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EVALUATION OF IRON AND ZINC ENRICHED RICE (*Oryza sativa* L.) GENOTYPES IN DIFFERENT LOCATIONS OF BANGLADESH

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Abstract

Malnutrition among women and children are extremely prevalent in Bangladesh. Bangladeshi children become stunted and underweight due to micronutrients deficiencies particularly iron (Fe) and zinc (Zn). Anemia is also highly prevalent among children and women in the country due to Fe deficiency. Biofortification of rice (*Oryza sativa* L.) with micronutrients is widely recognized as a sustainable strategy to alleviate human Fe and Zn deficiencies in Bangladesh where rice is the staple food. With this view, four advanced brown rice genotypes: IZSD-10, IZSD-26, IZSD-44 and IZSD-45 along with Binadhan-20 as check variety were analyzed for grain Fe and Zn concentration using energy Dispersive X-ray Fluorescence Spectrophotometer (ED-XRF). Advanced yield trial was conducted in three different locations of Bangladesh during Aman season of 2020 in a randomized complete block design (RCBD) with three replications in each location. The Fe concentration varied from 9 to 15 mg kg^{-1} and 1 to 4 mg kg^{-1} whereas Zn concentration ranged from 45 to 59 mg kg^{-1} and 29 to 40 mg kg^{-1} in unpolished and polished rice, respectively. Almost higher Fe loss (~60 to 94 %) was observed compared to Zn (~18 to 42%) at 10% polishing throughout the grain shape that was responsible due to loss of embryo, pericarp and aleurone layer. Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations. The genotype IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days with yield 5.36 t ha^{-1}). Considering earliness, Fe and Zn content and higher yield, the genotypes IZSD-26 and IZSD-10 might be recommended for further regional yield trial to develop Fe and Zn enriched varieties. Moreover, other genotypes IZSD-44 and IZSD-45 with high Zn concentration were identified, which have the potential to be used in rice improvement for bio fortification.

Key words: Advanced yield trial, Bangladesh, Biofortification, Iron (Fe), Zinc (Zn) Rice (*Oryza sativa* L.)

Introduction

Rice is the staple food for about 164.6 million people in Bangladesh (BBS, 2019) which inevitably meets most of the nutritional demand of the majority of its people (Shelley *et al.*, 2016). But rice can't provide all the nutrition a human being requires and many poor

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people throughout the world particularly in Asia face acute nutritional deficits. Millions of individuals have a danger of sickness, disease, growth failure and more simply, deficiencies in Fe, Zn and the vitamins needed. It is currently a significant problem in Bangladesh also (FAO, IFAD, UNICEF, WFP and WHO 2021). It is estimated that micronutrient deficiencies affect approximately 1.6 billion people globally, particularly children, pregnant and lactating women, in low and middle-income countries. (Chandu *et al.*, 2020). Biofortification can be used to improve nutritional quality by increasing the content of important micronutrients in rice (Hasanzadeh and Hazrati, 2020). Since rice fortifies a large part of the diet, it can be a revolutionary remedy for micronutrients needed. Afterwards, rice yield differs due to growth conditions, e.g., various locations, seasonal fluctuations, diverse planting dates and so on (Sarker, 2002). With this view, Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA) has developed some Fe and Zn enriched genotypes. Therefore, multi-locations trial is very important to evaluate the performance of rice varieties through appropriate cultural practices to get maximum yield and quality before releasing the rice promising genotypes as national varieties and passed on to the farmers as end users. The objectives of the present study were to identify brown rice genotypes with enriched Fe and Zn concentration using ED-XRF method and to evaluate the yield performance of those genotypes in different locations of Bangladesh.

Materials and Methods

Plant materials

Four advanced brown rice genotypes: IZSD-10, IZSD-26, IZSD-44, IZSD-45 were gained from crosses between Binadhan-7 and IR66946-3R-178-1-1 in the year of 2012 to develop iron and zinc enriched variety in Bangladesh Institute of Nuclear Agriculture (BINA). Binadhan-20 was used as check that was released as Fe and Zn enriched rice variety in Bangladesh.

Estimation of Fe and Zn concentration

Well dried 10g of paddy sample from each genotype was de-husked using non-metallic de-husker (Krishi international 810 de-husker) having a roller made of polymer to avoid Fe and Zn contamination. De-husked rice was cleaned by removing broken grains and debris and 5 g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Fe and Zn concentration in brown rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (ED-XRF) instruments (model X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) from IRRI, Bangladesh Office. Concentration of Fe and Zn was expressed in micrograms per gram ($\mu g\ g^{-1}$) or parts per million (ppm) and converted into milligrams per kilogram ($mg\ kg^{-1}$).

Advanced yield trial

The genotypes were evaluated in three locations such as BINA HQ Mymensingh, BINA sub-stations Jamalpur and Nalitabari as advanced yield trial during Aman season of 2020. The trial was replicated three times in each location. The unit plot size for each entry was 20 m^2 (5 m × 4 m). Seedling ages varied from 25 to 30 days at the locations due to different unavoidable circumstances during transplanting time. Seedlings were transplanted at 25 cm × 15 cm spacing on 5^{th} August with a single number. At the rate of 120, 20, 60, 20 and 4 kg by N, P, K, S and Zn containing fertilizers per hectare were applied, respectively. All fertilizers except urea were applied as a basal dose and whereas urea was applied in 3 equal splits at 15, 30 and 45 days after transplanting. Other standard management practices were followed as and when necessary. Appropriate measures were taken to control pests and diseases. Data on days to flowering, days to maturity, plant height, total tillers $hill^{-1}$, effective tillers $hill^{-1}$, panicle length, grains $panicle^{-1}$, sterile spikelets $panicle^{-1}$, 1000-grain weight and grain yield were recorded. For yield estimation, 10 m^2 sample area from each plot was harvested at maturity and grain yield was adjusted to 14% moisture content. The yield, yield contributing traits and other parameters were statistically analyzed using the software Statistix 10.

Results and Discussion

The mean value of grain Fe concentration of rice genotypes ranged from 9 to 15 mg kg^{-1} and 1 to 4 mg kg^{-1} in unpolished and polished rice, respectively (Fig. 1). Besides, the mean value of Zn concentration of rice genotypes ranged from 45 to 59 mg kg^{-1} and 29 to 40 mg kg^{-1} in unpolished and polished rice, respectively (Fig. 2). The lowest concentration of Fe was observed in IZSD-10 and Binadhan-20 with 9 mg kg^{-1} and that of Zn in IZSD-44 with 45 mg kg^{-1} while the highest grain Fe and Zn concentration was observed in IZSD-26 with 15 mg kg^{-1} and 59 mg kg^{-1}, respectively in unpolished rice. In polished rice, the lowest concentration of Fe was observed in IZSD-44 with 0.8 mg $kg^{-1}$and that of Zn in Binadhan-20 with 29 mg kg^{-1} while the highest grain Fe and Zn concentration was observed in IZSD-10 with 4 mg kg^{-1} and 40 mg kg^{-1}, respectively. Higher heterogeneity in Fe and Zn levels among the genotypes were observed following polishing where Fe loss (~60 to 94 %) was almost higher at 10% polishing throughout the grain shapes compared to Zn (~18 to 42%). More loss of Fe than Zn was caused during polishing that might be owing to a loss of embryo, pericarp and aleurone layer, partly or total, in the polishing process; because of embryo has more Fe followed by pericarp and aleurone layer with endosperm, variation in pericarp or aleurone layer thickness or both (Matres *et al.*, 2021; Cakmak and Kutman, 2018; Díaz-Benito *et al.*, 2018; Kawakami and Bhullar, 2018; Trijatmiko *et al.*, 2016; Gregorio, 2002). It happened due to mechanical damage and removing of outermost layer to make thin rice grain (Majumder *et al.*, 2019). Almost all the genotypes had higher Fe and Zn concentration than the check variety. Similar findings were found by Anuradha *et al.* (2012) from analyzed 126 accessions of brown rice genotypes for Fe (6.2-71.6 ppm) and Zn (26.2-67.3 ppm) concentration using Atomic Absorption Spectrophotometer (AAS). The analysis of 100 genotypes of rice for Fe and Zn content using ED-XRF method also performed by Chandu *et al.* (2020). The authors found Fe concentration varied from 1.6 to 15.2 ppm whereas Zn concentration ranged from 6.2 to 33.2 ppm of the tested germplasms. Banerjee *et al. (*2010) screened 46 rice lines including cultivated and wild accessions and showed that wild rice accessions have higher grain Fe and Zn concentration.

Significant differences were observed among the genotypes and the check variety for most of the yield and yield attributing characters for three individual locations and mean over locations from advanced yield trial presented in Table 1. The IZSD-26 performed better among the genotypes and check variety Binadhan-20 in terms of yield at all the locations. The highest yield was found on IZSD-26 at Nalitabari (5.71 t ha^{-1}) and the lowest was in IZSD-44 (4.27 t ha^{-1}) at Jamalpur. All the tested genotypes matured earlier than the check variety. Days to maturity ranged from 107-130 days for the three locations. From mean over locations, it was appeared that the IZSD-26 had significantly shorter duration (111 days) and higher number of filled grains (171.29) at all locations than the check variety followed by IZSD-45. Regarding plant height, significant differences among the genotypes were found ranging from 95-128 cm at all the locations. From mean over locations, check variety Binadhan-20 was the tallest (124.42 cm), while the genotype IZSD-45 was the shortest (102.40 cm) among the genotypes. There was no significant difference between the tested genotypes and check variety for the number of total tillers, number of effective tillers and panicle length. The lowest (22.06 g) 1000-grain weight was found in IZSD-44 and the highest was found in Binadhan-20 (24.03 g). Grain size was medium fine with red pericarp (Fig. 3). Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations than the check variety. But the genotypes IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days) (Table1). Kader *et al.* (2020) reported that a promising line which was later released as a Zn enriched variety BRRI dhan84 could produce 6.0-6.5 t ha^{-1} grain yield and mature 140 days in the dry (Boro) season. Besides, early maturing and high yielding varieties with good quality grain are chosen by farmers to cultivate (Mamin *et al.,* 2015). So, further yield trials in several years at different locations are needed to confirm the genotypes' stability (Inabangan-Asilo *et al.,* 2019) that was found in this experiment to release as a variety.

Conclusions

Based on the analysis, micronutrient enriched IZSD-26 and IZSD-10 genotypes contained higher Fe and Zn than the check variety Binadhan-20 both in unpolished and polished rice. Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations, also the genotypes IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days). Considering earliness, Fe and Zn content with higher yield, the genotypes IZSD-26 and IZSD-10 might be recommended for regional yield trial in next Aman season. Furthermore, other genotypes having higher Fe and Zn content can be used as plant materials for biofortification breeding programs in future.

Table 1. Agronomic performance of Fe and Zn enriched rice genotypes along with check variety at different locations of Bangladesh during Aman season 2020.

Locations	Genotypes	Days to flowering	Days to maturity	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Grains panicle^{-1} (no.)	Sterile spikelets panicle^{-1} (no.)	1000-grain weight (g)	Grain yield (t ha^{-1})
BINA HQ, Mymensingh	IZSD-10	88 b	119 b	100.13 b	11.33 a	10.0 a	24.87 a	160.00 bc	44.00 a	21.87 a	5.32 a
	IZSD-26	88 b	118 b	100.67 b	11.6 a	10.67 a	25.20 a	176.33 a	56.33 a	22.21 a	5.24 a
	IZSD-44	84 c	114 b	97.43 b	10.00 a	9.33 a	23.53 a	143.00 c	46.67 a	22.05 a	4.60 c
	IZSD-45	85 bc	117 b	96.40 b	11.40 a	10.40 a	23.00 a	175.00 ab	42.66 a	22.69 a	4.87 b
	Binadhan-20	97 a	129 a	123.67 a	11.67 a	10.00 a	23.67 a	165.33 bc	47.33 a	23.33 a	5.30 a
BINA Sub-station Jamalpur	IZSD-10	86 bc	108 b	112.67 b	10.67 a	10.33 a	26.00 a	155.67 ab	44.67 a	22.32 a	5.23 a
	IZSD-26	88 b	114 a	114.0 b	12.33 a	11.33 a	25.67 a	167.00 a	39.33 a	22.57 a	5.17 b
	IZSD-44	83 c	107 b	115.13 b	10.67 a	9.67 a	25.67 a	124.33 b	41.00 a	21.67 a	4.27 c
	IZSD-45	83 c	107 b	114.34 b	11.67 a	11.00 a	26.00 a	150.67 ab	40.67 a	22.23 a	5.12 b
	Binadhan-20	94 a	116 a	127.33 a	11.67 a	10.67 a	25.00 a	167.00 a	44.00 a	23.34 a	5.29 a
BINA Sub-station Nalitabari	IZSD-10	86 b	116 bc	100.00 b	11.13 a	10.33 ab	25.80 a	153.20 bc	31.00 a	24.36 ab	5.19 c
	IZSD-26	86 b	117 b	98.93 b	11.00 a	10.4 ab	26.73 a	170.53 a	44.73 a	23.33 bc	5.71 a
	IZSD-44	84 b	113 c	95.40 b	10.20 a	9.53 b	25.13 a	132.60 c	45.73 a	22.46 c	5.16 c
	IZSD-45	84 b	115 bc	96.47 b	11.13 a	10.7 a	27.00 a	158.73 bc	33.80 a	24.36 ab	5.59 ab
	Binadhan-20	98 a	129 a	128.27 a	11.60 a	106 ab	27.06 a	161.33 ab	35.80 a	25.43 a	5.50 b
Mean over locations	IZSD-10	87 bc	114 bc	104.27 b	11.04 a	10.33 a	25.56 a	159.62 ab	39.89 a	22.86 ab	5.22 a
	IZSD-26	87 b	116 b	104.53 b	11.93 a	10.8 a	25.87 a	171.29 a	46.80 a	22.74 ab	5.37 a
	IZSD-44	84 c	111 c	102.72 b	10.29 a	9.51 a	24.78 a	133.31 c	44.47 a	22.06 b	4.67 b
	IZSD-45	84 bc	113 bc	102.40 b	11.40 a	10.7 a	25.33 a	161.47 ab	39.04 a	23.13 ab	5.19 a
	Binadhan-20	96 a	129 a	124.42 a	11.64 a	10.42 a	25.24 a	164.56 a	42.30 a	24.03 a	5.36 a

In a column, values with the same letter(s) for individual location/combined means do not differ significantly at 5% level.

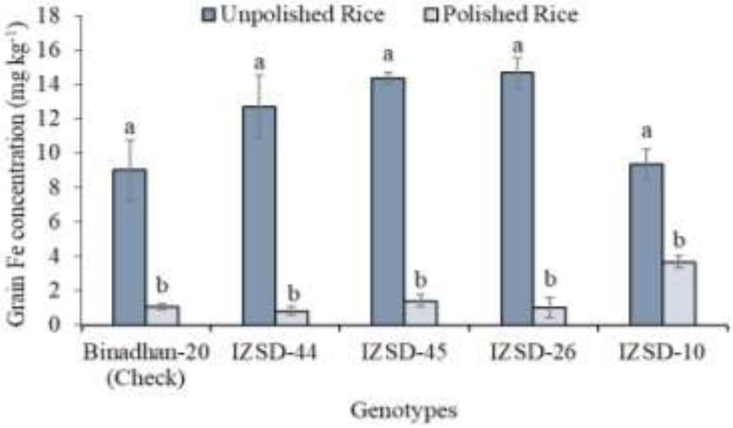

Fig. 1. Grain Fe concentration in unpolished and polished rice of the four genotypes along with check. Standard error indicated by error bars and lettering was done at 5 % level of Tukey's honest significant difference test.

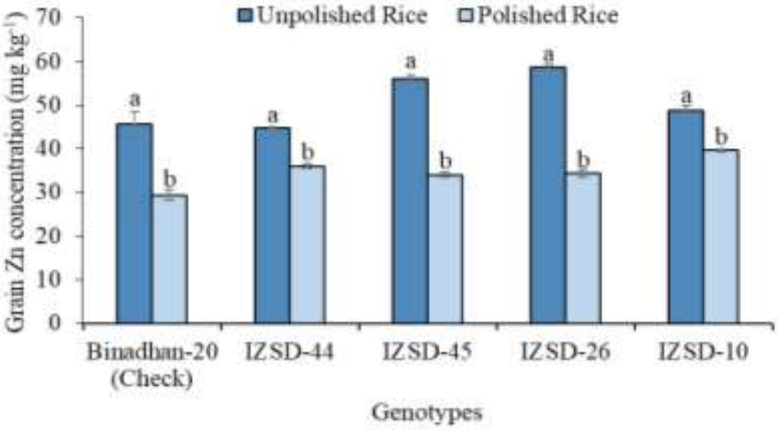

Fig. 2. Grain Zn concentration in unpolished and polished rice of the four genotypes along with check. Standard error indicated by error bars and lettering was done at 5 % level of Tukey's honest significant difference test.

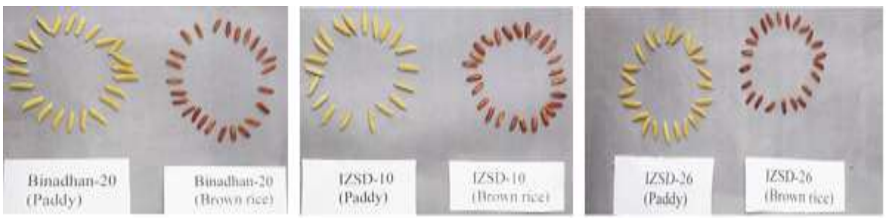

Fig. 3. Pictorial view of paddy with brown rice of Fe and Zn enriched genotypes (IZSD-10, IZSD-26) along with check variety Binadhan-20.

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