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Improvement of Nutrient Concentration in Rice Grain by Zinc Biofortification

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Authors' contributions

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

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ABSTRACT

The experiment was conducted in the research farm at Bangladesh Agricultural University (BAU) to investigate the nutrient 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. 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 increased with the rates of Zn application. Application of Zn increased the protein concentration in rice grain showing that zinc had helped protein synthesis. The grain Zn concentration increased with Zn application rates in a quadratic line which indicates that Zn concentration in rice grain was increased by Zn fertilization, but it attained a maximum value up to Zn6.0 treatment which was 12.2% increase over control.

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1. INTRODUCTION

Rice (*Oryza sativa L.*), is one of the agronomically and nutritionally important cereal crops and most important staple food among cereal grains for a large part of the world's human population mainly in the developing countries, being the major source of carbohydrate and even protein. Rice is the major staple food crop for about half of the world's population whose nutritional status is a topic of discussion in the research sector. This principal staple food crop contains a reduced quantity of many essential micro and macro elements such as vitamins, minerals, some phytochemicals, essential amino acids and fatty acids, which are indispensable to human health [1].

Zinc deficiency is recognized as one of the major nutrient disorders in human and its effects are more profound in children [2]. Dietary deficiency of Zinc (Zn) is a substantial global public health and nutritional problem [3]. One-third of the world population is at risk due to low dietary intake of Zn [4] [5]. Micronutrient deficiencies afflict more than two billion individuals or one in three people [6]. Increasing the Zn and Fe concentrations of food crop plants, resulting in better crop production and improved human health, is an important global challenge. It is well-known that deficiency or overexposure to various elements has noticeable effects on human health. The effect of an element is determined by several characteristics, including absorption, metabolism, and degree of interaction with physiological processes.

Iron (Fe) and Zinc (Zn) are essential mineral nutrients for humans. Deficiencies in both contribute to severe cases of malnutrition. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport.

Proteins are made up of many building blocks, known as amino acids. Our body needs dietary protein to supply amino acids for the growth and maintenance of our cells and tissues. Our dietary protein requirement changes throughout life. The European Food Safety Authority (EFSA) recommends adults consume at least 0.83 g of protein per kg body weight per day (e.g. 58 g/day for a 70 kg adult) [7].

For proper digestion of food and growth, the human body needs nitrogen. It is extremely important in the development of the human fetus. Cell replacement, tissue repair all requires nitrogen for the production of new cells. For making some other types of compounds that are not proteins, nitrogen is used like heme in haemoglobin which carries oxygen in red blood cells. Therefore, we come to know that nitrogen plays a crucial role in our life. It helps in protein synthesis. Our body eliminates ammonia by converting it to urea, which kidneys excrete it in the form of urine. In this way, nitrogen is returned back to the environment.

Biofortification, the enhancement of bioavailable micronutrients in the edible parts of stable food by either conventional plant breeding, biotechnology techniques, or agronomic approaches can help to alleviate malnutrition in the regions where the main source of calories and micronutrients come from staples [8,9].

Micronutrient levels in food need to raise for the sake of improved human and animal health. Preliminary studies indicate that zinc enrichment of seeds of cereals is possible through zinc fertilization and the magnitude depends on the crop and varieties. Further observation is that when seed zinc concentration increases, the protein concentration also increases. Thus, the prevailing situation makes micronutrient research a promising area of study in meeting the demand for higher food production with enhanced mineral nutritional value in this millennium.

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 24.75°N latitude and 90.50°E longitude at a height of 18m above the mean sea level. It was a medium high land. AEZ of this soil is Old Brahmaputra Floodplain (AEZ 9) with silt loamy 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).

Table 1. Physicochemical 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

The experiment was laid out in a split-plot design with three replications. There were two rice varieties such as BRRI dhan28 (V1) and BINA dhan16 (V2) and five Zn treatments such as T1: Zn0 (zinc @ 0.0 kg ha-1), T2: Zn1.5 (Zinc @ 1.5 kg ha-1), T3: Zn3.0 (Zinc @ 3.0 kg ha-1), T4: Zn4.5 (Zinc @ 4.5 kg ha-1), T5: 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 (44-46% P), K 75 kg ha-1 from MoP (60% K) and S 15 kg ha-1from 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 the soil by hand.

34 days aged seedlings were transplanted in the experimental plots maintained 3 seedlings per hill. The plots were weeded and irrigated whenever required. Insecticide and fungicide spray was 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 65°C 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 procedure [10].

The analysis of variance (ANOVA) for various crop characters and also for nutrient concentrations was done using the statistical programmed package STAR version 2.0.1 [11]. Mean comparisons of the treatments were adjudged by Duncan's Multiple Range Test [12]. Correlation statistics were performed to examine the relationship between nutrient (N, Zn & Fe) concentrations and zinc rates under study.

3. RESULTS

Rice grain was analyzed for the determination of N, Zn and Fe concentrations. The results of the experiment are presented in tabular and graphical forms and stated as text.

3.1 Nitrogen Concentration

The N concentration of rice grain was significantly influenced by different rates of Zn application (Table 2). The highest grain-N concentration (1.17%) was recorded by the treatment containing Zn at 3.0 kg ha-1 (Zn3.0) which was similar to other treatments except control (Zn0). There is increase in N content up to certain level increase in Zn concentration thereafter it decreases. Optimum concentration of 3.0 kg/ha Zn was found for maximum N content. However, protein content remained same after optimum dose of Zn i.e. 3.0 kg/ha. The control treatment had the lowest grain N concentration (1.0%). The grain-N concentration was little higher in BINA dhan16 (1.15%) than that in BRRI dhan28 (1.13%) (Table 3). There was a significant interaction between variety and zinc in case of grain-N concentration (Table 4). 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 2). The results clearly indicate that Zn helps protein synthesis.

3.2 Zinc Concentration

The grain Zn concentration was markedly influenced by the Zn fertilization (Table 2). Comparing the five rates of Zn application, Zn6.0 (6.0 kg Zn ha-1) demonstrated the highest Zn concentration (21.3 µg g-1) that was not significantly different from that observed with Zn4.5 treatment (4.5 kg Zn ha-1). The Zn control treatment had the lowest Zn concentration (19.0 µg g-1). 10.2% and 12.2% increase in Zn concentrations of rice grain over control were recorded due to higher doses of 4.5 kg Zn ha-1 and 6.0 kg Zn ha-1 respectively (Table 2). The highest Zn fortification of rice grain was obtained from treatment Zn6.0 (6.0 kg Zn ha-1). Zinc application linearly increased the grain Zn concentration and application of Zn at the rate of 1 kg ha-1 increased the Zn concentration in rice grain by 0.41µg g-1 (Fig. 1). The Zn concentration of rice grain show significant difference in two varieties (Table 3). It showing that the grain Zn content in BRRI dhan28 (18.3µg g-1) is lower than in BINA dhan16 (21.9µg g-1). The interaction effect of variety and zinc on Zn concentration was significant indicating that the influence of zinc application on grain-Zn concentration was affected by the varieties (Table 4).

3.3 Iron Concentration

Like N concentration, the Fe concentration of rice grain responded significantly to Zn application (Table 2). The highest grain-Fe concentration (6.67 µg g-1) was observed in the treatment having Zn at 3.0 kg ha-1 (Zn3.0). The Zn control treatment (Zn0) produced the lowest grain-Fe concentration (4.44µg g-1) (Table 2). Zinc application linearly increased the grain Fe concentration and application of Zn at the rate of 1 kg ha-1 increased the Fe concentration in rice grain by 0.25µg g-1 (Fig. 1). The rice varieties did not differ significantly based on grain-Fe concentration showing the order of BRRI dhan28 (5.95µg g-1) >BINA dhan16 (5.34µg g-1). Further, interaction effect of variety and zinc on grain-Fe concentration was also significant (Table 4). The grain-Fe concentration was maximum in V1 × Zn3.0 (7.28µg g-1), which was statistically similar to V1 × Zn4.5 (6.88µg g-1) and the lowest result in V1 × Zn0 (4.43µg g-1).

4. DISCUSSION

With the advancement of time accompanied with cultivation of modern varieties of crops and increasing cropping intensity, the soil fertility has declined and deficiency of some micronutrients has arisen in Bangladesh soil. Among the micronutrients, Zn deficiency is the most visible micronutrient disorder. A lot of field trials have been out with Zn fertilization, the objective of all those trials was to achieve yield benefits, not to achieve a higher nutrient concentration of crop production. The present study was done to improve the nutrient concentration in grains of rice varieties through biofortification. There is increasing evidence that improved growth, yield and grain Zinc concentration through Zn fertilization of many crops, including rice [13,14]. Application of Zn fertilizers greatly contributes to the biofortification of cereal grains with Zn [15]. By contrast, application of various inorganic and chelated Fe fertilizers remains ineffective for increasing grain Fe concentration. However, improving N nutritional status of plants promoted accumulation of Fe and Zn in grains. It appears that the N nutritional status of plants plays a critical role in the biofortification of cereal grains with Zn and Fe. Recently work on agronomic biofortification of zinc and iron in rice and wheat has been initiated [16].

Table 2. Nitrogen, protein, Zn and Fe concentrations of rice grain as influenced by zinc rates

Zinc rate	N (%)	Protein (%)	Zn($\mu g\ g^{-1}$)	% Zn increase over control	Fe($\mu g\ g^{-1}$)
Zn_0	1.07b	6.41b	19.0d	-	4.44d
$Zn_{1.5}$	1.13a	6.74a	19.3cd	1.5	5.19c
$Zn_{3.0}$	1.17a	6.91a	20.1bc	6.0	6.67a
$Zn_{4.5}$	1.16a	6.91a	20.9ab	10.2	6.04b
$Zn_{6.0}$	1.16a	6.91a	21.3a	12.2	5.88b
CV (%)	3.81	3.81	3.89	-	6.25
Level of significance	*	*	**	-	**

*In a column, the values having same letter do not differ significantly at 5% level by DMRT; Zn0 = Zinc @ 0.0 kg ha-1; Zn1.5 = Zinc @ 1.5 kg ha-1; Zn3.0 = Zinc @ 3.0 kg ha-1; Zn4.5 = Zinc @ 4.5 kg ha-1; Zn6.0 = Zinc @ 6.0 kg ha-1; ** = 1% level of significance; CV = Co-efficient of variation*

Biofortification is a feasible and cost-effective means of delivering micronutrients to populations that may have limited access to diverse diets and other micronutrient interventions [8].

Application of Zn fertilizer resulted in a significant influence on the rice crop which was assessed in terms of grain nutrient concentrations (%N, %Zn and %Fe) in rice grain. The increase in grain yield due to Zn fertilization might be the fact that Zn played an important role in biosynthesis of the IAA and initiation of primordia for reproductive parts and as a result of the favorable effect of zinc on the metabolic reactions within the plants.

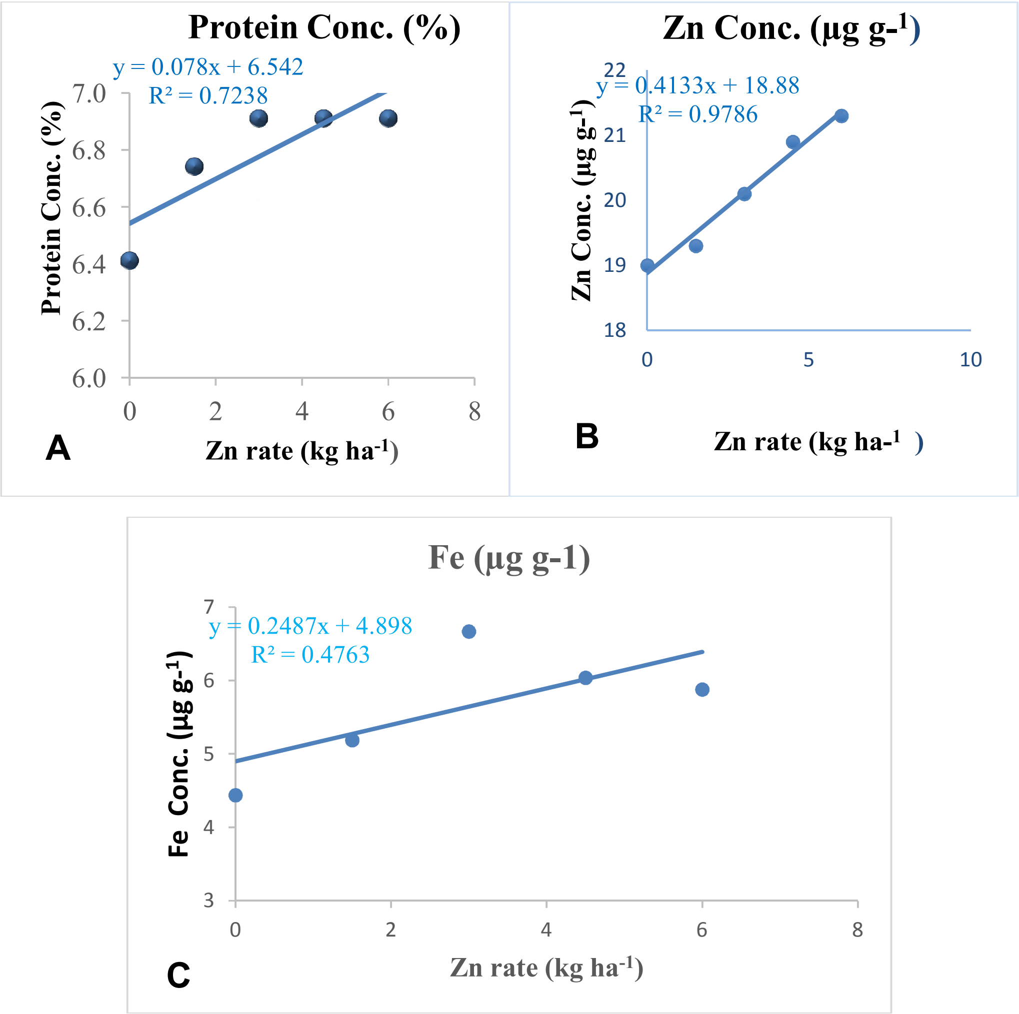

Fig. 1. Relationship between Zn rates and rice protein (A), zinc (B) and iron (C) Concentrations

Table 3. Nitrogen, protein, Zn and Fe concentrations of rice grain as influenced by varieties

Variety	N (%)	Protein (%)	Zn(µg g^{-1})	Fe(µg g^{-1})
V_1	1.13	6.70	18.3b	5.95
V_2	1.15	6.86	21.9a	5.34
CV (%)	6.85	4.85	3.46	14.8
Level of significance	NS	NS	**	NS

*In a column, the values having same letter do not differ significantly at 5% level by DMRT; V1 = BRRI dhan28; V2 = BINA dhan16; ** = 1% level of significance; NS = Not significant; CV = Co-efficient of variation*

Table 4. Nitrogen, protein, Zn and Fe concentrations of rice grain as influenced by variety × Zn interaction

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	18.7abA	21.5bA	7.28aA	6.04aB
$Zn_{4.5}$	1.06cB	1.26aA	6.33cB	7.49aA	20.0aA	21.8bA	6.88aA	5.19bB
$Zn_{6.0}$	1.15abA	1.17bA	6.83abA	7.00bA	18.4bcB	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; Zn0= Control; Zn1.5 = Zinc @ 1.5 kg ha-1; Zn3.0 = Zinc @ 3.0 kg ha-1; Zn4.5 = Zinc @ 4.5 kg ha-1; Zn6.0 = Zinc @ 6.0 kg ha-1; V1 = BRRI dhan28; V2 = BINA dhan16; ** = 1% level of significance; NS = Not significant; CV = Co-efficient of variation*

The N concentration of rice grain was significantly influenced by Zn treatments. The highest grain-N concentration (1.17%) was recorded by the treatment containing Zn at 3.0 kg ha-1 (Zn3.0) which was statistically identical to others except control treatment. Like grain N concentration, the grain Fe concentration increased with Zn rates and every increment of 1 kg ha-1resulted in an increase of 0.25μg g-1 Fe.

There was no significant difference between the varieties in accumulating N in rice grain and there was a significant difference between the two varieties of rice in accumulating Zn in rice grain. The probable reason might be that the tested rice varieties have different genetic makeup.

5. CONCLUSION

Biofortification, the process of adding nutrients to food crops through breeding and agronomic (fertilization) approaches, is important for human health and nutrition. The present study addressed the zinc fertilization approach intending to enrich nutrient concentration in rice grains. Chemical analysis of rice grains showed that the concentrations of N, Zn and Fe were significantly and positively influenced by the Zn treatments. The crop varieties did not differ significantly based on grain N and Fe concentrations. But, the grain Zn concentration was remarkably higher in BINA dhan16 than in BRRI dhan28.

The Zn application is increased the grain N content up to Zn 3.0 treatment and then decreased, but the zinc application linearly increased the protein content. Application of Zn increased the protein concentration in rice grain showing that zinc had helped protein synthesis. The grain Zn concentration increased with Zn application rates in a quadratic line which indicates that Zn concentration in rice grain was increased by Zn fertilization, but it attained a maximum value up to Zn6.0 treatment which was 12.2% increase over Zn control. As the people of under developing countries cannot afford to supplemented and diversified foods, research and development of nutrient-enriched biofortified crops should carried out to overcome this problem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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