Biofortification of Rice Grain as Affected by Different Doses of Zinc Fertilization

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MJ and MRI designed the study, wrote the protocol and conducted study with author KN. Author MJ performed the statistical analysis and author SK wrote the first draft of the manuscript. Authors MR and MTS managed the analyses of the study. Authors KN and SK managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The experiment was conducted in the research farm at Bangladesh Agricultural University (BAU) to investigate the zinc biofortification ability of rice grain at different doses of zinc fertilization. In this experiment two rice varieties (BRRI dhan28 and Binadhan-16) and five doses (0, 1.5, 3.0, 4.5 and 6.0 kg ha^{-1}) of zinc fertilization were used following split-plot design with three replications. Except 1000-grain weight and plant height, all other plant characters viz., tillers hill^{-1}, panicle length and grains panicle^{-1} were significantly influenced by zinc fertilization. The treatment receiving Zn at 4.5 kg ha-1 (Zn 4.5) produced the highest grain yield (7.70 t ha^{-1}) in BRRI dhan28 which was statistically similar with the yield obtained with Zn 3.0 treatments. The zinc control treatment (Zn 0) produced the lowest grain yield in both varieties. The concentrations of N, Zn and Fe were significantly and positively influenced by the Zn treatments. The crop varieties did not differ significantly in respect of N and Fe concentrations, but the grain Zn concentration was considerably higher in BINA dhan16 than in BRRI dhan28. The grain N content as well as grain

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protein content linearly increased with the rates of Zn application. Thus, application of Zn at the rate of 6.0 kg ha^{-1} demonstrated the highest Zn fortification in both varieties but maximum zinc fortification was observed in Binadhan-16 (24.1 µg g-1) in rice grain which was 12.2% higher over control treatment.

1. INTRODUCTION

Rice (*Oryza sativa*) is a main staple food of Bangladesh, adult eats about 400 g milled rice daily. Rice is the main source of Zn to human [1]. Unfortunately, rice is a poor source of metabolizable Zn, due to inherently low in Zn content and the bio-available Zn [2] that causees Zn deficiency for animals and humans. Zinc and iron deficiencies are more concentrated in developing countries where cereal-based foods are predominant sources of minerals and energy intake [3].

Increasing the Zn and Fe concentrations of food crop plants, resulting in better crop production and improved human health, is an important global challenge. Among micronutrients, Zn deficiency is occurring in both crops and humans [4-6]. Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally. According to a WHO [7] report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks the 11th among the 20 most important factors in the world and the 5th among the 10 most important factors in developing countries.

With advancement of time soil fertility in Bangladesh has deteriorated. The reasons can be attributed to increasing cropping intensity (143% in 1971-72 and 192% in 2014-15) [8] accompanied with increasing cultivation of modern varieties, with little addition of organic manure. Consequently, along with N, P, K and S, micronutrient deficiency has arisen in this country' soils. Moreover, farmers are paying less attention to the application of micronutrient (e.g. zinc) fertilizers in their farming. At present Zn deficiency is the most visible micronutrient deficiency of soils and crops. Zinc deficiency in Bangladesh was identified in late 1970s [9]. The Country Investment Plan of Bangladesh [10] has also addressed the problem of micronutrient deficiency in soils. More than 70% of cultivated soils in this country have Zn deficiency [11].

Biofortification of food crops with zinc by choosing cultivars for higher uptake efficiency or by using zinc fertilizers (soil and foliar application) and reducing inhibitors of zinc absorption, like phytate can be an effective strategy to address dietary zinc deficiencies in human [12,13,6]. Several studies have revealed that zinc fertilization not only increases crop yield and it also increases Zn concentration in grains [14,15].

Zinc biofortification, which aims to enhance Zn concentration as well as bioavailability of cereal grains, is considered as the more sustainable and economical solution to address human Zn deficiency [16]. Genetic and agronomic biofortifications are two important agricultural tools to improve cereal grain Zn concentration [17,18]. 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 [17,19,20,21]. On the contrary, agronomic biofortification through Zn fertilization (also referred to as ferti-fortification) results in increased grain production as well as higher Zn concentration in grains at the same time [22]. Also, even cultivars developed by genetic biofortification will need Zn fertilization.

It is hypothesized that Zn fertilization and appropriate varieties will help increase rice yield as well as Zn concentrations of rice grain. This will be helpful to increase nutritional property of rice grain and reduce malnutrition of people of Bangladesh.

The aim of this study is to determine the perfect Zn application dose to improve biofortification of zinc in rice grain.

2. MATERIALS AND METHODS

The experiment was conducted in the research field of Bangladesh Agricultural University (BAU), Mymensingh during January 2016 to May 2016. The experimental field is located at 24.75ºN latitude and 90.50ºE longitude at a height of 18 m above the mean sea level. It was a medium high land. AEZ of this soil is Old Brahmaputra Floodplain (AEZ 9) with silt loamy

Table 1. Physico chemical characteristics of soil and interpretation of their status

Physical properties				
Soil type	Clay	Sand	Silt	Textural class
Properties (%)	22	60	18	Silt loam
Chemical properties and interpretation				

pH	OM (%)	Total N (%)	Exch. K (me%)	Available status (mg kg^{-1})				
				P	S	Zn	Fe	B
6.51	3.10	0.170	0.237	4.60	12.43	0.780	55.4	0.240
N	M	L	M	VL	L	L	VH	L

OM- Organic Matter, N-Neutral, M-Medium, L- Low, VL- Very Low, VH – Very High

soil. Before the start of experiments, puddle layer (0–15 cm top soil) soil samples were taken from five random spots of the field and analyzed for various physico-chemical properties (Table 1).

The experiment was laid out in a split-plot design with three replications. There were two rice varieties such as BRRI dhan28 (V_1) and BINA dhan16 (V_2) and five Zn treatments such as T_1: Zn_0 (Zinc control), T_2: $Zn_{1.5}$ (Zinc @ 1.5 kg ha^{-1}), T_3: $Zn_{3.0}$ (Zinc @ 3.0 kg ha^{-1}), T_4: $Zn_{4.5}$ (Zinc @ 4.5 kg ha^{-1}), T_5: $Zn_{6.0}$ (Zinc @ 6.0 kg ha^{-1}).

Fertilizers were applied to each plot as per treatments. Nitrogen was applied @ 140 kg ha^{-1} from urea (46% N), P @ 20 kg ha^{-1} from TSP (20% P), K @ 75 kg ha^{-1} from MoP (50% K) and S @ 15 kg ha^{-1} from gypsum (18% S). The one-third dose of urea and the full dose of all other fertilizers were applied as basal to the individual plots during final land preparation. The second split of urea was applied after 25 days of sowing (active tiller stage) and the third split was after 55 days (panicle initiation stage). Zinc sulphate (23% Zn) as a source of zinc was applied to the respective plots as per treatments and mixed with soil prior to sowing. Fertilizers were incorporated into soil by hand.

34 days aged seedling were transplanted in the experimental plots maintain 3 seedling per hill. The plots were weeding and irrigated whenever required. Insecticide and fungicide spray were done to keep the crop free from any insect and pathogen attack.

After physiological maturity, the crop was harvested from each plot. The yield contributing parameters were recorded from 10 plants of each plot. The grain and straw yields were recorded plot-wise and the weight in g plot^{-1} was converted to kg ha^{-1}.

The grain samples were dried in an oven at 65ºC for about 48 hours and then ground by mottle pestle. The ground plant materials (grain) were stored in paper bags in a desiccator. The grain samples were analyzed for determination of N, Zn and Fe concentrations. The N content of rice grain was analyzed following the Kjeldahl method and Zn and Fe concentrations were determined by the following Yoshida, et al. [23] procedure.

The analysis of variance (ANOVA) for various crop characters and also for nutrient concentrations was done using statistical programmed package STAR version 2.0.1 [24]. Mean comparisons of the treatments were adjudged by the Duncan's Multiple Range Test [25]. Correlation statistics was performed to examine the relationship between nutrient (N, Zn & Fe) concentrations and zinc rates under study.

3. RESULTS AND DISCUSSION

3.1 Yield Contributing Parameters

In Table 2 the result showed that incase of plant height maximum plant height was observed in Binadhan-16 at the dose of $Zn_{(4.5)}$ (93.6 cm) and minimum plant height was observed in same variety at dose of $Zn_{(0)}$ (86.5 cm). Binadhan-16 produced taller plant at different doses of Zinc except control compare to BRRI dhan28.

The number of tillers hill^{-1} was significantly influenced by the Zn treatments. It was observed that at $Zn_{(3.0)}$ recorded the maximum number of tillers hill^{-1} (13) in BRRI dhan28 (13). The lowest number of tillers hill^{-1} (9.97) was found in control treatment in BRRI dhan28. Tillering ability also higher in Binadhan-16 at different doses of Zinc compare to BRRI dhan28 (Table 2).

High Panicle length was observed in Binadhan-16 (26.1 cm) at the treatment having Zn at 3.0 kg ha^{-1} ($Zn_{3.0}$) and low panicle length was observed in BRRI dhan28 (20.9 cm) at the control treatment having Zn at 0 kg ha^{-1} (Zn_0). Panical

length also high in Binadhan-16 at different doses of Zink (Table 2).

The Zn treatments resulted in a significant improvement in the number of filled grains per panicle Table 2). Among the treatments, Zn $_{(1.5)}$ (1.5 kg Zn ha^{-1}) demonstrated the highest number of grains panicle^{-1} in both varieties. Maximum grain number per panicle was observed in BRRI dhan28 at the treatment of Zn $_{(1.5)}$ that is statistically similar in all treatment in Binadhan-16 except control (Table 2). The Zn treated plots produced significantly higher number of grains compared to control. Such result, thus, indicates that decrease of grain production in rice occurs due to Zn deficiency and it is possible to overcome the problem by Zn application to soil.

There was no significant effect of Zn application on 1000-grain weight of rice. In case of BRRI dhan28 1000-grain weight decrease at different doses of Zn as compare to control but in case of n-16 1000-grain weight was higher at different doses of Zn as compare to control treatment (Table 2).

3.2 Yield Performance of Rice

The grain yield of rice was significantly influenced by Zn application (Table 3). Comparing the performances of five doses of Zn application, the four doses $Zn_{1.5}$, $Zn_{3.0}$, $Zn_{4.5}$ and $Zn_{6.0}$ were equally effective in correcting Zn deficiency and producing grain yield. The highest grain yield (7.70 t ha^{-1}) was obtained in BRRI dhan28 at the treatment $Zn_{4.5}$ which was statistically identical to $Zn_{3.0}$ (7.60 t ha^{-1}) (Table 3). Maximum grain yield observed in Binahan-16 at the treatment $Zn_{1.5}$ (6.78 t ha^{-1}) which was statistically identical to $Zn_{4.5}$ (6.67 t ha^{-1}).

The straw yield of rice increased significantly due to Zn application to soil for both varieties. Maximum straw yield observed in BRRI dhan28 at the treatment $Zn_{4.5}$ (8.29 t ha^{-1}) and incase of

Binadhan-16 produce high straw yield at the treatment $Zn_{1.5}$ (7.39 t ha^{-1}).

Harvest index of rice was not influenced by Zn application (Table 3). It is noted that treatment $Zn_{3.0}$ (3.0 kg Zn ha^{-1}) gave the highest harvest index and Zn control treatment (Zn_0) had the lowest harvest index for both varieties. There was no significant difference between two varieties at different doses of zinc application except control treatment (Table 3).

3.3 Biofortification of Rice Grain

The N concentration of rice grain was significantly influenced by different rates of Zn application (Table 4). The highest grain-N concentration (1.26%) was recorded in Binadhan-16 by the treatment containing Zn at 4.5 kg ha^{-1} ($Zn_{4.5}$) which was statistically different to other treatment. The control treatment had the lowest grain N concentration in both varieties. The grain-N concentration was higher in Binadhan-16 than that in BRRI dhan28. Protein concentration in rice grain was calculated as %N × 5.95; thus the grain protein concentration like grain N concentration was similarly influenced by the Zn treatments (Table 4). The results clearly indicate that Zn helps protein synthesis.

The grain Zn concentration was markedly influenced by the Zn fertilization (Table 4). Comparing the five rates of Zn application, $Zn_{6.0}$ (6.0 kg Zn ha^{-1}) demonstrated the highest Zn concentration in both varieties that was statistically different from other treatment. The Zn control treatment had the lowest Zn concentration. Binadhan-16 grain was content higher amount of zinc compare to BRRI dhan28. This result clearly mentioned that the highest Zn fortification of rice grain was obtained in Binadhan-16.

Like N concentration, the Fe concentration of rice grain responded significantly to Zn application (Table 4). The highest grain-Fe

Table 2. Yield contributing characters of two rice varieties at 5 doses of zinc application

Zinc rate	Plant height (cm)		Tillers hill^{-1} (no.)		Panicle length (cm)		Grains panicle^{-1} (no.)		1000-grain weight (g)	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
Zn_0	87.8	86.5	9.97	10.6	20.9	22.3	78.4	74.8	20.1	16.9
$Zn_{1.5}$	89.8	92.1	12.4	12.2	22.9	25.5	100	95.8	19.7	20.1
$Zn_{3.0}$	92.4	92.7	13.0	12.1	24.0	26.1	93.9	92.6	20.1	19.1
$Zn_{4.5}$	89.4	93.6	11.9	12.8	22.9	23.8	83.6	93.3	19.3	18.1
$Zn_{6.0}$	87.7	91.9	12.8	12.2	21.6	23.8	84.3	90.3	19.3	17.4

Table 3. Grain yield, straw yields and harvest index of two rice varieties at 5 doses of zinc application

Zinc rate	Grain yield (t ha^{-1})		Straw yield(t ha^{-1})		Harvest index (%)	
	V$_1$	V$_2$	V$_1$	V$_2$	V$_1$	V$_2$
Zn$_0$	6.11	6.14	6.92	6.99	46.7	46.7
Zn$_{1.5}$	7.03	6.78	7.46	7.39	48.5	47.9
Zn$_{3.0}$	7.60	6.55	7.99	7.09	48.7	48.0
Zn$_{4.5}$	7.70	6.67	8.29	7.34	48.1	47.5
Zn$_{6.0}$	7.37	6.24	8.03	7.03	47.8	47.0

Table 4. Nitrogen, protein, Zn and Fe concentrations of grain of two rice varieties at 5 doses of zinc application

Zinc rates	N (%)		Protein (%)		Zn (µg g^{-1})		Fe (µg g^{-1})	
	V$_1$	V$_2$	V$_1$	V$_2$	V$_1$	V$_2$	V$_1$	V$_2$
Zn$_0$	1.09bcA	1.06cA	6.49bcA	6.33cA	17.2cB	20.7bA	4.43cA	4.45cA
Zn$_{1.5}$	1.14abA	1.12bcA	6.83abA	6.66bcA	17.2cB	21.3bA	4.94cA	5.45abA
Zn$_{3.0}$	1.17aA	1.15bA	7.00aA	6.83bA	18.7bA	21.5bA	7.28aA	6.04aB
Zn$_{4.5}$	1.06cB	1.26aA	6.33cB	7.49aA	18.5bA	21.8bA	6.88aA	5.19bB
Zn$_{6.0}$	1.15abA	1.17bA	6.83abA	7.00bA	20.4aB	24.1aA	6.23bA	5.52abB
CV (%)	3.81		2.93		3.89		5.25	
Level of Significance	**		**		**		**	

The small letters in a column and capital letters in a row for Fe concentration do not differ significantly at 5% level by DMRT

concentration (7.28 µg g^{-1}) was observed in BRRI dhan28 at the treatment having Zn at 3.0 kg ha^{-1} (Zn$_{3.0}$) which was statistically similar at the treatment Zn$_{4.5}$ in same varieties.

4. CONCLUSION

BRRI dhan28 produce highest grain yield (7.70 t ha^{-1}) at the dose of Zn 4.5 kg ha^{-1} which was statistically similar with the yield obtained with Zn 3.0 treatments. The zinc control treatment (Zn0) produced the lowest grain yield in both varieties. The concentrations of N, Zn and Fe were significantly and positively influenced by the Zn treatments. The crop varieties did not differ significantly in respect of N and Fe concentrations, but the grain Zn concentration was considerably higher in BINA dhan16 than in BRRI dhan28. The grain N content as well as grain protein content linearly increased with the rates of Zn application. Thus, application of Zn at the rate of 6.0 kg ha^{-1} demonstrated the highest Zn fortification in both varieties but maximum zinc fortification was observed in Binadhan-16 (24.1 µg g^{-1}) in rice grain which was 12.2% higher than control treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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