Original Research Article

Optimizing growth and zinc bioavailability in rice (*Oryza sativa* L.) cultivars through agronomic biofortification

Commented [A1]: Enhancing Growth and Zinc Enrichment in Rice (*Oryza sativa* L.) Cultivars through Innovative Agronomic Biofortification Strategies

Abstract

A field experiment was conducted using four rice varieties *viz.,* Uma, Pournami, Gouri and DRR Dhan 45 under varying levels of $ZnSO_4$ foliar application (control, 0.1 per cent, 0.5 per cent and 1.0 per cent) in a randomized complete block design. $ZnSO_4$ @ 1.0 per cent recorded taller plants at panicle initiation (80.5 cm) and harvest stages (113 cm) and was comparable with $ZnSO_4$ @ 0.5 per cent. Higher tillers per hill and dry matter production were also recorded at panicle initiation and harvest stages with $ZnSO_4$ @ 1.0 per cent which was statistically similar to $ZnSO_4$ @ 0.5 per cent. Zinc application at 0.5 per cent and 1.0 per cent enhanced Zn concentration in rough rice, brown rice, white rice, and bran. The highest Zn accumulation in white rice (21.2 mg kg^{-1}) was achieved with 1.0 per cent $ZnSO_4$ foliar spray, which was comparable to 0.5 per cent $ZnSO_4$ spray. The application of 1 per cent $ZnSO_4$ during maximum tillering and milk stage led to substantial reductions in the phytate: Zn molar ratio in rough rice (28.3), brown rice (32.7), white rice (6.31), and rice bran (25.9). Dhan 45 treated with 1.0 per cent $ZnSO_4$ achieved the lowest ratios in rough rice (18.9) and brown rice (21.8). Although the 1.0 per cent treatment yielded greater reductions in the phytate: Zn molar ratio, the 0.5 per cent $ZnSO_4$ treatment also produced notable decrease in rough rice (30.2), brown rice (34.8), white rice (6.72), and rice bran (27.6) making it a viable option for lower Zn input. Overall, foliar application of Zn improved Zn bioavailability in both whole grains and milled rice, aligning phytate: Zn molar ratios closer to optimal levels for human nutrition.

Keywords: Bioavailability, Foliar application, $ZnSO_4$, Phytate: Zn molar ratio, Rice grain fractions

1. INTRODUCTION

The crucial role of micronutrients in health and nutrition is undeniable, and zinc (Zn) stands out as a vital element whose health benefits are becoming more recognized. Zinc deficiency can significantly contribute to the development of various diseases. Since the human body lacks long term storage mechanism for Zn, regular dietary intake is essential, particularly during childhood, adolescence, and pregnancy (Gibson and Anderson, 2009) [1]. Zinc deficiency has significantly impacted approximately 33 per cent of the global population, predominantly in nonurban communities. Reports indicate that Zn deficiency is linked to approximately 1 lakh 16000 deaths each year across the globe (Galetti, 2018) [2]. Lower serum Zn levels (below 65 µg/dL) have been observed in Indian children under five years old, with the highest prevalence reported in Orissa, followed by Uttar Pradesh, Gujarat, Madhya Pradesh and Karnataka (Kapil and Jain, 2011) [3]. The recommended dietary advice suggested that adult women should consume 8 mg of Zn daily, while adult men should intake 11 mg daily (NIH, 2021) [4].

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Zinc malnutrition in humans is more prevalent in regions where cereals such as rice and wheat are primary food sources, particularly in areas where these crops are grown in Zn deficient soils. It is also estimated that roughly half of the world's cereal producing regions have Zn deficient soils (Cakmak and Kutman, 2018) [5]. Biofortification of cereal and staple crops provides a sustainable way to fulfil the human body's micronutrient needs, supporting better health. The effectiveness of applying Zn based fertilizers to crops varies depending on factors such as the method of application (e.g., soil, foliar, seed priming, or combinations), the type of Zn used, the timing of the application, as well as the crop's genetic makeup and the environmental conditions in which it is grown (Yaseen and Hussain, 2021 [6] and Prasad *et al*., 2014 [7]). Agronomic biofortification involves the use of micronutrient enriched fertilizer and is an easy and rapid way to enhance the nutritional content of crops. Consuming these fortified crops helps to improve human nutrition (Cakmak and Kutman, 2018) [8]. In similar growing conditions, rice cultivars differ in their ability to accumulate micronutrients in grain. At the International Rice Research Institute, about 1,000 rice genotypes were examined and has discovered that Zn content in rough rice ranged from 15.9 to 58.4 mg kg^{-1} (Graham *et al*., 1999) [9]. Foliar fertilized Zn can be remobilized from the leaves to the grain by most of the rice genotypes, however the capacity to remobilize Zn from leaves to grain varies depending on the plant genetic composition and soil Zn availability (Mabesa *et al*., 2013) [10].

The enrichment of Zn in grains should be evaluated in relation to changes in other key nutritional traits of the grain, such as concentrations of iron (Fe), phytic acid, the phytic acid to zinc molar ratio, and protein levels (Cakmak *et al*., 2010 [11] & Hussain *et al*., 2012 [12]). These compositional changes in grains or cooked rice can influence Zn bioavailability, as it is well recognized that the two main factors affecting Zn absorption in adults are the levels of Zn and phytate in the diet (Miller *et al*., 2007) [13]. Bioavailability conveys the fact that not all of the consumed Zn is really absorbed by human body. Nutrient bioavailability is useful in improving diets and it provides suitable dietary nutrient recommendations for people (Schönfeldt *et al*., 2016) [14]. Zinc bioavailability refers to the portion of absorbed Zn in the blood stream that is available for use in regular physiological functions (La Frano *et al*., 2014) [15]. The bioavailability of Zn in rice grains is affected by the Zn content in the grain, and enriching rice grains with Zn has significantly increased the amount of bioavailable Zn. However, certain antinutrients like phytic acid can lower Zn bioavailability by binding to Zn and forming indigestible complexes in the human body. In recent years, various strategies have been suggested to enhance Zn bioavailability in grains by boosting their Zn content. The present study was conducted to assess the impact of Zn foliar application on growth, Zn concentration and bioavailability in grains of selected rice cultivars.

2. MATERIALS AND METHODS

A field trail in rice was conducted on a farmer's field located at 8° 43' N latitude and 76° 45' E longitude, at an elevation of 52 meters above sea level in the southern coastal plains of Kerala during the rainy (kharif) season of 2020-21. The area received 935.8 mm of seasonal rainfall over 50 rainy days, which proved beneficial for crop growth and grain development. The average seasonal temperature ranged from a maximum of 30.3° to 32.7°C and a minimum of 24.5° to 25.9°C. The field experiment was designed using a two factor factorial randomized complete block layout, with three replications and 16 treatment combinations. The treatment included four medium duration rice varieties as factor (V), V_1: Uma, V_2: Pournami, V_3: Gouri and V_4: DRR Dhan 45, and four levels of foliar Zn application as factor (F), F_1: control, F_2: $ZnSO_4$ @ 0.1 per cent, F_3: $ZnSO_4$ @0.5 per cent and F_4: $ZnSO_4$ @ 1 per cent. The study involved both biofortified varieties and conventional rice varieties. The key characteristics of the rice varieties used in the study were given in Table1. Foliar Zn application of the rice crop was applied in two stages with a uniform spray concentration. The initial spray was given during the maximum tillering and the second spray on the milk stage.

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Table 1. Characteristics of the rice cultivars tested in the experiment

Cultivars	Station	Parentage	Duration in days	Zn in grains (mg kg^{-1})	Phytic acid in grains (g kg^{-1})
Uma (MO-16)	Rice Research Station, Monkompu	Cul.12814/ Mo.6	120-135	13.8	6.12
Pournami	Rice Research Station, Monkompu	Mo.4/ Cul. 25331	115-120	15.4	5.99
Gouri (MO-20)	Rice Research Station, Monkompu	KAUM 109-1-2-1/ IET 23739	120	21.5	6.06
DRR-Dhan 45	Indian Institute of Rice Research, Hyderabad	IR 73707-45-3-2-3/ IR 77080-B-34-3	125	28.6	6.28

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Before the field experiment, a composite soil sample was collected from a depth of 0-15 cm and analysed for its physio chemical properties. The experimental soil was clay loam in texture, very strongly acidic (pH 5.4), high in organic carbon (1.32 per cent), sufficient in Zn (1.05 mg kg^{-1}), medium in available nitrogen (282 kg ha^{-1}), phosphorus (12.7 kg ha^{-1}) and potassium (187 kg ha^{-1}). In accordance with treatments, the spray solution for foliar Zn fertilization was applied to the crop in the late afternoon, continuing until the solution just started to drip off the leaves, following the recommendations of Cakmak *et al.* (2010) [11]. At reaching maturity, the rice plants from each individual plots were harvested and manually threshed to separate the paddy from the straw. The growth parameters viz., plant height, tillers per hill and dry matter production were recorded at maximum tillering, panicle initiation and harvest stages. The data were subjected to statistical analysis, and critical difference at 5 per cent significance level was calculated for each parameter.

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2.1 Collection and analysis of rice grains

The rough rice from each plot was cleaned to eliminate foreign matter, washed to remove dust, air dried, and then oven dried to a constant weight. A representative sample of rough rice obtained from various Zn fertilization and cultivars was then dehulled to produce brown rice and husk. The entire amount of husk was collected, weighed and set aside for analysis. The total quantity of brown rice produced was milled into white rice and bran, and the white rice was used to prepare cooked rice. During milling both the white rice and bran were collected, weighed and stored for analysis. The dehulling and milling processes were conducted using a compact mill, resulting in husk, bran and white rice. These along with rough rice and brown rice were later analysed for Zn and phytate using the standard procedures.

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2.2 Cooking of the processed grains

A 100 g portion of white rice was washed twice with 250 ml of water and then soaked in 250 ml of distilled water for 30 minutes before cooking. The water was completely drained, and the soaked rice grains were cooked on a hot plate at 380^0 C with 600 ml of water. Cooking was stopped when a few cooked kernels showed no white kernel left behind when pressed between two glass slides (opaque core of cooked rice just disappeared). The cooked rice and decanted rice water were separated. They were dried in hot air oven at 60^0 C to a constant weight, grounded using pestle and mortar, sieved using 0.5 mm sieve and stored in an air tight polyethene bag in room temperature prior to digestion (S

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uman, 2011) [16.] The samples were analysed for Zn as per the standard procedures.

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2.3 Zinc and phytate analysis and Zinc bioavailability

Zinc can be analysed by nitric-perchloric acid (9:4) digestion and atomic absorption spectrometry (Jackson, 1973) [17]. Phytate was extracted with trichloroacetic acid and subsequently precipitated as ferric salt. The iron concentration of the precipitate was measured calorimetrically using a spectrophotometer at 480 nm. This value was used to compute the phytate concentration, assuming a constant 4Fe: 6P molecular ratio in the precipitate (Sadasivam and Manickam, 2016) [18].

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Phytate: Zn molar ratio was calculated using the following formula (Murphy *et al*., 1992 [19]; Gibson, 2005 [20]).

$$\text{Phytate: Zinc molar ratio} = \frac{Phytic\ acid\ concentration\ (mg\ kg^{-1})/\ 660}{Zn\ content\ in\ (\ mg\ kg^{-1})/65.4}$$

where, 660 is molecular weight of phytic acid and 65.4 is atomic weight of Zn. The inhibitory effect of phytate on Zn bioavailability in humans i.e., the absorbability of dietary Zn in humans, can be predicted from phytate: Zn molar ratio in human diet (Gibson, 2005 [20]). Algorithm of Murphy and co-workers to estimate bioavailability of Zn in humans, based on work of Murphy *et al*. (1992) [19] is given as follows

Zn bioavailability estimate (per cent)	Phytate: Zn molar ratio
55	0-5: 1
35	5-15: 1
15	15-30: 1
10	>30: 1

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3. Result and Discussion

3.1 Plant height

In rice cultivars plant height was significantly affected with foliar fertilization with Zn during panicle initiation and harvest stage (Table 2.). Plant height was not significantly influenced by the effect of varieties and their interaction with fertilization at maximum tillering, panicle initiation and harvest stages. The plant height was greater with 1.0 per cent $ZnSO_4$ and was comparable to 0.5 per cent $ZnSO_4$ at both the panicle initiation stage (80.5 cm) and harvest stage (113 cm). The lowest plant heights across

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all the growth stages were observed in the control. The enhancement in height can be attributed to the sufficient supply of Zn, which likely improved the availability and uptake of other essential nutrients leading to better crop growth. Sudha and Stalin (2015) [20] reported that foliar application of micronutrients significantly increased plant height, attributing to the enhanced enzymatic activity and auxin metabolism in the plants. Shivay *et al.* (2016) [21] noted that the application of $ZnSO_4$ at 0.5 per cent resulted in taller plants.

Table 2. Effect of Zn foliar fertilization on plant height

Treatment	Panicle Initiation	Harvest
No $ZnSO_4$ application	63.6	82
$ZnSO_4$ @ 0.1 per cent	66.4	86
$ZnSO_4$ @ 0.5 per cent	79.1	110
$ZnSO_4$ @ 1 per cent	80.5	113
Sem ((±) F	2.0	4
CD (0.05)	5.85	10.9

3.2 Tillers per hill

Data related to tillers per hill under the influence of different rootstock has been shown in Table 3. Foliar fertilization of Zn had significant effect on the tiller count per hill. At the panicle initiation stage higher number of tillers per hill (20.2) were registered with $ZnSO_4$ at 1.0 per cent, which was comparable to 0.5 per cent $ZnSO_4$ treatment. Similarly, at harvest the higher tiller count was recorded with 1 per cent $ZnSO_4$ application. The absence of Zn foliar fertilization resulted in lowest tiller number across all the growth stages. Mustafa *et al.* (2013) [22] stated that optimum quantity of Zn enhanced number of tillers at all growth stages. Increase in number of tillers due to Zn application was also reported by Slaton *et al.* (2005) [23].

Table 3. Effect of Zn foliar application on tillers per hill

Treatment	Panicle Initiation	Harvest
No $ZnSO_4$ application	12.9	13.9

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$ZnSO_4$ @ 0.1 per cent	14.0	14.1
$ZnSO_4$ @ 0.5 per cent	19.0	15.4
$ZnSO_4$ @ 1 per cent	20.2	15.7
Sem ((±) F	0.8	0.3
CD (0.05)	2.16	0.71

3.3 Dry Matter Production

The data presented in Table 4. revealed that at panicle initiation and harvest stages dry matter production was significantly influenced by Zn fertilization and was higher with $ZnSO_4$ @ 1.0 per cent. In both the stages it was on par with $ZnSO_4$ @ 0.5 per cent. This was owing to the reason that dry matter generation in plant depends on potential photosynthetic capacity which in turn depends on leaf area, nutrient consumption and favourable environmental circumstances (De Datta, 1981) [24]. Higher dry matter production with varied Zn treatments could be ascribed to increased plant height and leaf area index, as Zn is essential for auxin and enzyme synthesis. Increase in dry matter production due to Zn application was also observed by Tetarwal *et al*. (2011) [25], Kumar *et al*. (2011) [26] and Ravi *et al*. (2012) [27].

Table 4. Effect of Zn foliar fertilization on dry matter production

Treatment	Panicle Initiation	Harvest
No $ZnSO_4$ application	8.73	20.8
$ZnSO_4$ @ 0.1 per cent	9.54	22.4
$ZnSO_4$ @ 0.5 per cent	11.40	27.3
$ZnSO_4$ @ 1 per cent	12.00	28.9
Sem ((±) F	0.36	0.9
CD (0.05)	1.067	2.53

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3.4 Effect of Zn application on Estimated Zn bioavailability in rice grain fractions

3.4.1 Zinc

The results of the study illustrated that the accumulation of Zn in white rice increased with the application of $ZnSO_4$ at 0.5 per cent (19.5 mg kg^{-1}), which was statistically similar to the level obtained with the application of $ZnSO_4$ at 1 per cent (21.2 mg kg^{-1}). Additionally, $ZnSO_4$ at 0.5 per cent enhanced the Zn content in harvested rough rice, brown rice, rice bran and cooked rice 22, 26,75.3, and 12.8 mg kg^{-1} respectively, reaching levels comparable to those achieved with $ZnSO_4$ at 1 per cent (Fig.1.). The interaction effect between rice varieties and Zn foliar application was not significant. However, foliar application of $ZnSO_4$ consistently increased Zn content across all rice cultivars (Fig.2.). Notably, Dhan 45 showed higher Zn content when treated with 1 per cent $ZnSO_4$ followed by 0.5 per cent application as the next most effective treatment. Zinc applied through foliar spray is effectively absorbed by the leaves and can be translocated to the grains (Boonchuay *et al*., 2013) [28]. The timing of foliar spray is also a key factor for enhancing Zn content in grains. Phuphong *et al*. (2018) [29] reported that applying Zn foliar sprays after flowering significantly increases the Zn concentration in both polished and brown rice.

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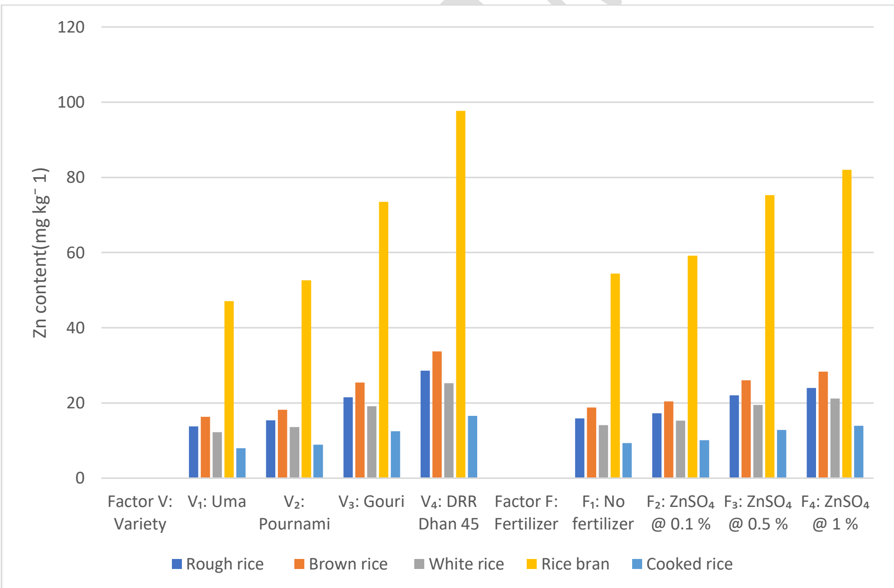

Fig.1. Effect of Zn foliar fertilization on Zn content in rice grain fractions of various rice varieties

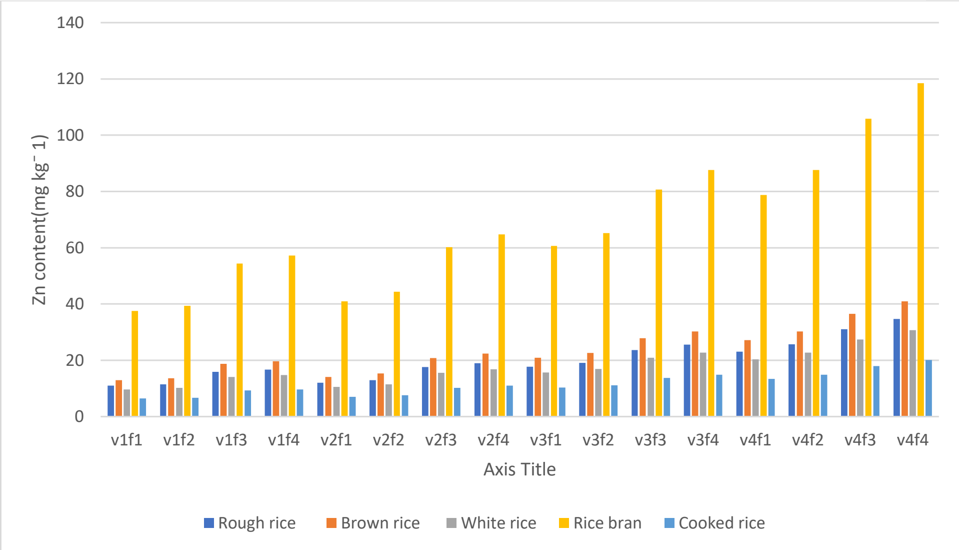

Fig.2. Interaction effect between rice varieties and Zn foliar fertilisation on Zn content of rice varieties

3.4.2 <u>Phytate</u>

The study found that varieties, Zn fertilization and their interactions had no significant effect on phytate concentrations in whole grain and milled fractions, in contrast to prior findings that showed that soil or foliar Zn application reduced phytate concentrations in rice grain considerably (Mabesa *et al*., 2013 [30]; Imran *et al*., 2015 [31]). Possible explanation could be that foliar Zn application inhibits the conversion of inorganic phosphorus to phytate in rice grain.

3.4.3 <u>Phytate: Zn molar ratio</u>

The results of the study illustrated that Zn application significantly reduced the phytate: Zn molar ratio in polished and brown rice for all rice cultivars over control, the lowest phytate: Zn molar ratio was noted in foliar application with $ZnSO_4$ @ 1.0 per cent which was followed by foliar application with $ZnSO_4$ @ 0.5 per cent (Table 5.). These findings align with the established recommendation that a phytate: Zn molar ratio below 20 is optimal for Zn nutrition in human diets (Weaver and Kannan, 2002 [32]). Irrespective of the varieties and Zn fertilization levels, the phytate: Zn molar ratio in polished white rice consistently remained below 20, indicating optimum Zn nutrition in human diets. Among the rice varieties evaluated, the genetically biofortified Dhan 45 recorded significantly lower phytate: Zn molar ratios in rough rice (22.1), brown rice (25.5), white rice (5.12), and rice bran (21.3). Fertilization with $ZnSO_4$ @ 1.0 per cent at maximum tillering and milk stage resulted in significantly lower phytate: Zn molar ratios in rough rice (28.3), brown rice (32.7), white rice (6.31), and rice bran (25.9) respectively. In the case of rough rice (18.9) and brown rice (21.8), foliar application of $ZnSO_4$ @ 1.0per cent in Dhan 45 recorded significantly lower phytate: Zn molar ratio. This suggests that varieties, in combination with Zn fertilization can further enhance Zn bioavailability. While the 1per cent concentration achieved the greatest reduction in the phytate: Zn molar ratio, the 0.5 per cent treatment provided a substantial improvement, for instance the phytate: Zn molar ratio decreased to 30.2 in rough rice, 34.8 in brown rice, 6.72 in white rice, and 27.6 in rice bran with the 0.5 per cent $ZnSO_4$ treatment. This makes it a viable alternative where lower Zn input is preferred. Fertilization with higher Zn concentrations in whole grain and milled fractions resulted in decreased phytate: Zn molar ratios, bringing phytate: Zn molar ratios closer to desirable reference levels for improved Zn bioavailability. The result is in consonance with the observation of Hussain *et al*. (2012) [33} who reported that foliar Zn fertilization increased the estimated Zn bioavailability and decreased the molar ratio of phytate: Zn in rice.

The distribution of phytic acid within the rice kernel further explains the variations observed in the phytate: Zn molar ratio across different rice fractions. In all the rice varieties, the sub surface layer holds roughly 23-25per cent of total phytic acid, accounting for 3.4-4.5per cent of total weight. In the middle section of the kernel, less than 2per cent of total phytic acid was found. The mesocarp of brown rice contained remaining 40-50 per cent of phytic acid, accounting for 13-15 per cent of the kernel weight. The distribution of phytic acid in rice types and sections of the rice kernel varies. The outermost layer of rice kernels contains the majority of the phytic acid (Liang *et al*., 2007) [34]. Except for the high Zn density in the embryo, Zn distribution is similar in different rice varieties and reasonably even in rice kernels. This pattern was evident in all varieties and Zn fertilization levels tested in this study.

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Table 5. Effect Zn foliar fertilization on Phytate: Zn molar ratio of rough rice, brown rice, rice bran and white rice

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Treatment	Rough rice	Brown rice	Rice Bran	White rice
Factor V: Variety				
V1	45.5	52.6	42.3	10.21
V2	39.6	45.7	37.5	9.19

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V3	28.3	32.7	27.3	6.56
V4	22.1	25.5	21.3	5.12
SEm(±)	0.1	0.1	0.3	0.08
CD (0.05)	0.26	0.30	0.90	0.247
Factor F: Fertilizer				
F1	38.0	43.9	38.6	9.29
F2	39.1	45.1	36.3	8.77
F3	30.2	34.8	27.6	6.72
F4	28.3	32.7	25.9	6.31
Sem (±)	0.1	0.1	0.3	0.08
CD (0.05)	0.26	0.30	0.90	0.247
Interaction (VxF)				
v1f1	54.9	63.3	51.0	12.31
V1f2	52.2	60.3	48.5	11.82
V1f3	38.0	43.9	35.3	8.46
V1f4	37.1	42.8	34.4	8.27
V2f1	43.7	50.5	46.5	11.19
V2f2	46.5	53.7	43.2	10.39
V2f3	35.3	40.7	31.2	7.88
V2f4	32.8	37.8	29.1	7.31
V3f1	29.7	34.3	31.8	7.65
V3f2	32.5	37.6	30.3	7.26
V3f3	26.4	30.5	24.6	5.89
V3f4	24.5	28.2	22.7	5.44
V4f1	23.7	27.4	25.1	6.03
V4f2	25.1	29.0	23.3	5.61
V4f3	20.8	24.0	19.3	4.64
V4f4	18.9	21.8	17.5	4.20

Sem (±)	0.2	0.2	0.62	0.171
CD (0.05)	0.53	0.61	1.80	0.495

4. Conclusion

It can be concluded that foliar fertilization of Zn enhanced growth parameters such as plant height, tillers per hill and dry matter production in rice varieties and was comparable between 0.5 per cent and 1.0 per cent $ZnSO_4$ concentrations. Foliar fertilization with 1.0 per cent and 0.5 per cent $ZnSO_4$ reduced the phytate: Zn ratios. Although the 1.0 per cent $ZnSO_4$ concentration resulted in greater reductions, the 0.5 per cent $ZnSO_4$ treatment proved to be the optimal, achieving significant zinc enrichment with minimal phytate interference. The results suggest that brown rice can be processed into white rice to produce desired phytate: Zn ratio for optimum Zn nutrition in humans.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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REFERENCES

1. Gibson, R. S., & Anderson, V. P. (2009). A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc. Food and Nutrition Bulletin, 30(1), 108–143.

2. Galetti V. (2018). "Zinc deficiency and stunting," in Handbook of Famine, Starvation, and Nutrient Deprivation. Eds. Preedy V., Patel V. B. (Cham: Springer International Publishing;), 1–23. doi: 10.1007/978-3-319-40007-5_93-1

3. Kapil, U. and Jain, K. (2011) Magnitude of zinc deficiency amongst under five children in India. Indian Journal of Pediatrics 78, 1069-1072.

4. NIH (National Institute of Health). (2021). Zinc– Health professional fact sheet. Available: https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/

5. Cakmak, I., & Kutman, U. Á. (2018). Agronomic biofortification of cereals with zinc: a review. European journal of soil science, 69(1), 172-180.

6. Yaseen, M. K., & Hussain, S. (2021). Zinc-biofortified wheat required only a medium rate of soil zinc application to attain the targets of zinc biofortification. Archives of Agronomy and Soil Science, 67(4), 551-562.

7. Prasad, R., Shivay, Y. S., & Kumar, D. (2014). Agronomic biofortification of cereal grains with iron and zinc. Advances in agronomy, 125, 55-91.

8. Cakmak, I., & Kutman, U. Á. (2018). Agronomic biofortification of cereals with zinc: a review. European journal of soil science, 69(1), 172-180.

9. Graham, R., Senadhira, D., Beebe, S., Iglesias, C., & Monasterio, I. (1999). Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field crops research, 60(1-2), 57-80.

10. Mabesa, R. Á., Impa, S. Á., Grewal, D., & Johnson-Beebout, S. E. (2013). Contrasting grain-Zn response of biofortification rice (Oryza sativa L.) breeding lines to foliar Zn application. Field Crops Research, 149, 223-233.

11. Cakmak, I., Kalayci, M., Kaya, Y., Torun, A. A., Aydin, N., Wang, Y., ... & Horst, W. J. (2010). Biofortification and localization of zinc in wheat grain. Journal of Agricultural and Food Chemistry, 58(16), 9092-9102.

12. Hussain, S., Maqsood, M. A., Rengel, Z., & Aziz, T. (2012). Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. Plant and Soil, 361, 279-290.

13. Miller, L. V., Krebs, N. F., & Hambidge, K. M. (2007). A Mathematical Model of Zinc Absorption in Humans As a Function of Dietary Zinc and Phytate, 2. The Journal of nutrition, 137(1), 135-141.

14. Schönfeldt, H. C., Pretorius, B., & Hall, N. (2016). Bioavailability of nutrients. Caballero, B, P Finglas and F Toldrá, F (eds) The Encyclopedia of Food and Health, 1, 401-406.

15. La Frano, M. R., de Moura, F. F., Boy, E., Lönnerdal, B., & Burri, B. J. (2014). Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops. Nutrition reviews, 72(5), 289-307.

16. Suman, (2011). Effect of cooking methods on nutritional quality of rice (Oryza sativa L.) varieties. MSc thesis, Chaudhary Charan Singh Haryana Agricultural University, Haryana,103p.

17. Jackson, M. L. (1973). Soil Chemical Analysis (2nd Ed.). Prentice-Hall of India (Pvt) Ltd, New Delhi, 498p.

18. Murphy, S. P., Beaton, G. H., & Calloway, D. H. (1992). Estimated mineral intakes of toddlers: predicted prevalence of inadequacy in village populations in Egypt, Kenya, and Mexico. The American journal of clinical nutrition, 56(3), 565-572.

19. Gibson, R.S. (2005). Dietary strategies to enhance micronutrient adequacy: experiences in developing countries. In: Anderson, P., Tuladar, J.K., Karki, K.B., Maskey, S.L. (eds), Micronutrients in South and South East Asia. Proceedings of an

international workshop, Kathmandu, Nepal. International Centre for Integrated Mountain Development, Nepal, pp.3-7.

20. Sudha, S., & Stalin, P. (2015). Effect of zinc on yield, quality and grain zinc content of rice genotypes. International journal of farm sciences, 5(3), 17-27.

21. Shivay, Y.S., Prasad, R., Kaur, R., & Pal, M. (2016). Relative efficiency of zinc sulphate and chelated zinc on zinc biofortification of rice grains and zinc use-efficiency in Basmati rice. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 86(4): 973-984.

22. Mustafa, G., Ehsanullah, Akbar, N., Qaisrani, S.Q., Iqbal, A., Khan, H.Z., Jabran, K., Chattha, A.A., Trethowan, R., Chattha, T. and Atta, M.B. (2013). Effect of zinc application on growth and yield of rice (Oryza Sativa L.). IJAVMS. 5(6): 530-535.

23. Slaton, N. A., Gbur, E. E., Wilson, C. E., & Norman, R. J. (2005). Rice response to granular zinc sources varying in water-soluble zinc. Soil Science Society of America Journal, 69(2), 443-452.

24. De Datta, S.K. (1981). Principles and practices of rice production. John Wiley & sons, Inc, 485p.

25. Tetarwal, J. P., Ram, B., & Meena, D. S. (2011). Effect of integrated nutrient management on productivity, profitability, nutrient uptake and soil fertility in rainfed maize (Zea mays). Indian journal of Agronomy, 56(4), 373-376.

26. Kumar, V., Bhatia, B.K., & Shukla. U.C. (2011). Effect of different levels of zinc on growth and yield of amaranth. Soil Science.131: 151-155.

27. Ravi, N., Basavarajappa, R., Chandrashekar, C. P., Harlapur, S. I., Hosamani, M. H., & Manjunatha, M. V. (2012). Effect of integrated nutrient management on growth and yield of quality protien maize.

28. Boonchuay, P., Cakmak, I., Rerkasem, B., & Prom-U-Thai, C. (2013). Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. Soil science and plant nutrition, 59(2), 180-188.

29. Phuphong, P., Cakmak, I., Dell, B., & Prom-u-thai, C. (2018). Effects of foliar application of zinc on grain yield and zinc concentration of rice in farmers' fields. Chiang Mai University Journal of Natural Sciences, 17(3), 181-190.

30. Mabesa, R. Á., Impa, S. Á., Grewal, D., & Johnson-Beebout, S. E. (2013). Contrasting grain-Zn response of biofortification rice (Oryza sativa L.) breeding lines to foliar Zn application. Field Crops Research, 149, 223-233.

31. Imran, M., Kanwal, S., Hussain, S., Aziz, T., & Maqsood, M. A. (2015). Efficacy of zinc application methods for concentration and estimated bioavailability of zinc in grains of rice grown on a calcareous soil. Pakistan Journal of Agricultural Sciences, 52(1).

32. Weaver, C.M. and Kannan, S. (2002). Phytate And Mineral Bioavailability. In: Reddy, N.R. and Sathe, S.K (eds) Food phytate. CRC Press, Boca Raton, 223p.

33. Hussain, S., Maqsood, M. A., Rengel, Z., & Aziz, T. (2012). Biofortification and estimated human bioavailability of zinc in wheat grains as influenced by methods of zinc application. Plant and Soil, 361, 279-290.

34. Liang, J., Han, B. Z., Han, L., Nout, M. R., & Hamer, R. J. (2007). Iron, zinc and phytic acid content of selected rice varieties from China. Journal of the Science of Food and Agriculture, 87(3), 504-510.