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Loading of zinc and iron in grains of different wheat genotypes in the calcareous and floodplain soils of Bangladesh

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ABSTRACT

Major malnutrition in Bangladesh is zinc (Zn) and iron (Fe) deficiency as most people commonly depend on cereals, chiefly rice and wheat. The main objectives are to enhance Zn and Fe concentrations through the use of selected varieties and the application of respective fertilizers. Field experiments were conducted at Bangladesh Agricultural University (BAU) farm, Mymensingh (AEZ 9, non-calcareous soil) and at Bangladesh Institute of Nuclear Agriculture (BINA) substation, Ishwardi (AEZ 11, calcareous soil) for two consecutive wheat seasons (2014–15 and 2015–16) with 10 varieties and 15 advanced lines. Varieties BARI Gom 25, 27, 28 & 29 and breeding lines Vijay, HPYT-5, 15 & 21 and BL-1883 have been recognized as Zn-enriched wheat varieties (24–30 μg g^{-1}). Among the genotypes, Zn further increased by 4–8 μg g^{-1} due to Zn fertilization. Concerning Fe-enriched wheat genotypes (24–30 μg g^{-1}), five varieties viz. Shatabdi, Prodip, BARI Gom 25 & 28 and Sufi, and four lines such as HPYT-12, BL-1883, BL-1040 and Fery-60 have been identified. The grain Fe concentration of wheat genotypes increased when Fe was added, the increment being 6–12 μg g^{-1}. A positive relationship between Zn and N is observed with increased protein content. The grain yield of wheat was increased by 3.8–25.7% due to Zn application over the varieties and locations but Fe addition had no effect. The result of the current study showed that a potential breeding line with appropriate fertilization can improve Zn and Fe levels in wheat grain, without incurring loss to wheat yield.

1. Introduction

More than 40% of the World population is directly affected by zinc (Zn) deficiency [1] and it is very deep in Bangladesh due to the high consumption of cereals [2,3]. The low content of Zn in cereals results in a deficiency in general people [4]. Cereals provide 60% Zn and 55% Fe in Bangladesh [5] and anemia caused by Fe deficiency is highly prevalent among children and women in this country.

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The process of increasing micronutrients in cereals grain known as biofortification could help reduce Zn deficiency among poor people WHO's are not capable of fortified food [6,7]. The 'baby zinc table has a huge contribution to reducing child mortality caused by diarrhoea in Bangladesh [8]. Iron is an important nutrient that has a deficiency problem in plants and human as well as a major challenge for health concerns [9].

Wheat (*Triticum aestivum*) is a salient cereal crop in Bangladesh. It has a high nutritive value (>75% starch and >11% protein). The requirement of wheat grain is about 24 kg/capita/year, so more than 50% of wheat is imported from foreign countries to Bangladesh. These foods usually contain low amounts of zinc which causes dietary zinc deficiency in humans and animals. Among the cereals, wheat has a 28% contribution [10]. It is assumed that Zn and Fe fertilization and selection of wheat varieties/genotypes will increase the yield as well as Zn, Fe and other mineral contents of food grains, and thus would help increase the food grain production and reduce the malnutrition of people of Bangladesh. It is necessary to trace out the potential wheat cultivars that have a higher accumulation of micronutrients by fertilizer application as well as remove the unbearable condition of Zn and Fe uptake [11–14]. This present study was executed to increase Zn and Fe concentration by selecting potential wheat varieties and fertilizer applications.

2. Materials and methods

2.1. Experimental site and location

This study was executed at the Soil Science Field Laboratory of BAU, Mymensingh and BINA substation, Ishwardi for two consecutive wheat seasons, 2014–2015 and 2015–2016. The two locations belong to the AEZ 9 (Old Brahmaputra Floodplain) and AEZ 11 (High Ganges River Floodplain), respectively. A major contrast between the two AEZs is – the soils of AEZ 9 are non-calcareous and that of AEZ 11 are calcareous. Texturally, BAU farm Mymensingh soils are silt loam and fall under the Sonatala series. This location is medium-high land and as per FAO Soil Unit is *Chromic-Eutric Gleysols* under USDA Soil Taxonomy Aeric Haplaquept. The Ishwardi soil is silty clay loam in texture and the name of the series is Sara. This location is medium-high land and soil as per FAO Soil Unit is*Chromic-Eutric Gleysols* under USDA Soil Taxonomy Aeric Haplaquept and both soil of the site namely "Gleysols" according to the world references base [15]. Soil characteristics both morphological and chemical are shown in Table 1. The site of the experiment is in a sub-tropical climate and the temperature is high (20–39 °C) in summer (April–September) compare to winter (October–March) is low (5–19 °C) in the rabi season.

2.2. Crop variety and season

During 2014–15 and 2015–16, there were ten wheat varieties, and fifteen advanced lines were used as crop varieties. Varieties are Shatabdi (V_1), Sufi (V_2), Bijoy (V_3), Prodip (V_4), BARI Gom 25 (V_5), BARI Gom 26 (V_6), BARI Gom 27 (V_7), BARI Gom 28 (V_8), BARI Gom 29 (V_9) and BARI Gom 30 (V_{10}) and advanced line (Rawal 87 (L_1), Vijay (L_2), BAW 917 (L_3), Fery 60 (L_4), BL 1040 (L_5), KRLI-4 (L_6), BL 1883 (L_7), BAW (L_8), HPYT-5 (L_9), HPYT-9 (L_{10}), HPYT-12 (L_{11}), HPYT-14 (L_{12}), HPYT -15 (L_{13}), HPYT -21 (L_{14}), and HPYT -24 (L_{15}).

2.3. Design and treatments

The fertilizer treatments for wheat were $T_1 = Zn_0Fe_0$, $T_2 = Zn_3Fe_0$ and $T_3 = Zn_3Fe_4$, subscripts representing the dose in kg ha^{-1}. Zinc and iron were added as $ZnSO_4{\cdot}7H_2O$ (23% Zn) and $FeSO_4{\cdot}7H_2O$ (19% Fe), respectively. The rate of Zn was 3 kg ha^{-1} and that for Fe was 4 kg ha^{-1}. The other nutrients were used as N_{120}, P_{30}, K_{50}, S_{12} and $B_{1.5}$ kg ha^{-1} from urea, triple super phosphate, murate of potash, gypsum and boric acid respectively as a recommended rate for all plots. These experiments were designed as split-plot and replicated thrice. Seeds were sown continuously along the lines with 20 cm from line to line distance. The wheat seeds were sown on November 23, 2014 and matured on March 21, 2015. In 2015–16, sowing was done on November 17, 2015 which was harvested on March 12, 2016. Intercultural operations were done whenever necessary. The crops were harvested when they attained full maturity. Whole plot

Table 1
The initial soil characteristics of the experimental site.

Soil Characteristics	BAU farmMymensingh	BINA substation Ishwardi
Textural Class	Silt loam	Silty clay loam
Organic carbon (%)	1.14	1.47
pH	6.5	7.5
Total N (%)	0.11	0.12
Available P (mg kg^{-1})	7.5	14.6
Exchangeable K (cmol kg^{-1})	0.12	0.11
Available S (mg kg^{-1})	14.0	19.3
Available Zn (mg kg-^{1})	0.78	0.97
Available B (mg kg^{-1})	0.24	0.35
Available Fe (mg kg^{-1})	55.4	24.0
Available Cu (mg kg^{-1})	3.0	5.0
Available Mn (mg kg^{-1})	19.4	2.6

areas were harvested to record grain and straw yields. A sub-sample weighting 100 g grain sample from every plot was taken to the laboratory for chemical analysis of N, Zn and Fe.

2.4. *Soil and plant analysis*

The initial soil samples were done before starting the experiment at 0–15 cm depth. The grain sample was collected and kept in paper bags in desiccators and was analysed for nitrogen, zinc and iron concentration.

2.5. *Statistical analysis*

Plant parameters (growth, yield and yield components) and plant analysis data were analysed by using a computer-based program ensuring basic principles [16]. Analysis of variance (ANOVA) and significance was done accordingly at 5% by Duncan's Multiple Range Test (DMRT).

3. Results

3.1. *Bioavailability of Zn in wheat grain*

3.1.1. *Genotype effect*

The different varieties and advanced lines of wheat had different Zn uptake potentials and markedly differed with genotypes, in both locations. As recorded in BAU farm experiment, the Zn concentration (2 years' average) varied between 18.7 and 30.2 μg g^{-1}, with an average of 26.5 μg g^{-1} Zn (Table 2) in control (No Zn & Fe). The highest and the lowest grain Zn concentration were found in advanced lines Rawal 87 and BAW, respectively. For the Ishwardi experiment, the BL 1883 obtained the maximum Zn concentration (31.7 μg g^{-1}) and BAW did the lowest result (20.0 μg g^{-1} Zn), the genotype average being 24.9 μg g^{-1} Zn (Table 3).

When the grain Zn concentrations of three treatments are pooled, it reveals that the mean Zn concentration lies between 21.6 and 36.1 μg g^{-1}, the grand average being 31.1 μg g^{-1} which is 4.5 μg g^{-1} higher than the control value that found from BAU experiment. Concerning the BINA sub-station experiment, the mean value of grain Zn concentration comes to be 22.4–33.8 μg g^{-1}, the grand average being 29.8 μg g^{-1} which is found adjacent to the BAU experimental result.

Table 2
Grain Zn concentration (μg g^{-1}) of different genotypes of wheat at BAU farm, Mymensingh during 2014–15 and 2015-16.

Genotypes	2014–15			2015–16		
	Control	Zn	Zn + Fe	Control	Zn	Zn + Fe
V_1: Shatabdi	29.8 a-c	35.4 b-f	36.7 c-g	20.7 g-i	25.50ij	25.8 h-k
V_2: Sufi	27.7 a-d	34.4 b-f	31.5 hi	20.1 hi	25.4 ij	22.6 jk
V_3: Bijoy	32.6 a	33.9 c-f	38.7 c-e	18.6 i	27.8 g-i	26.2 h-j
V_4: Prodip	28.3 a-d	39.1 ab	37.2 c-f	19.6 i	30.8 f-h	29.6 e-h
V_5: BARI Gom 25	31.7 ab	39.0 ab	44.7 ab	22.7 d-i	27.7 g-i	33.3 a-g
V_6: BARI Gom 26	27.1b-d	31.6 ef	38.7 c-e	25.6 b-g	33.1 b-f	31.5 d-g
V_7: BARI Gom 27	28.1a-d	36.7 a-d	32.9 f-i	21.1 f-i	27.2 hi	26.3 h-j
V_8: BARI Gom 28	28.3 a-d	35.9 a-e	34.1 d-i	22.4 e-i	32.6 c-g	29.1 f-i
V_9: BARI Gom 29	29.8 a-c	35.2 b-f	31.0 hi	21.5 f-i	31.4 e-h	28.3 g-i
V_{10}: BARI Gom 30	27.4b-d	31.9 d-f	32.5 f-i	22.0 e-i	32.2 d-h	24.4 i-k
L_1: Rawal 87	28.8a-d	35.0 b-f	48.6 a	31.7 a	36.4 a-e	36.1 a-d
L_2: Vijay	28.3 a-d	35.9 a-e	40.0 bc	24.9 c-h	30.7 f-h	34.7 a-d
L_3: BAW 917	28.7 a-d	40.6 a	33.0 f-i	25.4 b-g	36.2 a-e	36.6 a-c
L_4: Fery 60	29.0 a-d	38.1 a-c	35.1 c-h	30.4 ab	38.3 a	34.0 a-f
L_5: BL 1040	27.6 a-d	31.5 e-f	29.3 ij	30.1 ab	37.4 a-c	37.2 ab
L_6: KRLI-4	25.9 cd	34.4 b-f	33.8 e-i	30.2 ab	36.9 a-d	31.8 c-g
L_7: BL 1883	29.0 a-d	34.5 b-f	34.6 d-h	30.4 ab	37.9 ab	37.5 a
L_8: BAW	17.2 e	24.5 g	25.7 j	20.2 hi	21.4 j	20.9 k
L_9: HPYT-5	27.9 a-d	38.9 a-c	33.9 e-i	30.3 ab	33.8 a-f	35.3 a-d
L_{10}: HPYT-9	24.4 d	30.6 f	39.0 cd	27.9 a-c	38.2 a	36.3 a-d
L_{11}: HPYT-12	27.3b-d	33.9 c-f	37.2 c-f	26.7 a-e	34.8 a-f	36.0 a-d
L_{12}: HPYT-14	28.5 a-d	34.6 b-f	33.4 f-i	26.7 a-e	37.2 a-d	34.3 a-e
L_{13}: HPYT-15	29.0 a-d	36.3 a-e	31.8 g-i	26.0 b-f	36.5 a-d	33.8 a-f
L_{14}: HPYT-21	25.6 cd	32.0 d-f	32.4 f-i	27.7 a-d	34.7 a-f	32.2 b-g
L_{15}: HPYT-24	27.6 a-d	37.2 a-c	32.3 f-i	28.7 a-c	37.8 ab	35.7 a-d
F-test (0.05)	**	**	**	**	**	**
Max	31.7	40.6	48.6	31.7	38.3	37.5
Min	17.2	24.5	25.7	18.6	21.4	20.9
Mean	27.6	34.8	35.1	25.3	32.9	31.6

Genotypes V_1–V_{10} = varieties and L_1 - L_{15} = advanced breeding lines. **, significance at 1% level of probability.

Table 3

Grain Zn concentration (µg g^{-1}) of different genotypes of wheat at BINA substation, Ishwardi during 2014–15 and 2015-16.

Genotypes	2014–15			2015–16		
	Control	Zn	Zn + Fe	Control	Zn	Zn + Fe
V_1: Shatabdi	18.6 k	28.5 kl	27.3f	21.7 b-e	31.4 a-e	29.8 b-d
V_2: Sufi	20.0 jk	38.5 c-g	23.9 fg	25.7 a-c	31.1 a-f	31.6 bc
V_3: Bijoy	23.0h-k	39.6 c-e	26.6 ef	23.0 a-e	32.5 a-c	30.1 b-d
V_4: Prodip	29.6 b-e	39.2 c-f	27.6 ef	19.7 e	26.4 e-h	31.9 b
V_5: BARI Gom 25	25.2 e-i	40.4 b-e	31.3 d	22.9 a-e	30.9 a-f	30.7 b-d
V_6: BARI Gom 26	32.6 bc	30.2 jk	29.1 de	27.5 a	35.7 a	32.1 b
V_7: BARI Gom 27	28.7b-g	40.0 b-e	28.2 e	19.8 e	32.4 a-c	32.8 b
V_8: BARI Gom 28	26.8d-h	33.1 h-k	32.1 cd	22.8 a-e	32.0 a-d	29.2 b-d
V_9: BARI Gom 29	29.4 b-f	45.5 a	24.0 fg	24.2 a-e	33.2 ab	29.3 b-d
V_{10}: BARI Gom 30	31.1b-d	33.9 g-j	31.9 d	25.0 a-d	33.2 ab	31.4 b-d
L = : Rawal 87	28.7b-g	40.6 a-e	31.4 d	23.7 a-e	28.9 b-g	30.4 b-d
L_2: Vijay	33.1 b	31.7 i-k	26.3 ef	22.6 a-e	26.6 e-h	30.2 b-d
L_3: BAW 917	27.8 c-h	41.2 a-e	42.6 a	20.2 de	29.7 b-g	32.9 b
L_4: Fery 60	23.7 g-j	36.2 e-i	28.9 el	21.2 b-e	28.0 c-g	26.5 de
L_5: BL 1040	28.6b-g	31.5 i-k	35.2 bc	20.1 de	26.3 f-h	28.0 b-d
L_6: KRLI-4	21.3 i-k	42.5 a-c	31.9 d	19.4 e	26.7 e-h	30.3 b-d
L_7: BL 1883	38.3 a	34.4f-j	34.8 bc	25.0 a-d	29.2 b-g	30.3 b-d
L_8: BAW	19.8 jk	24.3 l	25.5 ef	20.1 de	22.3 h	22.2 e
L_9: HPYT-5	33.2 b	44.8 ab	40.1 ab	26.2 ab	25.6 gh	29.3 b-d
L_{10}: HPYT-9	26.5d-h	33.2 h-k	39.3 b	19.3 e	30.8 a-f	28.4 b-d
L_{11}: HPYT-12	24.4 f-j	38.6 c-g	40.2 a	22.7 a-e	26.7 e-h	32.3 b
L_{12}: HPYT-14	26.1 d-i	39.1 c-f	36.5 b	20.7 c-e	32.6 a-c	38.3 a
L_{13}: HPYT-15	28.4b-g	37.0 d-h	26.4 ef	22.2 b-e	27.1 d-h	26.7 c-e
L_{14}: HPYT-21	26.1 d-i	38.0 c-h	28.0 e	25.7 a-c	28.2 b-g	30.3 b-d
L_{15}: HPYT-24	30.8b-d	41.4 a-d	40.4 a	22.3 b-e	28.4 b-g	28.4 b-d
F-test (0.05)	**	**	**	**	**	**
Max	38.3	45.5	62.7	27.5	35.7	38.3
Min	18.6	24.3	23.9	19.3	22.3	22.2
Mean	27.3	36.9	32.8	22.5	29.4	30.1

Genotypes V_1–V_{10} = varieties and L_1 - L_{15} = advanced breeding lines. **, significance at 1% level of probability.

The grain Zn concentrations of different varieties of wheat were presented in four groups (Fig. 1 (A) and (B)). As observed in BAU farm (Fig. 1(A)), only 1 genotype (BAW) falls under <20 µg g^{-1} Zn, 2 varieties (Sufi and Prodip) under 20.1–24 µg g^{-1} Zn, 15 varieties & lines 24.1–28 µg g^{-1} Zn and the rest 7 genotypes (1 variety and 6 lines) > 28 µg g^{-1} Zn. In Ishwardi (Fig. 1(B)) (BINA substation), the number of varieties under the four stated Zn groups is noted as 1, 9, 11 and 4, respectively.

The results revealed that the same varieties did not fall always under the same group; some are common, and some are different between the two locations (Fig. 1(A) and (B),. The location common genotype under <20 µg g^{-1} Zn group is BAW (line), that under 20.1–24 µg g^{-1} Zn group is Sufi, those under 24.1–28 µg g^{-1} Zn group are BARI GOM 25, 27, 28 & 29, Vijay (line) and HPYT-15 & 21 (line) and genotypes under the last Zn group (>28 µg g^{-1} Zn) are BL-1883 & HPYT-5 (Fig. 1(A) and (B)).

3.1.2. *Fertilizer effect*

A positive effect was observed in grain Zn concentration, although the magnitude varied with varieties, locations and years. The BAU farm experiment shows that Zn concentration in grain in 2014–15 ranged from 17.2 to 32.6 µg g^{-1} in control plots and 24.5–40.6 µg g^{-1} in Zn-treated plot and in 2015–16, the control treatment recorded 18.6–30.4 µg g^{-1} and the Zn treatment did 21.4–38.2 µg g^{-1} over the genotypes (Table 2). The result was somewhat different for the Ishwardi experiment where the grain Zn concentration of different genotypes in 2014–15 was found 19.8–38.3 µg g^{-1} and 24.3–44.8 µg g^{-1} in control and Zn treatment, respectively. The grain Zn concentration as noted in 2015–16 was 19.3–26.2 and 22.3–33.2 µg g^{-1} in T_1 (Control) and T_2 (Zn addition) treatments (Table 3). The breeding line BAW showed always the lowest grain Zn concentration, but the highest value varied with genotypes between locations.

The grain Zn concentration of different genotypes as an effect of Zn fertilization varied from 22.9 to 38.4 µg g^{-1}, the average being 33.9 µg g^{-1} (average of 2 years), as noted for BAU farm (Fig. 1 (A)). The advanced line BAW 917 recorded the highest Zn concentration. The Zn concentration results due to Zn treatment are similar to that due to Zn + Fe treatment showing a range of 23.3–42.4 µg g^{-1}, an average of 33.0 µg g^{-1}. The addition of Fe with Zn had no additional influence to accumulate Zn in grain over sole Zn incorporation.

At Ishwardi (Fig. 1 (B)), the Zn concentration of different genotypes due to added Zn ranged from 23.3 to 39.3 µg g^{-1}, the average being 33.2 µg g^{-1}; the highest Zn concentration recorded by BARI Gom 29 (Fig. 1). For the Zn + Fe treatment, the Zn concentration range was 23.9–46.5 µg g^{-1}, and the mean value of 31.4 µg g^{-1}, where KRLI-4 performed the best. On an average, the grain Zn concentration was increased to an extent of 4–8 µg g^{-1} across the tested wheat genotypes.

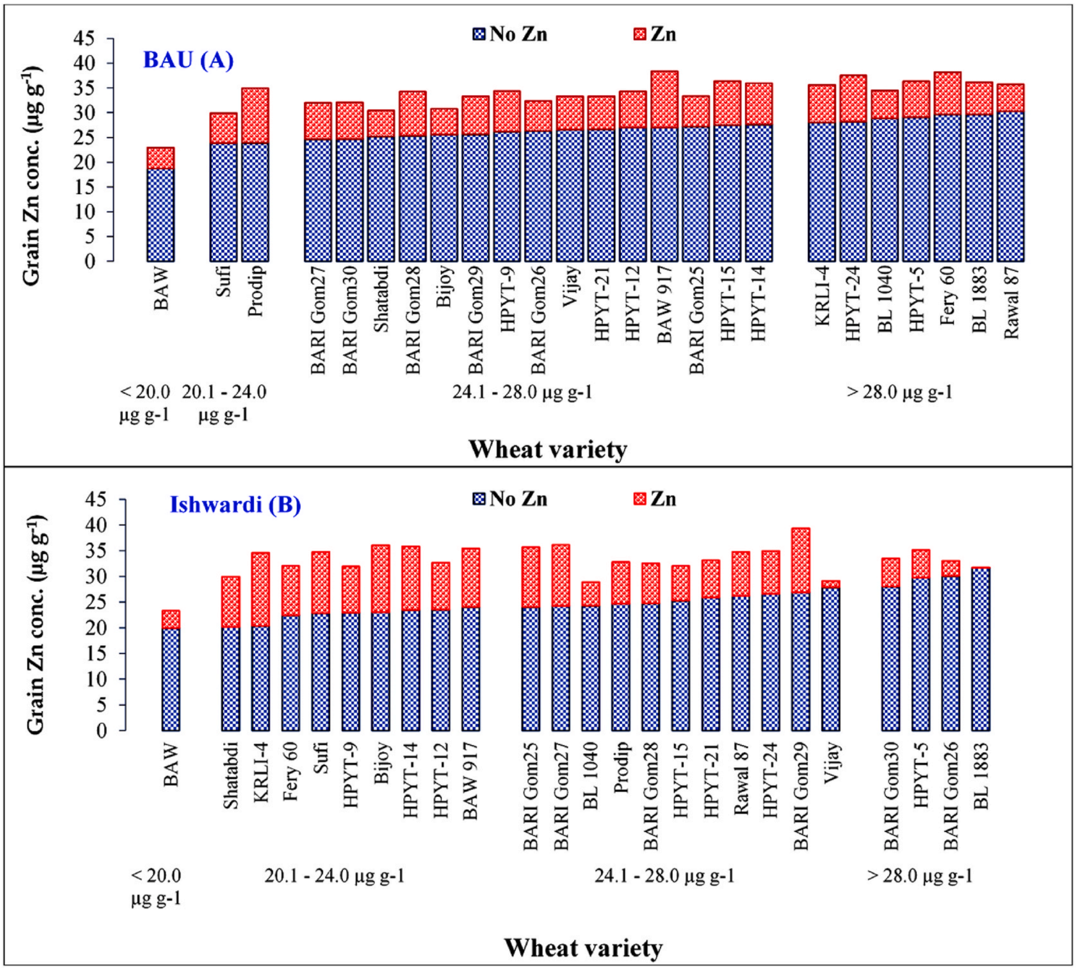

Fig. 1. Grain Zn concentration of different genotypes of wheat at (A) BAU (Bangladesh Agricultural University) and (B) Ishwardi (results are the average of 2 years).

3.1.3. *Zinc efficiency of wheat genotypes*

Among the varieties, BARI Gom 26 & 30, and among the breeding lines, BL 1040, HPYT-5, BAW, Vijay and BL 1883 were found to be Zn efficient or moderately Zn efficient wheat genotypes indicating that those genotypes react minimum even when grown in soil with Zn deficient. Of the 10 selected genotypes, 4 varieties such as BARI Gom 25 & 26, Shatabdi and Bijoy, and of the breeding lines, 5 breeding lines viz. HPYT-21, BAW, BL 1883, BL 1040 and Rawal 87 can be regarded as Zn efficient or moderately Zn efficient.

3.2. *Bioavailability of iron*

3.2.1. *Genotype effect*

The Fe concentration (2 years' average) of wheat grain at BAU farm varied between 19.6 and 30.0 μg g^{-1}, an average of 26.0 μg g^{-1} Fe and at Ishwardi, this was 19.8–29.1 μg g^{-1}, mean 24.0 μg g^{-1} (Table 4 and Fig. 2(A) and (B)).

Four Fe concentration categories are made with a gap of 4 μg g^{-1}. At BAU farm (Fig. 2(A)), of the 25 tested varieties, only 1 genotype (BAW) belongs to <20 μg g^{-1} Fe, 3 varieties to 20.1–24 μg g^{-1} Fe, 17 varieties to 24.1–28 μg g^{-1} Fe and the rest 4 varieties belong to >28 μg g^{-1} Fe. For Ishwardi (Fig. 2(B)) experiment 2, 9, 12 and 2 genotypes fall under the four Fe groups, respectively. The results indicate that the varieties of the same group are not always common over the two locations (Fig. 2 (A) and (B)), showing a location variation. The commonly found genotype under the 4th group (>28 μg g^{-1} Fe) was noted as HPYT-12 (line), those under the 3rd group (24.1–28 μg g^{-1} Fe) are Shatabdi, Prodip, BARI Gom 25 & 28, Sufi, BL-1883, BL-1040 and Fery-60, under the 1st group <20 μg g^{-1} Fe) is BAW and no any common genotype under the 2nd group (20.1–24 μg g^{-1} Fe).

3.2.2. *Fertilizer effect*

The result of grain Fe concentration of wheat due to Fe fertilization has been estimated by the variation between the result due to Zn + Fe addition and that due to only Zn addition. The grain Fe concentration had increased for Fe fertilization. It appears that the

Table 4
Grain Fe concentration (μg g^{-1}) of different genotypes of wheat at BAU farm, Mymensingh during 2014–15 and 2015-16.

Genotypes	2014–15			2015–16		
	Control	Zn	Zn + Fe	Control	Zn	Zn + Fe
V_1: Shatabdi	31.3 a-c	31.9 a-c	38.6 a-c	18.9 hi	22.5 ef	31.2 e-h
V_2: Sufi	28.3 a-f	29.7 a-e	36.9 a-e	24.1 c-g	26.7 b-e	27.2 h
V_3: Bijoy	27.5 c-f	30.0 a-e	33.9 d-f	23.0 e-h	23.4 c-f	33.4 b-f
V_4: Prodip	28.2 b-f	28.3 c-e	38.1 a-d	24.6 b-g	27.1 b-d	29.9 f-h
V_5: BARI Gom 25	30.0 a-e	28.7 b-e	38.7 a-c	21.6 g-i	20.7 fg	34.8 a-e
V_6: BARI Gom 26	26.9 c-f	30.7 a-e	35.9 b-e	18.4 i	23.7 c-f	31.7 d-h
V_7: BARI Gom 27	30.0 a-e	28.7 b-e	30.8 f	25.4 a-g	24.5 b-f	28.7 gh
V_8: BARI Gom 28	27.2 c-f	28.5 c-e	36.8 a-e	21.3 g-i	23.9 c-f	31.2 e-h
V_9: BARI Gom 29	29.2 a-e	26.2 ef	34.3 c-f	23.0 e-h	23.7 c-f	32.0 d-g
V_{10}: BARI Gom 30	26.6 d-f	29.8 a-e	33.3 ef	19.0 hi	20.4 fg	34.0 b-f
L_1: Rawal 87	28.5a-f	28.8 b-e	37.8 a-e	27.1a-e	29.0 ab	39.0 a
L_2: Vijay	24.6 f	27.3 de	35.6 b-e	27.8 a-c	27.5 bc	34.3 b-f
L_3: BAW 917	29.1 a-f	26.2 ef	36.5 b-e	22.5 f-i	25.6 b-e	32.9 c-g
L_4: Fery 60	31.1 a-d	33.6 a	33.8 d-f	23.2 d-h	25.5 b-e	34.5 a-e
L_5: BL 1040	30.0 a-e	33.2 ab	39.2 ab	24.4 c-g	17.0 g	37.7 ab
L_6: KRLI-4	27.0 c-f	28.1 c-e	36.9 a-e	25.3 a-g	24.4 c-f	34.1 b-f
L_7: BL 1883	29.7 a-e	31.5 a-d	41.1 a	22.4 f-i	24.8 b-f	32.2 d-g
L_8: BAW	19.8 g	17.9 g	29.8 fg	19.5 hi	20.8 fg	20.4 i
L_9: HPYT-5	32.2 ab	30.1 a-e	39.1 ab	27.7 a-d	25.4 b-e	35.4 a-e
L_{10}: HPYT-9	27.3 c-f	29.6 a-e	37.5 a-e	29.8 a	32.3 a	37.2 a-c
L_{11}: HPYT-12	32.8 a	29.3 a-e	36.4 b-e	26.6 a-f	24.8 b-f	33.5 b-f
L_{12}: HPYT-14	29.8 a-e	29.2 a-e	39.4 ab	29.0 ab	26.7 b-e	34.2 b-f
L_{13}: HPYT-15	19.1 g	22.6 f	26.1 g	23.1 e-h	22.9 d-f	31.1 e-h
L_{14}: HPYT-21	28.1 b-f	29.0 b-e	37.5 a-e	26.7 a-f	24.1 c-f	36.0 a-d
L_{15}: HPYT-24	25.7 ef	30.1 a-e	36.9 a-e	24.7 b-g	23.7 c-f	33.4 b-f
F-test (0.05)	**	**	**	**	**	**
Max	32.2	33.6	41.1	29.8	32.3	39
Min	19.8	17.9	26.1	18.4	17	20.4
Mean	28.3	28.7	36.0	24.0	24.4	32.8

Genotypes V_1–V_{10} = varieties and L_1 - L_{15} = advanced breeding lines. **, significance at 1% level of probability.

range of grain Fe concentration at BAU farm in 2014–15 was 19.1–32.2 μg g^{-1} (Control) and 26.1–39.4 μg g^{-1} in Zn + Fe treatment and in 2015–16 the results were 18.4–29.8 and 20.4–37.2 μg g^{-1} for the two treatments, respectively (Table 5).

At Ishwardi, the variation of grain Fe concentration in 2014–15 was 20.6–31.8 μg g^{-1}in Zn control and 30.4–47.1 μg g^{-1} in Zn treatment and in 2015–16 the Fe concentration was found as 16.0 to 27.5 and 22.4–36.2 μg g^{-1} for the Zn and Zn + Fe treatments as well. The result shows that the Fe concentration of wheat grain at the BAU farm ranged from 25.1 to 38.5 μg g^{-1}, average of 34.2 μg g^{-1} Fe, as recorded in Zn + Fe treated plots (Table 5). At Ishwardi, the grain Fe concentration varied from 25.4 to 40.8 μg g^{-1}, the average being 34.7 μg g^{-1} Fe (Table 6). This result indicated that the grain Fe concentration rise up due to Fe addition over the locations. It also noticed a synergistic relationship between Fe addition and its impact on grain Fe concentration.

3.3. *Nitrogen and protein content*

3.3.1. *Genotype effect*

The grain N concentration of wheat, as recorded with BAU farm experiment, varied between 1.32 and 1.49%, the mean value being 1.40% in control plots. Variety BARI GOM 30 demonstrated the highest grain N%. At Ishwardi, the grain N concentration ranged from 1.31 to 1.47% with an average of 1.37%. The highest result was obtained with KRLI-4 and the lowest result with BAW 917, HPYT-14 & HPYT- 15. The protein content of wheat grain over the genotypes varied from 7.72 to 8.70%, mean value of 8.21% at the BAU farm and 7.66–8.60% having an average of 8.03% at the BINA substation (Table 6).

3.3.2. *Fertilizer effect*

Zinc fertilization helps to increase the uptake of nitrogen in grain in all varieties and advanced lines. But Fe application had no effect (Table 6). At BAU farm, the N concentration of wheat grain in Zn fertilized plots showed an increase of 0.11% as a minimum and 0.23% as the maximum, an average value was noted as 0.21% on the top of the control result. Breeding line BL 1040 recorded the highest grain N concentration and Fery 60 did the lowest. The results of the Ishwardi experiment showed an increment of 0.10–0.28% with a mean of 0.23% grain N concentration. In this location, variety Prodip contained the highest grain N% and HPYT-24 had the lowest grain N concentration.

The average protein of 9.44% was found in Zn-treated treatment T_2 which ranged from 8.37 to 10.06% at the BAU farm whereas it lay between 8.27 and 10.26% with a mean of 9.39% at the BINA substation (Table 6) which also showed that Zn and N uptake occurred in plant in proportionate quantity.

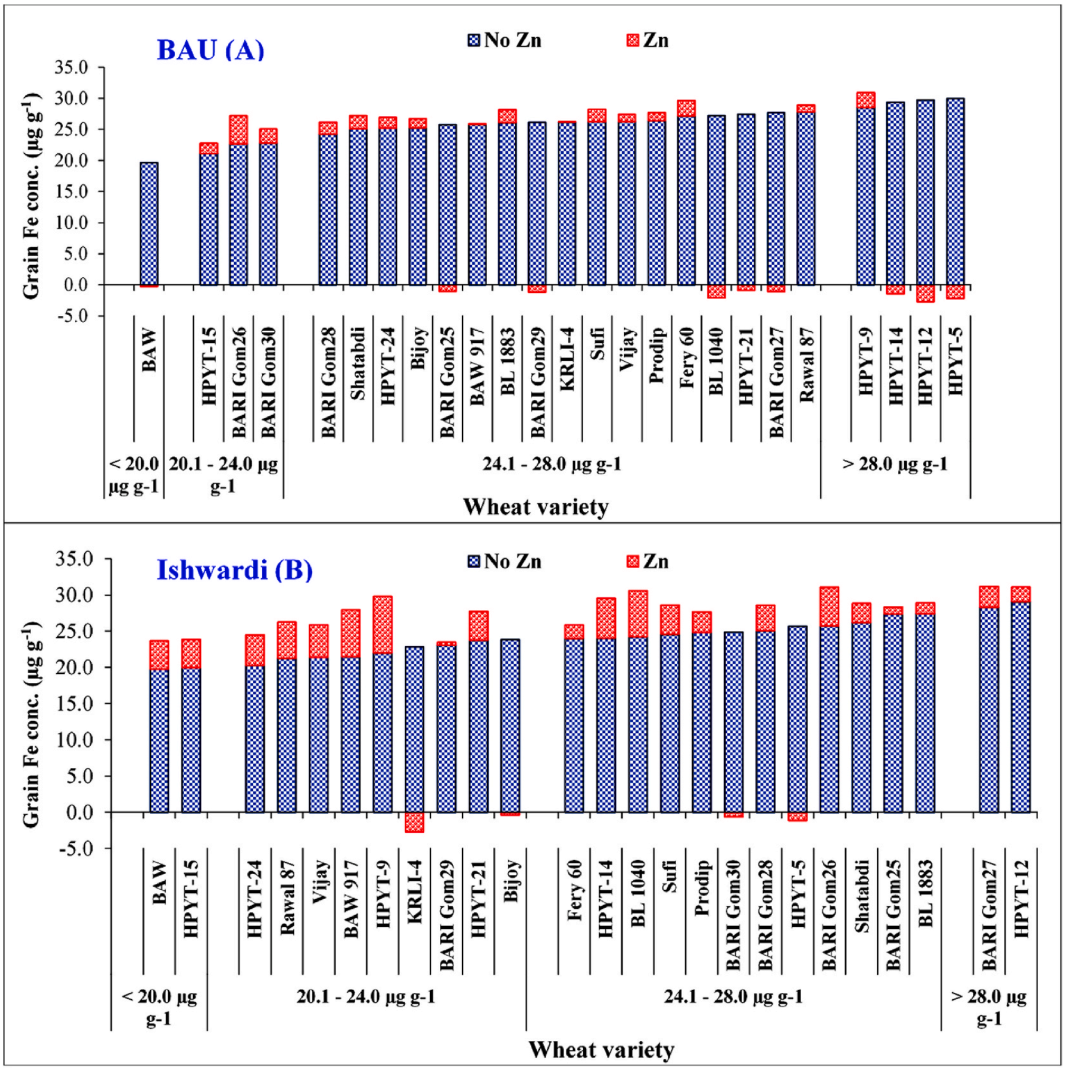

Fig. 2. Grain Fe concentration of different genotypes of wheat at the locations of (A) BAU (Bangladesh Agricultural University) and (B) Ishwardi as influenced by added Zn (results are the average of 2 years).

3.4. Relationship between grain yield and grain Zn concentration

The relationship between grain yield and grain Zn concentration for control (upper graph) and Zn treatment (lower graph) at the location of BAU (Bangladesh Agricultural University) and Ishwardi have been presented in Fig. 3(A) and (B). A positive relationship was observed between grain yield and grain Zn concentration in both locations for control and Zn treatments.

4. Discussion

The amount of Zn in the soil (depth 0–15 cm) appears to affect grain nutrition and both experiment sites had low Zn content but higher in Fe (Table 1). The soil availability affects the amount of Zn and Fe in wheat grain [17]. In the grain of wheat, the Zn content was lower than the Fe content. This is due to reduced Zn content and similar results have been documented (18). The Zn and Fe are relatively mobile in plant systems and less Zn and Fe are translocated from sources to sink [18].

Micronutrient concentration of food crops needs to increase and the only means is by genetic improvement as well as agronomic procedure. The Zn concentration is not enough that cultivated by the farmers of Bangladesh which is not able to solve the deficiency. Agronomic biofortification is suitable for ready mitigation for Zn insufficiency in cereals [19]. The findings of the study express that the Zn concentration increased 4–8 µg g^{-1} Zn among the 25 genotypes whereas 6–12 µg g^{-1} increment of Fe was observed never-the-less no effect was found in grain yield for Fe addition even though the soil was not insufficient. There are several genotypes has proven as Zn enriched (24.1–30 µg g^{-1} grain Zn) like BARI Gom 25, 27, 28 & 29, Vijay, HPYT-5, 15 & 21 and BL-1883 (line). Shatabdi, Prodip,

Table 5

Grain Fe concentration (μg g^{-1}) of different genotypes of wheat at BINA substation, Ishwardi during 2014–15 and 2015-16.

Genotypes	2014–15			2015–16		
	Control	Zn	Zn + Fe	Control	Zn	Zn + Fe
V_1: Shatabdi	26.1b-g	27.6 e-i	37.0 e-j	26.2 a-d	30.0 a	31.9 c-h
V_2: Sufi	24.2 d-i	28.3 d-h	35.1 h-k	24.9 a-f	28.9 ab	32.8 c-g
V_3: Bijoy	26.9b-g	25.4 h-j	36.2 f-j	20.8 f-j	21.4 g-k	28.9 g-i
V_4: Prodip	29.3 a-c	34.6 ab	34.5 i-l	20.3 g-k	20.6 g-k	31.2 e-h
V_5: BARI Gom 25	28.5 a-e	32.3 a-d	35.1 h-k	26.2 a-d	24.4 b-h	30.2 e-i
V_6: BARI Gom 26	24.1 e-i	33.2 a-c	34.0 i-l	27.2 ab	28.9 ab	33.6 b-f
V_7: BARI Gom 27	29.6 ab	32.3 a-d	33.5 j-l	27.0 a-c	30.1 a	32.5 c-h
V_8: BARI Gom 28	24.8 c-i	26.7 g-j	43.2 ab	25.3 a-f	30.4 a	36.1 a-d
V_9: BARI Gom 29	23.4 g-i	22.8 j	39.4 b-h	22.7 b-h	24.1 c-i	37.8 ab
V_{10}: BARI Gom 30	28.1 a-f	23.5 ij	34.5 i-l	21.6 e-i	24.9 b-g	31.4 e-h
L_1: Rawal 87	23.9 f-i	31.0 b-g	35.4 g-k	18.6h-k	21.5 f-k	29.7 f-i
L_2: Vijay	26.1b-g	31.9 a-e	38.4 c-i	16.7 jk	19.8 ijk	31.1 e-h
L_3: BAW 917	25.2b-h	34.3 ab	37.6 d-j	17.8 i-k	21.6 e-k	28.0 hi
L_4: Fery 60	28.0 a-f	29.4 c-h	41.1 b-e	20.0 g-k	22.4 d-j	40.6 a
L_5: BL 1040	26.2b-g	33.5 a-c	41.2 b-e	22.3 d-i	27.7 a-c	31.2 e-h
L_6: KRLI-4	28.7 a-d	22.8 j	39.7 b-g	17.0 jk	17.6 k	31.5 e-h
L_7: BL 1883	31.5 a	31.7 a-f	42.2 bc	23.3 a-g	26.1 a-e	36.2 a-c
L_8: BAW	20.6 i	27.2 f-j	30.4 l	18.9 g-k	20.1 h-k	20.5 k
L_9: HPYT-5	23.8 f-i	22.8 j	36.7 e-j	27.5 a	26.3 a-d	30.5 e-h
L_{10}: HPYT-9	24.5 d-i	35.9 a	42.0 b-d	19.4 g-k	23.7 c-i	34.5 b-e
L_{11}: HPYT-12	31.8 a	35.4 ab	40.5 b-f	26.4 a-d	26.8 a-d	33.5 b-f
L_{12}: HPYT-14	22.4 g-i	33.0 a-c	47.1 a	25.6 a-e	26.0 a-f	25.8 ij
L_{13}: HPYT-15	23.8 f-i	29.1 c-h	31.0 kl	16.0 k	18.5 jk	22.4 jk
L_{14}: HPYT-21	25.0 c-i	31.0 b-g	41.0 b-e	22.5 c-h	24.5 b-h	30.5 e-h
L_{15}: HPYT-24	21.3 hi	29.3 c-h	39.2 b-h	19.2 g-k	19.6 i-k	31.6 d-h
F-test (0.05)	**	**	**	**	**	**
Max	31.8	35.9	47.1	27.5	30.4	40.6
Min	20.6	22.8	31	16	17.6	20.5
Mean	25.9	29.8	38.2	22.1	24.2	31.4

Genotypes V_1–V_{10} = varieties and L_1 - L_{15} = advanced breeding lines.

BARI Gom 25 & 28, Sufi, BL-1040 & 1883, HPYT-12 and Fery 60 are found as Fe-enriched wheat genotypes (24.1–30 μg g^{-1}). The important issue is the extent of 4–8 μg g^{-1} Zn over the tested genotypes and an increment of 11.7 (control) to 26.9 μg g^{-1} Zn concentration was found with Zn fertilization [20]. Although the yield and Zn concentration of grain are not significant, correlate negatively in both BAU farm-Mymensingh and BINA substation Ishwardi (Fig. 2 (A) and (B)) indicating the variation of genetic makeup. It was observed that grain yield correlated negatively with the Zn concentration which was found to diminish course significantly [22].

The increase in grain yield due to Zn fertilization could be to Zn's role in the biosynthesis of IAA (Indole-3 Acetic Acid) and the initiation of primordia for reproductive parts, as well as its beneficial effect on metabolic reactions within the plants, but this was not investigated in the current study. Zinc fertilization increased yield by 1.2–25.7% with a mean of 15.2% at BAU farm and 6.3 to 25.7 with an average value of 15.9% at Ishurdi. However, little is known about the genetic control and molecular physiological mechanisms that contribute to high Zn and other micronutrient accumulation in grains of various genetic materials [21–23]. Wheat yield response to Zn treatment has been well documented in Bangladesh and India [24–26]. Numerous research have demonstrated that applying Zn to Zn-deficient soils increases grain yield (9–256%) and grain Zn content (9–912%) of wheat [27–29].

There are many wheat varieties which have higher yield potential containing Zn (15–35 μg g^{-1}) and Fe (20–60 μg g^{-1}) is observed [30]. The total grain Zn concentration increase was 14.6 μg g^{-1} and the average Zn concentration in wheat grain is 34.3% and 33.9% at Ishwardi and Mymensingh, respectively, which is noticeable. This study showed that the average Fe increased in 6–12 μg g^{-1} due to the addition of Fe fertilizer in both locations (Fig. 2 (A) and (B)). Enriching commonly used compound fertilizers with Zn is a fertilizer practice that can help increase plant Zn concentration [31]. The addition of Fe to Zn had no further effect on grain Zn content over the use of only Zn.

Apart from grain Zn and Fe content, the protein concentration (%N × 5.85) has shown a synergistic relationship. Zinc enhances protein synthesis by influencing different enzyme that helps the synthesis of RNA, DNA and glutenin. An adequate amount of Zn increases nitrogen uptake which in turn to the accumulation of higher Zn in grain [32] that suggests a proper combination of N and Zn applications. Nitrogen fertilizer improves Zn and Fe concentrations in grain, which could be related to N form chelation with Zn and Fe, which promotes their transport through phloem tissue into the grain [33]. Other researchers [34,35] have revealed similar findings on how N nutrition aids in the enhancement of Zn and Fe content in grain.

Genetic biofortification is in conjunction with an agronomic strategy increasing the prospect of developing new cultivars that are more effective at accumulating minerals in the edible section [36]. For the immediate less costly approach of increase, Zn in grain is agronomic means which can meet up the present malnutrition situation [37].

Table 6

Grain protein concentration (%) of different genotypes of wheat at BAU farm, Mymensingh and BINA substation, Ishwardi during 2015–16.

Genotypes	BAU farm				BINA substation			
	Control	Zn	Zn + Fe	Mean	Control	Zn	Zn + Fe	Mean
V_1: Shatabdi	8.23	9.87	10.06	9.39	8.25	9.85	9.77	9.29
V_2: Sufi	7.84	9.18	9.03	8.68	7.82	9.23	9.22	8.76
V_3: Bijoy	8.21	9.61	9.46	9.09	7.94	9.52	9.34	8.93
V_4: Prodip	8.03	9.09	8.95	8.69	7.76	8.90	8.87	8.51
V_5: BARI Gom 25	8.35	9.73	9.57	9.22	8.33	10.26	10.2	9.60
V_6: BARI Gom 26	8.27	9.91	9.75	9.31	8.09	9.66	9.61	9.12
V_7: BARI Gom 27	8.52	9.96	10.0	9.49	8.23	9.74	10.34	9.44
V_8: BARI Gom 28	8.39	9.85	9.91	9.38	8.13	9.93	10.02	9.36
V_9: BARI Gom 29	8.6	9.93	10.1	9.54	8.21	9.81	9.69	9.24
V_{10}: BARI Gom 30	8.7	10.06	9.83	9.53	8.29	10.11	10.26	9.55
L_1: Rawal 87	7.94	9.24	9.07	8.75	7.76	9.21	9.11	8.69
L_2: Vijay	8.44	9.56	9.38	9.13	8.23	10.13	9.52	9.29
L_3: BAW 917	8.46	9.67	9.22	9.12	7.68	9.25	9.26	8.73
L_4: Fery 60	8	8.37	8.62	8.33	8.15	8.54	8.50	8.40
L_5: BL 1040	8.5	10.06	10.02	9.53	8.11	9.79	9.40	9.10
L_6: KRLI-4	8.58	8.93	8.64	8.72	8.60	9.11	8.93	8.88
L_7: BL 1883	8.19	9.50	9.36	9.02	8.23	9.93	9.36	9.17
L_8: BAW	7.76	9.01	9.09	8.62	7.82	8.58	9.07	8.49
L_9: HPYT-5	7.72	8.83	8.97	8.51	7.90	9.58	9.34	8.94
L_{10}: HPYT-9	8.19	9.38	9.22	8.93	8.11	9.36	9.23	8.90
L_{11}: HPYT-12	8.35	9.54	9.17	9.02	8.09	9.34	9.24	8.89
L_{12}: HPYT-14	7.98	8.68	8.44	8.37	7.68	8.56	7.55	7.93
L_{13}: HPYT-15	8.11	9.48	9.57	9.05	7.66	9.03	9.93	8.87
L_{14}: HPYT-21	8.05	9.63	9.36	9.01	7.82	8.97	9.65	8.81
L_{15}: HPYT-24	7.96	8.93	9.07	8.65	7.78	8.27	8.13	8.06
F-test (0.05)	**	**	**	**	**	**	**	**
Max	8.70	10.06	10.1	9.62	8.60	10.26	10.26	9.71
Min	7.72	8.37	8.44	8.18	7.66	8.27	7.55	7.83
Mean	8.21	9.44	9.35	9.00	8.03	9.39	9.34	8.92

Genotypes V_1–V_{10} = varieties and L_1 - L_{15} = advanced breeding lines.

5. Conclusion

Zinc and iron deficiency is common in developing countries like Bangladesh and to combat this malnutrition bioavailability of these nutrients is necessary for cereals grain. It is necessary to find potential genotypes that can assemble micronutrients (Zn & Fe) in grain. Among the tested varieties BARI Gom 25, 27, 28 & 29 are found potential (24–30 μg g^{-1}Zn) for Zn enrichment. Regarding Fe, Shatabdi, Prodip, BARI Gom25 & 28 and Sufi (24–30 μg g^{-1}) are best for enrichment as an agronomic approach and found potential genotypes for breeding concern. Nitrogen content increases as well with the Zn application thus helping protein synthesis.

Author contributions statement

Mahbubur Rahman Khan, Md. Jahiruddin, Md. Abdullah Al Mahmud, Md. Mahbubul Alam Tarafder, Md. Habibur Rahman and Shilpi Das conceived and designed the experiment. Mahbubur Rahman Khan, Md. Jahiruddin, Md. Abdullah Al Mahmud, Md. Mahbubul Alam Tarafder, Md. Habibur Rahman and Shilpi Das performed the experiment.

Mahbubur Rahman Khan, Bassem M. Raafat, Ahmed Gaber and Akbar Hossain analysed and interpreted the data.

Mahbubur Rahman Khan, Bassem M. Raafat, Ahmed Gaber and Akbar Hossain contributed reagents, materials, analysis tools or data.

Mahbubur Rahman Khan, Md. Jahiruddin, Md. Abdullah Al Mahmud, Md. Mahbubul Alam Tarafder, Md. Habibur Rahman, Shilpi Das, Bassem M. Raafat, Ahmed Gaber and Akbar Hossain wrote and edited the paper.

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Data availability statement

Most of the data are available in all Tables and Figures of the manuscripts.

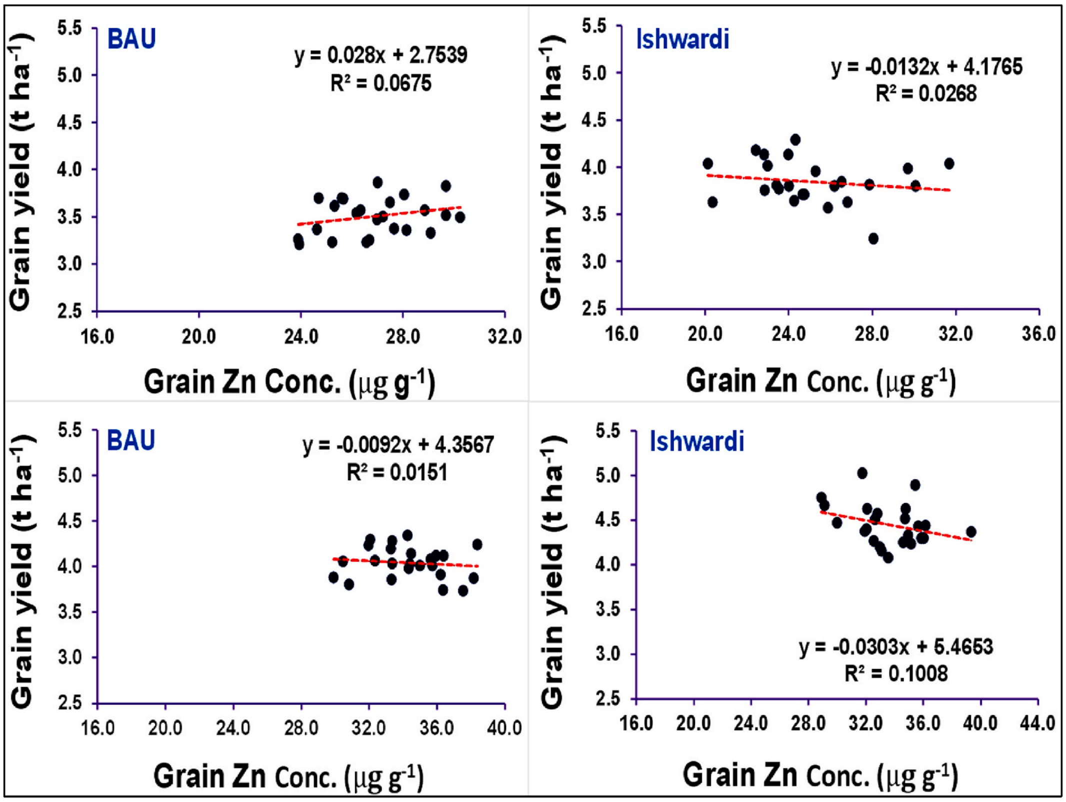

Fig. 3. Relationship between grain yield and grain Zn concentration for control (upper graph) and Zn treatment (lower graph) at the location of (A) BAU (Bangladesh Agricultural University) and (B) Ishwardi (results are the average of 2 years).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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