



Agronomic Bio-fortification of Rice and Maize with Iron and Zinc: A Review

**Bisma Jan¹, Tauseef A. Bhat¹, Tahir A. Sheikh¹, Owais Ali Wani^{1*},
M. Anwar Bhat¹, Ajaz Nazir¹, Suhail Fayaz¹, Tabish Mushtaq¹,
Anees Farooq¹, Suffiya Wani¹ and Aabiroo Rashid¹**

¹Faculty of Agriculture, Wadura Sopore, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, 193201, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors BJ and OAW compiled and drafted this review others assisted in collecting literature relating to this study. All authors read and approved the final manuscript.

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ABSTRACT

Earlier, the agriculture system was oriented more towards achieving higher agronomic yields than the nutritional quality of food. Green revolution significantly enhanced the crop production primarily rice, wheat and maize production was boosted to meet the energy needs of growing population. As a consequence of the predominance of cereal-based staples that are fundamentally low in micronutrients, specifically Zn and Fe, more than 2 billion people worldwide suffer from an insidious type of deficiency known as micronutrient malnutrition. Just moderate amounts of micronutrient malnutrition can affect cognitive development, reduce disease resistance and increase the risk of women dying during childbirth. The approach to micronutrient fertilization has been shown to improve the yield and nutritional content of the staples. Agronomic biofortification provides an immediate and effective method to enhance accumulation of micronutrients especially Zn and Fe in cereals. An adequate amount of plant available micronutrients is a prime requisite to ensure adequate nutrient uptake. Most of the cereals are grown in soils deficit in Zn and under reduced conditions of rice ecosystem, its availability is decreased due to formation of less soluble Zn complexes with sulphate and carbonate. The form of fertilizer used, timing and method of

*Corresponding author: E-mail: owaisagrisoil@gmail.com, owaisaliwani@gmail.com;

application is critical for the enhancement of the grain quality of Zn and Fe. The effectiveness of agronomic biofortification can be enhanced by application of synthetic chelated micronutrient fertilizers and/or organic fertilizers fortified with micronutrients in combination with NPK ensuring proper nourishment of crops with adequate nutrient supply by slow release of nutrients in soil solution. Further, the response of foliar application has shown better results than soil application. Previous studies suggest that Zn fertilization not only enhances Zn concentration in grain but also improves the overall performance of maize crop. Agronomic biofortification of crops is advantageous in terms of accessibility, rapid result, ease in application and high sustainability.

Keywords: Agronomic approaches; biofortification; iron; maize; rice; zinc.

1. INTRODUCTION

The era of “Green revolution” primarily focused on the staple crops viz., rice, wheat and maize to meet the energy requirements of rapidly growing population. However, micronutrient deficiencies were subsequently noticed in many parts of the world with the introduction of high yielding varieties (HYVs) [1]. Such micronutrient shortages were due to the high yield capacity and high nutritional needs of these high yielding genotypes, which resulted in improper usage of high analytical fertilizers, no introduction of organic manures, imbalanced fertilization, etc [2]. These micronutrients are not only essential to plants, but help in sustaining human as well as animal health. Inadequate consumption or lack of essential micronutrients limits plant growth by affecting the metabolic biochemical reactions, leading to reduced yield and quality [3]. Micronutrient deficiency has affected more than 2 billion people, mostly in Africa and South Asia [4]. It is noteworthy that the problem of malnutrition occurs in major parts of the globe predominantly in developing countries where people consume few starchy staple food crops that are poor in micronutrient [5]. To order to resolve the issue of hunger, therefore, food protection policies would put adequate emphasis on 'Micronutrient Safety,' i.e. efficient development of healthy, secure food. In spite of this, biofortification has developed as one of the world's leading methods for tackling micronutrient deficiency. Biofortification is an action technique [6]. Although, biofortified crops cannot meet the nutritional requirements of people on daily basis as that of commercially fortified food or supplements but it provides a way to reach the population where these means may be difficult to implement and/or adopt due to geographical or financial problems [7]. Moreover, it can help to improve the daily adequacy of micronutrient intakes. Thereby reducing the widespread gap between micronutrient requirements and intake.

2. BIOFORTIFICATION VIA AGRONOMIC PRACTICES

Agronomic bio fortification is the application of a micronutrient containing mineral fertilizer to soil and/or plant leaves (foliar) to improve the micronutrient quality of the edible portion of food crops [8]. It temporarily increases the nutritional status of the crop, thereby fulfilling the human nutritional requirement [9]. It is also termed as ferti-fortification (Prasad 2009). This method is beneficial for growing micronutrients that can be directly absorbed by the plant, such as zinc, but not so for micronutrients that are synthesized in the plant and cannot be absorbed directly by the plant [10].

The effectiveness of agronomical biofortification is calculated by a variety of variables. The state of soil micronutrients and their availability for plant absorption, fertilization methods, soil alteration, cropping systems, etc. are essential factors that affect the system's nutrient production [11]. Furthermore, bioavailability of nutrients at different stages of crop growth, nutrient-allocation and retranslocation of nutrients to the edible parts of crop also influence the nutritional status of crop [6]. Soil applications are stated to be more frequent and efficient in higher levels of nutrients needed. Nonetheless, micronutrient foliar fertilization also stimulates more nutrient uptake and effective allocation of edible plant parts than soil fertilization, particularly in cereals and leafy vegetables [12]. In case of foliar fertilization, high surface area is primary requisite for absorbing the applied nutrient solution in sufficient amount and at times need to go for more than one application depending upon the intensity of nutritional deficiency [13]. Although, foliar application proves to be more efficient pathway but an immediate rainfall can easily wash-off the applied fertilizers, thus adding to the input cost [14]. According to Cakmak et al. [15] soil application is more efficient in increasing grain yields, but Zn

accumulation is much more pronounced in foliar application than soil application [16]. Therefore, combination of soil and foliar application proves to be a reliable method and more advantageous [17].

Soil factors such as pH, soil aeration, organic matter content, soil moisture and interaction among various nutrients influence the bioavailability and uptake of micronutrients by plants from the soil [18,19]. Furthermore Marschner et al. [20], higher plants exude a range of organic acids or protons (H^+ ions) into the soil, thus modifying their rhizosphere to increase the micronutrient availability in the soil [21]. Various interactions among nutrients in soil are another determinant influencing the effectiveness of agronomic biofortification. Several studies showed positive interaction of Fe and Zn content in grains with nitrogen fertilizer [22]. As reported by Singh and Prasad [23] addition of nitrogen is able to increase the further iron content in rice grain. On the other hand, phosphorus helps plants to grow prolifically therefore reduces the toxicity of Fe and Zn by decreasing their concentration due to dilution effect [24]. Phosphorus also promotes Zn deficiency by precipitating Zn in soil [25].

3. AGRONOMIC BIOFORTIFICATION IN RICE

Rice plays an important role in food security for its wider adaptability while being a staple food crop for more than a billion people [26]. However, it is a poor source of essential micronutrients such as Zn and Fe [27,28], thus clearly highlighting the immense need of biofortification in rice. Soil Zn availability in rice is affected by the factors including soil redox potential, sulphur content and soluble bicarbonate. As rice is cultivated under flooded conditions (reduced) where Zn is bound to Sulphur and carbonate, thus becomes unavailable to the crop [29]. For the biofortification of cereals with Zn, it is critical to maintain an adequate level of plant available Zn in soil. However, increased rate of Zn fertilizer may increase the Zn uptake, but this could prove uneconomical approach as the cost of cultivation increases [30]. Alternatively, use of synthetic chelates and/or foliar fertilization (no fixation) can be effective as well as practical approach [6]. Application of zinc to soil as fertilizer in addition to foliar spraying proves to be an effective technique for growing the zinc grain content of rice grown in soils with a low zinc context [31].

The timing of foliar Zn fertilizer application is an important determinant of its effectiveness in terms of biofortification [6]. Foliar application of Zn at later stages of crop development is particularly effective in enriching the grain with Zn [15]. For the reason that immature leaves are physiologically incapable of exporting nutrients until they mature. As reported by Saha et al. [32] that foliar application of Zn at late stage (flowering) yielded significantly more Zn-dense grains than that at early stage (maximum tillering) (Table 1).

The effectiveness of foliar Zn fertilization on Zn concentration of brown and polished rice varied with sources of Zn fertilizer. In spite of supplying the lowest amount of zinc through Zn EDTA treatment, highest yield and Zn mobilization efficiency was reported as compared to other Zn sources [33]. In an experiment, among various forms tested for foliar application, foliar Zn-Amino-acid and $ZnSO_4$ had significant impact on improving the Zn concentration in brown rice and polished rice than Zn-EDTA and Zn-citrate (Fig. 1) [34]. And with respect to N and K uptake in grain as well as straw Zn-EDTA gave better response among the various sources (Zinc sulphate, Zinc oxide, Zinc enriched urea, Zinc FYM incubated and Zn-EDTA) being at par with zinc enriched urea and Zn-FYM incubated but significantly higher than ZnO and $ZnSO_4$ [35] (Syed et al., 2017). Similarly, Naik and Das [34] also found better response of Zn-EDTA over $ZnSO_4$ because application of chelated-Zn facilitates greater absorption and maintains Zn in soil at a steady rate as compared to $ZnSO_4$. Evaluation of different Zn concentrations (0, 5, 10, 15 and 20 mg Zn kg^{-1} soil) in four rice genotypes (Jhelum, SR1, China 1007 and China1039) in a pot experiment showed that Zn concentration in brown rice due to the progressive increase in Zn levels increased significantly only upto 15 mg Zn kg^{-1} soil (Table 2) [36].

Among various approaches chosen to increase the Fe concentration in rice grains, fertilization especially foliar is considered as a rapid and efficient method [20,37]. Generally, the solubility of Fe compounds is relatively low but reduced conditions and/or lowering of pH favours conversion of insoluble ferric oxides (Fe^{3+}) to plant available ferrous oxides (Fe^{2+}). Biofortification of rice plants by foliar spray of Fe is an effective way to promote Fe concentration in rice grains [38]. Application of Zn fertilizer had a significant effect on Fe content of grain, which

increased drastically as the concentration of zinc fertilizer increased from 0 to 0.45 kg ha⁻¹. The result suggests simultaneous biofortification of Zn and Fe can be achieved. Sperotto et al. [22] demonstrated that foliar sprays of different forms of Fe at the anthesis stage remarkably increased the Fe concentration of polished rice with elevating effects of FeSO₄ and DTPA-Fe than those of HEDTA-Fe (hydroxyethylethylenediaminetriacetic acid) and EDTA-Fe (Ethylenediaminetetraacetic acid) Na (Fig. 2). Fe fertilizer via foliar application of FeSO₄.7H₂O significantly increases the yield and

Fe concentration in rice grain as well. Different cultivars showed differential response to foliar spray of 0.5 and 1% of FeSO₄.7H₂O at different growth stages (maximum tillering, pre-anthesis and post-anthesis) in terms of yield, uptake and concentration of Fe in rice crop (Table 3) [39]. The concentration of nutrients varies with the part of plant and age of leaf during the panicle differentiation stage in rice crop [40]. Foliar application enhance Fe concentration, particularly if applied at later stages of development, preferably during post anthesis [39].

Table 1. Effect of Zn application on Zn concentration (mg kg⁻¹) in grains of the tested rice cultivars [32]

Treatments/Cultivars	C	S	SF _t	SF _r	F _{ff}	SF _{ff}	Mean
Gobindobhog	18.6	23.9	29.7	33.2	34.8	37.9	29.7 ^a
GB 1	20.6	29.7	32.4	38.0	40.0	40.0	33.5 ^a
MTU 7029	21.0	26.1	31.3	36.4	38.8	42.0	32.6 ^a
KRH 2	19.8	25.0	32.1	34.8	36.5	41.7	31.7 ^a
Satabdi	20.8	27.1	29.9	40.1	42.9	41.2	33.7 ^a
Lalat	28.1	31.1	31.3	34.8	38.8	41.9	34.3 ^a
Mean		21.5 ^b	27.2 ^b	31.2 ^{ab}	36.2 ^a	38.6 ^a	40.8 ^a

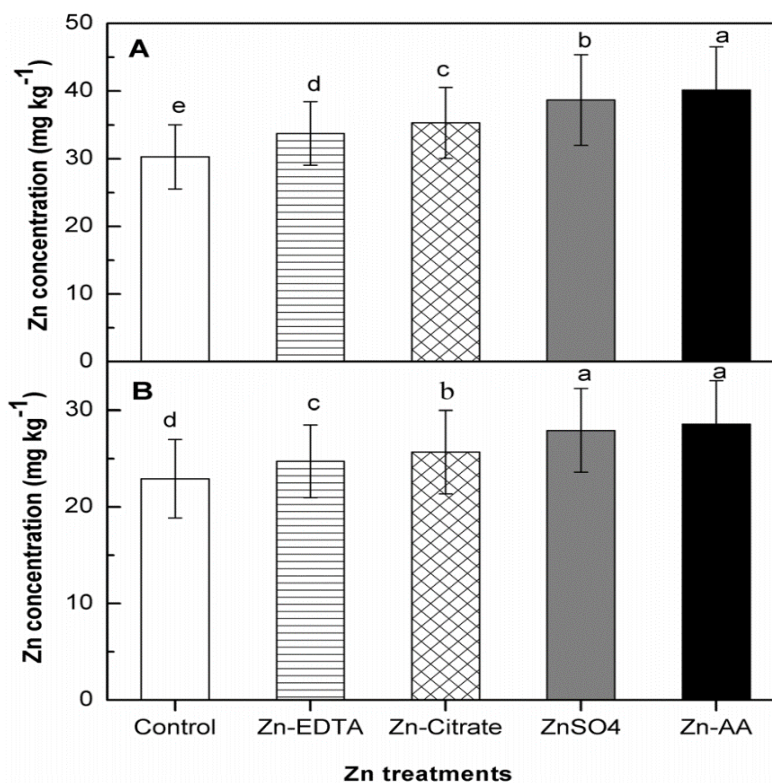
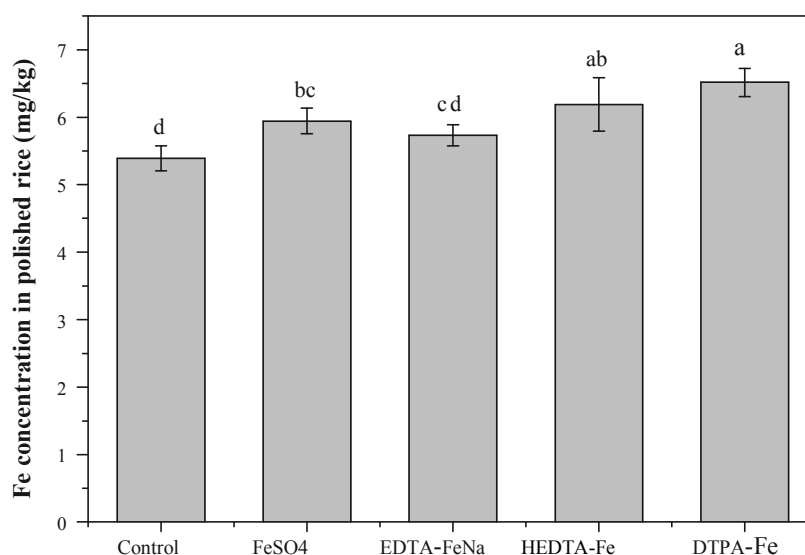


Fig. 1. Effect of different forms of foliar Zn fertilization on Zn concentration in rice grain. (A) Zn concentration in brown rice. (B) Zn concentration in polished rice [21] (a- Control, b- Zn EDTA, c- Zn Citrate, d- ZnSO₄, - Zn AA)

Table 2. Effect of zinc levels on Zn concentration (mg kg⁻¹) of different parts in rice genotypes [36]

Treatment	Brown rice		Hulls		Straw		Roots	
	2011	2012	2011	2012	2011	2012	2011	2012
Zinc levels (mg/kg)								
0	30.2	32.9	36.3	31.5	44.7	42.9	59.3	56.6
5	49.1	48.53	44.5	47.8	66.6	62.3	93.6	88.8
10	58.5	53.55	59.1	56.2	81.1	74.5	104.5	104.5
15	61.2	57.54	62.5	59.1	85.7	79.9	119.4	113.1
20	61.7	58.89	63.4	60.2	89.5	84.1	122.1	116.4
SEm±	1.45	1.42	1.30	1.25	1.70	1.64	1.20	1.16
C.D (p≤0.05)	2.93	2.87	2.63	2.57	3.43	3.22	2.43	2.35

**Fig. 2. Iron concentration of polished rice affected by different forms of foliar Fe applications [30]****Table 3. Concentration of Fe (mg kg⁻¹) in different rice cultivars [39]**

Treatments	Rice cultivars				
	PR113	PR116	PR118	PR120	PAU 201
Fe concentration in panicles at pre-anthesis stage					
Control	41.7	45.8	44.4	28.8	40.0
0.5% FeSO ₄	45.1	50.8	60.0	72.8	61.6
% Increase over control	8.2	10.9	35.1	152.7	54.0
1% FeSO ₄	52.4	53.8	64.9	78.2	83.0
% Increase over control	25.6	17.4	46.1	171.5	107.5
CD (P=0.05)	NS	NS	13.3	15.4	6.5
Interaction (Cultivars x Treatments)					
Fe concentration in panicles at post anthesis stage					
Control	38.7	35.0	33.6	34.6	32.2
0.5% FeSO ₄	49.7	62.0	40.3	67.7	69.0
% Increase over control	28.4	77.1	19.9	95.6	114.2
1% FeSO ₄	68.9	84.7	58.6	102.0	122.0
% Increase over control	78.0	142.0	74.4	194.7	278.8
CD (P=0.05)	9.5	9.9	3.7	14.4	10.5
Interaction (Cultivars x Treatments)	7.4				

4. AGRONOMIC BIOFORTIFICATION IN MAIZE

Maize is one of the popular cereal crop due to its diverse functionality as source of food for humans and animals as well [4,41]. It is an exhaustive crop that requires major nutrients as well as micronutrients particularly Zn for better yield [12]. However, maize grains are inherently poor in Zn, particularly when cultivated on soils deficient in Zn [1]. Recent studies showed that it is possible to increase the Zn concentrations in maize grain via various agronomic strategies of soil application or seed priming and that the crop responds substantially to Zn fertilization [42,43]. Combined application of soil and foliar Zn fertilizers under field conditions is highly effective and practical way to maximize uptake and accumulation of Zn in whole grain and yield than a single fertilization approach [44,45]. Results of a field study showed that the combined application of soil and foliar produced significantly more Zn concentration in corn grain as well as in stover. Similarly, foliar Zn spray combined with broadcasting or banding of Zn fertilizer significantly enhanced the crop yield as well as Zn concentration in grains (Table 3) [45]. It might be due to more auxin production as Zn is required in tryptophan synthesis, which is precursor of IAA and plays important role in internodal elongation [30,46]. In the same experiment, it was also recorded that Zn fertilization significantly reduces grain phytic acid concentration in maize grain due to dilution effect

of increased plant growth and yield revealed that priming of seeds with Zn sulphate solution increased the maize grain yield. However, combined application of Zn as seed priming (2% Zn solution) and foliar spray (2% Zn solution) in maize hybrids increased the Zn grain content [47].

Zn that is deposited in vegetative tissues particularly leaves and then re-translocated into grain during the reproductive stage determines Zn concentration in cereal grains [9]. Hence, among the various methods of Zn fertilization, foliar application is more effective than soil application to increase grain Zn accumulation. According to Zou et al. [27], foliar spray was found to enrich maize grains with Zn more effectively than soil application. Compared with the control, foliar spray of $ZnSO_4 \cdot 7H_2O$ significantly increased the Zn concentration of maize grain from 24.9 to 36.8 $mg\ kg^{-1}$ and enhanced the Fe concentration significantly by 25.0% (Table 4) [41]. Even though the relationship between Zn and Fe concentrations in major cereal crops is still uncertain, but in the aforementioned study, foliar Zn spraying simultaneously improved the concentrations and bioavailability of Zn and Fe in maize grains to some extent. Foliar application of nutrients is a quick responsive way to improve the nutritional status of the crop. As consolidated by the foliar application of Zn and Fe significantly increased the nutrient content in maize grain (Table 5). Soil application of vermicompost enriched with $ZnSO_4$

Table 4. Effect of various Zn treatments on the nutritional attributes in maize grain [45]

Zn treatments	Zinc concentration in grain ($mg\ kg^{-1}$)	Zinc content in grain ($\mu g\ seed^{-1}$)	Zinc concentration in stover ($mg\ kg^{-1}$)
Control	22.3±0.37 f	5.2±0.12 f	13.9±0.08 f
Surface broadcasting	26.7±0.15 e	6.9±0.09 e	22.9±0.05 d
Foliar	30.1±0.14 d	7.6±0.05 d	19.0±0.07 e
Subsurface banding	34.0±0.11 c	9.0±0.08 c	24.6±0.10 c
Surface broadcasting + foliar	37.4±0.17 b	10.4±0.04 b	28.0±0.15 b
Subsurface banding + foliar	41.9±0.15 a	12.3±0.11 a	30.3±0.09 a

Table 5. Effects of different foliar treatments on Zn and Fe concentrations, contents in maize grains in the field experiment at Licheng

Treatments	Grain Zn concentration ($mg\ kg^{-1}$)	Zn contents ($mg\ plant^{-1}$)	Grain Fe concentrations ($mg\ kg^{-1}$)	Fe Contents ($mg\ plant^{-1}$)
Deionized water	24.9 ± 0.8 b	3.6 ± 0.1 b	24.0 ± 2.8 b	3.4 ± 0.3 b
Sucrose	23.8 ± 0.6 b	3.3 ± 0.1 b	23.1 ± 0.5 b	3.2 ± 0.1 b
$ZnSO_4 \cdot 7H_2O$ (Zn)	36.8 ± 2.0 a	5.3 ± 0.3 a	30.0 ± 3.9 a	4.3 ± 0.5 a
Sucrose + Zn	34.8 ± 0.8 a	5.3 ± 0.2 a	27.7 ± 1.2 ab	4.2 ± 0.2 a

Table 6. Effect of Zn and Fe application on the zinc and iron contents of maize grain [50]

Treatments	Fe content (mg kg ⁻¹)	% increase	Zn content (mg kg ⁻¹)	% increase
NPK	74.1	--	14.3	--
NPK+10 kg Zn & Fe	91.6	23.6	18.3	28.0
NPK+20 kg Zn & Fe	107.6	45.2	23.2	62.2
NPK+30 kg Zn & Fe	122.7	65.6	25.1	75.5
NPK+ 0.1 % foliar spray of Zn & Fe	153.6	107.3	31.8	122.4

and FeSO₄ (10 kg each ha⁻¹) along with foliar spray of ZnSO₄ and FeSO₄ (1% each) had a positive impact on grain Zn and Fe concentrations in sweet corn [48]. Micronutrient uptake and concentration was improved due to fixation of externally added inorganic Zn and Fe into organically bound and naturally chelated form of Zn and Fe that favoured their availability and hence increased uptake. Chhagan et al. [49] reported similar findings that application of FYM fortified with different levels of ZnSO₄ and FeSO₄ increased the Zn and Fe iron in maize grain. Slow release of nutrients into the soil solution facilitates the higher nutrient uptake and prevents from leaching losses [50].

5. CONCLUSION

In conclusion, the deficiency of Zn and Fe in human nutrition continues to give rise to micronutrient malnutrition, which is a grave health care concern. Agronomic interventions are likely to prove effective approach of enrichment of food crops with these micronutrients. Recent development in this area, conclude that enhanced fertilization with micronutrients can enrich grains to greater extent. It is evident that using combination of strategies — seed priming, soil application, and/or foliar spray of Zn and Fe are having prominent effect on uptake and accumulation in plants. It is offering a truly feasible strategy to combat the problem of micronutrient deficiency, especially for those who rely on staple food crops inherently low in micronutrient content. Thus, agronomic biofortification provides a reliable, short-term and sustainable approach for nutrition of susceptible human population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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