding is more excellent to increase the grain yield and micronutrient concentration in grain part and has low cost as compared to other chemical methods. However, foliar sprays should be done at proper stages of crop growth. Priming seeds in Zn-containing solutions is an alternative way to increase seed Zn prior to sowing. High seed Zn concentrations ensure good root growth and contribute to better protection against abiotic and biotic stress like soil borne pathogens. Seedlings grown on coal ash based media also showed hyper accumulation of Zn as compared to plants grown on normal soil thus coal combustion residues can be treated Zn fertilizers especially desirable in various developing countries. Moreover, intercropping and crop rotation systems also contribute to grain Zn concentrations and the combined applications of Zn fertilizers together with organic materials (like farmyard manure and green manures) are effective in facilitating Zn uptake by roots and correcting Zn deficiency. There are also promising cultivars for higher accumulation of Zinc. Agronomic experiments need to be planned and conducted for developing micronutrient fertilizer doses, methods and timings for their application for increasing not only yield but also the concentration of micronutrient in grains.

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How to cite this article:

Sabia Akhter, Rubia Rasool, Ambreen Nabi, Vaseem Yousuf, Nasir A. Dar and Khurshid Ahmad Sofi, Rafia Munshi and Mir Nadeem. 2020. Agronomic Bio fortification in Rice with Zn. *Int.J.Curr.Microbiol.App.Sci.* 9(10): 2995-3008.
doi: https://doi.org/10.20546/ijcmas.2020.910.361