

ARTICLE

# Yield performance and seed quality of wheat under different levels of foliar zinc application at vegetative and reproductive stages

Salma Akter<sup>1</sup>, M Abdullah Al Mamun<sup>1,\*</sup>, Erin Zaman<sup>1</sup>, M Saiful Alam<sup>2</sup>, M Abdul Karim<sup>1,3</sup>

<sup>1</sup>Department of Agronomy, Gazipur Agricultural University, Gazipur 1706, Bangladesh

<sup>2</sup>Department of Soil Science, Gazipur Agricultural University, Gazipur 1706, Bangladesh

<sup>3</sup>College of Agricultural Sciences, International University of Business Agriculture and Technology, Dhaka 1230, Bangladesh

**ABSTRACT** Zinc (Zn) deficiency in soil affects wheat productivity and nutritional quality as well. Agronomic biofortification of zinc in wheat crops presumably lessens the problem. An experiment was conducted to evaluate the interactive effects of wheat varieties (BARI Gom-25, BARI Gom-30, and BARI Gom-33) and foliar Zn application (0, 3, and 6 g Zn L<sup>-1</sup>) at vegetative (VS) and reproductive stage (RS) on growth and yield performances, seed quality, and grain Zn concentration. The application of 6 g Zn L<sup>-1</sup> at RS markedly improved yield components (19.0 spikelets spike<sup>-1</sup> in BARI Gom-25 and 52 grains spike<sup>-1</sup> in BARI Gom-33), grain yield and Zn concentration, particularly in BARI Gom-25 and BARI Gom-33. The highest grain yield (5.07 t ha<sup>-1</sup>) produced by BARI Gom-25 with 6 g Zn L<sup>-1</sup> applied at the RS. Grain Zn concentration ranged from 35.48 to 65.69 ppm, with the highest value recorded in BARI Gom-33 receiving 6 g Zn L<sup>-1</sup> applied at the RS. Zinc application also reduced seed electrical conductivity and improved germination percentage and vigor index. Overall, Zn application at RS at higher doses proved to be more effective than application at VS for improving both productivity and nutritional quality of wheat.

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\*Corresponding author

E-mail: [aamamun@gau.edu.bd](mailto:aamamun@gau.edu.bd)  
[akarim1506@gmail.com](mailto:akarim1506@gmail.com)

## Introduction

Zinc (Zn) deficiency is one of the most widespread micronutrient disorders affecting both crop productivity and human nutrition worldwide. Nearly one-third of the global population suffers from inadequate Zn intake, particularly in developing countries where cereal-based diets dominate (Cakmak 2008; Alloway 2009). Wheat (*Triticum aestivum* L.), being a staple food for more than 35% of the world's population, contributes substantially to daily calorie intake but inherently contains low concentrations of bioavailable Zn, especially when grown on Zn-deficient soils (Cakmak and Kutman 2018). This dual challenge of agronomic Zn deficiency and dietary Zn malnutrition has made Zn biofortification of wheat a global research priority.

Agronomic biofortification through Zn fertilization in soil, seed, and particularly foliar application has emerged as a rapid and cost-effective strategy to enhance grain Zn concentration while simultaneously improving crop growth, yield, and seed quality (Cakmak 2008). Numerous studies have demonstrated that foliar Zn application

is more efficient than soil application, especially under calcareous, alkaline, or high-pH soils where Zn availability is severely limited (Cakmak 2008). Foliar Zn application at critical growth stages such as tillering, booting, and grain filling has been shown to significantly increase Zn translocation to developing grains, thereby enhancing grain Zn density without yield penalties (Phattarakul et al. 2012).

Literature highlights that the timing of Zn application plays a decisive role in determining grain Zn accumulation (Cakmak et al. 2010; Zhang et al. 2012; Ajiboye et al. 2015). Zn application at vegetative stages (VS) improves plant vigor, photosynthetic efficiency, and spike development, whereas application at reproductive stage (RS) or post-anthesis stages enhances Zn remobilization and direct loading into grains (Cakmak et al. 2010). Moreover, split Zn applications at both VS and RS have been reported to produce synergistic effects on grain yield, seed quality, and Zn concentration, compared with single-stage application (Wang et al. 2015).

Beyond yield enhancement, Zn biofortification positively influences seed quality traits, including seed weight, germination percentage, seedling vigor, and enzymatic

**Table 1.** Physico-chemical properties of experimental soil

| Soil properties            | Unit                           | Value      |
|----------------------------|--------------------------------|------------|
| Textural class             | -                              | Sandy loam |
| Sand                       | %                              | 53.63      |
| Silt                       | %                              | 35.26      |
| Clay                       | %                              | 12.11      |
| pH                         | -                              | 5.28       |
| Organic carbon (OC)        | %                              | 1.68       |
| Total nitrogen (N)         | %                              | 0.086      |
| Available phosphorus (P)   | $\mu\text{g g}^{-1}$           | 14.69      |
| Exchangeable potassium (K) | $\text{meq } 100\text{g}^{-1}$ | 0.218      |
| Available sulfur (S)       | $\mu\text{g g}^{-1}$           | 14.10      |
| Available zinc (Zn)        | $\mu\text{g g}^{-1}$           | 0.857      |

activity, which are critical for sustainable wheat production. Higher Zn concentration in seeds improves early seedling establishment and stress tolerance, creating a positive feedback loop for crop productivity in Zn-deficient environments (Cakmak 2008).

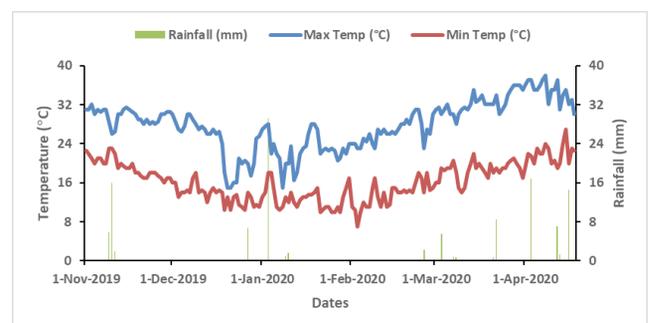
In Bangladesh, wheat is the second most important cereal crop after rice. However, Zn deficiency in agricultural soils is widespread due to intensive cropping, low organic matter, and imbalanced fertilizer use (FRG 2018). National soil surveys indicate that more than 50% of cultivated soils in Bangladesh are deficient or marginal in available Zn, posing serious constraints to wheat yield and nutritional quality. Although blanket Zn fertilizer recommendations exist (FRG 2018), field-level adoption remains low, and scientific evidence on growth-stage-specific Zn management for wheat biofortification under Bangladeshi agro-ecological conditions is limited.

Previous studies in Bangladesh have primarily focused on soil-applied Zn or single foliar spray, often emphasizing yield response rather than comprehensive assessment of grain Zn enrichment and seed quality. Moreover, varietal differences in Zn uptake, translocation efficiency, and grain loading among newly released high-yielding wheat varieties (e.g., BARI Gom-25, BARI Gom-30, and BARI Gom-33) remain poorly explored. This creates a critical knowledge gap in identifying optimal Zn dose, application stage, and genotype interactions for effective Zn biofortification. The integrated evaluation of foliar Zn biofortification strategies across vegetative stage (VS) and reproductive stage (RS) in Bangladesh new wheat varieties will give a new dimension of Zn enrichment. Unlike earlier reports (Hui et al. 2025), this study simultaneously examines grain yield, seed quality parameters, and grain Zn concentration in response to varying Zn doses and application timings. By comparing Zn application at jointing (VS) and post-anthesis (RS), the study provides mechanistic insights into Zn uptake dynamics

and grain Zn loading under local soil and climatic conditions. Furthermore, this work contributes new empirical evidence for Bangladesh by identifying stage-specific and variety-specific Zn management practices that can enhance both productivity and nutritional quality of wheat. The specific objective of the present study was conducted to evaluate the interactive effects of wheat variety, Zn dose, and stage of Zn application on growth, yield attributes, seed quality, and grain Zn concentration. The hypothesis was that the application of Zn at VS and RS of wheat would improve the grain Zn content. The findings are expected to support national biofortification initiatives, refine fertilizer recommendations, and contribute to sustainable strategies for combating Zn malnutrition through food-based approaches.

## Materials and methods

The experiment was conducted at the premises of the Department of Agronomy, Gazipur Agricultural University, Gazipur, Bangladesh from December 2019 to March 2020. The textural class was sandy loam, containing 12.11% clay, 35.26% silt, and 53.63% sand, having a pH of 5.28 soil organic carbon 1.68%, total N 0.086%, available P  $14.69 \mu\text{g g}^{-1}$ , exchangeable K  $0.218 \text{ meq } 100 \text{ g}^{-1}$  soil, and available S of  $14.10 \mu\text{g g}^{-1}$  and available Zn of  $0.857 \mu\text{g g}^{-1}$  (Table 1). The experimental land was first opened with a tractor on 4 December 2019. The land was prepared by repeated ploughing and cross-ploughing with a tractor drawn disc plough and then harrowed. Each ploughing was done followed by laddering for breaking the big clods, levelling the lands and collecting the stubbles and removing the weeds to obtain a desirable tilth. The highest rainfall was 29 mm during growth period; and maximum and minimum air temperatures were  $35 \text{ }^\circ\text{C}$  and  $7 \text{ }^\circ\text{C}$ , respectively (Fig. 1).



**Figure 1.** Rainfall pattern and air temperature of experimental site during growing period

### Treatments and design

Treatment consisted of three factors; Factor A (wheat varieties): BARI Gom-25, BARI Gom-30 and, BARI Gom-33; Factor B (Zn level): control (no Zn), 3 g ZnSO<sub>4</sub>·H<sub>2</sub>O L<sup>-1</sup> water (1.1% Zn), 6 g ZnSO<sub>4</sub>·H<sub>2</sub>O L<sup>-1</sup> water (2.2% Zn); Factor C (growth stages for Zn fertilizer application): vegetative stage (VS, jointing stage), reproductive stage (RS, after anthesis). The source of Zn was ZnSO<sub>4</sub>·H<sub>2</sub>O, which contained 36% Zn. To prepare 1.1% Zn solution, 8.1 g of ZnSO<sub>4</sub>·H<sub>2</sub>O was weighed accurately and dissolved it in a 270 ml of clean water to spray 9 m<sup>2</sup> (3 m × 3 m) plot, while 300 L water was needed for 1 ha land. Similarly, a 16.2 g ZnSO<sub>4</sub>·H<sub>2</sub>O solution corresponding to 2.2% Zn (equivalent to 5.0 kg Zn ha<sup>-1</sup>) was prepared by accurately weighing 16.2 g of ZnSO<sub>4</sub>·H<sub>2</sub>O and dissolving it in a volume of 270 ml of clean water. The chemical is added gradually with continuous stirring to ensure complete dissolution, after which the solution volume is made up to the required level by adding water. The solution is mixed thoroughly to obtain a uniform ZnSO<sub>4</sub>·H<sub>2</sub>O solution, ready for application. To avoid leaf burning and maximize absorption, spraying was done in the field during late evening. The experiment was conducted in factorial randomized complete block design (RCBD) having three replications.

### Seed sowing

Healthy seeds were sown in each plot maintaining uniform spacing having seed rate of 120 kg ha<sup>-1</sup> in each plot on 13 December 2019. Light irrigation was given after sowing by using the water cane to ensure uniform germination. The distance was maintained 20 cm from line to line, and the seeds were sown continuously in the line.

### Fertilizer application

The plots were fertilized with 260, 150, 150, 120 and 6 kg ha<sup>-1</sup> urea, triple super phosphate (TSP), muriate of potash (MoP), gypsum and boric acid, respectively (FRG 2018). Total amount of TSP, gypsum and MoP was applied at the time of final land preparation. Urea was applied in two splits. At final land preparation two thirds were applied and another one third urea was applied at 22 days after first application. The Zn was applied according to the treatment combinations.

### Intercultural operations

Intercultural operations were done uniformly in each plot to ensure normal growth of the crop. Thinning was done for uniform and healthy plants in each plot. Weeding was done intensively to keep the plots weed free. Irrigation was applied with tap water in all the plots for ensuring normal growth of the crop as and where necessary.

### Harvesting and data collection

The crop was harvested on 27 March 2020 when the flag leaf and spikes turned yellow. Threshing, cleaning and drying of grain were done separately according to the treatments and replications. The growth duration was calculated from days required from sowing to maturity. Ten wheat plants were collected from each plot to measure plant height, ear length and yield components. The plants were cut at the ground level and height was measured by a meter scale (100 cm). The heights of sample plants were measured from the base of the cut plants to the tip of main shoot and averaged. Ear length was measured by a small scale (30 cm). After collecting all the spikes, three spikes of each variety were selected randomly according to replication, and the length was measured and mean value was recorded. Total number of spikelets were counted from three spikes and averaged to calculate number of spikelets spike<sup>-1</sup>. Total number of seeds were counted from three spikes and averaged to find number of seeds spike<sup>-1</sup>. Total number of seeds from three spikes were counted and weight to find the weight of 1000-seed. Total seeds from each replication were weighed with an electrical balance (Model CA-2202). The moisture content of seeds was measured by a hand-held moisture meter (Model GMK-303RS). The weight was corrected at 14% moisture content by using the formula written below and the average value was used (Ahsan et al. 2023):

$$\text{Adjusted weight} = \frac{(100 - M_1)}{(100 - M_2)} \times W$$

Where, W is the fresh weight; and M<sub>1</sub> and M<sub>2</sub> were the fresh and adjusted moisture percent (14%) of the grain, respectively.

All the plants from 1.8 m<sup>2</sup> area were harvested to record the grain and straw yield. The collected plants were threshed and seeds from the harvest plants were collected. The collected seeds were cleaned and air dried. Seeds from 1.8 m<sup>2</sup> area were weighted and converted to t ha<sup>-1</sup>. Straw obtained from 1.8 m<sup>2</sup> area was dried in sun and weighed to record the final straw yield plot<sup>-1</sup> and finally converted to t ha<sup>-1</sup>. The harvest index was determined using equation written below (Mamun et al. 2022):

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Grain yield} + \text{Straw yield}} \times 100$$

### Zinc measurement (ppm)

After harvest, yield was recorded. Samples of grain-straw were collected and processed for drying and grinding. Ground material (0.5 g) was taken in a conical flask and 10 ml of tri-acid mixture (HNO<sub>3</sub>: HClO<sub>4</sub>: H<sub>2</sub>SO<sub>4</sub> in 10:4:1)

was added. It was kept in a digestion chamber till complete digestion. The residue dissolved in double-distilled water and after filtration (Whatman filter paper no. 42) its final volume was made to 50 ml. Total Zn content in grain and straw of wheat and DTPA-extractable Zn was extracted by DTPA-CaCl<sub>2</sub> solution in soil and determined with the help of atomic absorption spectrophotometer.

#### **Electrical conductivity**

Electrical conductivity of seed was determined to assess seed membrane integrity following the standard conductivity test. About 10 g of seeds were first cleaned to remove dust and surface contaminants. The seeds were soaked in a 10 ml of distilled water and kept in dark for 24 h at room temperature. Subsequently, the electrical conductivity of the solution was measured with the help of EC meter. The results were expressed as  $\mu\text{S cm}^{-1} \text{g}^{-1}$  of seed. Lower electrical conductivity values indicated better membrane stability and higher seed vigor, whereas higher values reflected greater electrolyte leakage due to membrane damage or seed deterioration.

#### **Seed quality analysis**

To determine the seeds quality attributes, 100 seeds were taken from each replication according to treatment. The seeds were germinated in petri dish. There were 100 seeds in one petri dish from each replication and each treatment. Seeds were kept for seven days for germination and seedling evaluation. Speed of germination was calculated by the following formula:

$$\text{Speed of germination} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \dots$$

Where, n = number of germinated seeds, d = number of days.

The germination index (GI) was calculated by using the following formula:

$$\text{G.I.} = \frac{n}{d}$$

where, n = number of seedlings emerging on day 'd', d = day after planting.

Seed vigor index (SVI) was calculated by using following formula:

$$\text{SVI} = \frac{\text{seedling height (cm)} \times \text{germination percentage}}{100}$$

Seed germination percentage was calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds incubated}} \times 100$$

#### **Statistical analysis**

The collected data were compiled and analyzed statistically using the analysis of variance with the help of Crop Stat 7.2 statistical package program. The mean differences were adjusted by Duncan's multiple range test at a significant level of 0.05 (Gomez and Gomez 1984). Graphs were prepared from Excel software.

## **Results and discussion**

#### **Growth duration, plant height and ear length of wheat**

Growth duration varied slightly among varieties, with BARI Gom-33 showing the longest growth period across Zn treatments. Zinc application had no pronounced effect on crop duration, indicating that Zn primarily influenced physiological efficiency rather than phenological development when applied at stem elongation and after anthesis. However, the longer growth duration was observed in BARI Gom-33 (114 days), when Zn was applied during RS at Zn<sub>6g</sub>. On the other hand, BARI Gom-30 took short duration (102 days), when Zn<sub>0g</sub> applied at VS (Table 2).

Plant height increased modestly with Zn application, particularly at Zn<sub>3g</sub> and Zn<sub>6g</sub> doses, which was consistent with Zn's role in auxin synthesis and cell elongation (Hafeez et al. 2013). BARI Gom-33 consistently produced the tallest plants, reflecting its varietal genetic potential. Plant height ranged from 92.67 to 93.67 cm in case of BARI Gom-33 and 83.34 to 86.34 cm for BARI Gom-30 (Table 2). Similar trends have been reported in wheat under Zn fertilization by Alloway (2008) and Cakmak (2017). The increase in plant height might be due to the involvement of Zn in different physiological processes like enzyme activation, stomatal regulation, etc. which ultimately increase the plant height (Yaseen et al. 2011). Chaure et al. (2019) observed that applying ZnSO<sub>4</sub> markedly influenced plant height of wheat.

Ear length was significantly influenced by varietal differences, with BARI Gom-33 produced the longest ear (12.24 cm) during application of Zn<sub>6g</sub> at RS, while BARI Gom-30 gave the smallest one (10.17 cm) when no Zn applied at VS (Table 2). Zinc application marginally improved ear length, especially when applied at the reproductive stage, likely due to improved assimilate partitioning during spike development. Ferdous et al.

**Table 2.** Interaction effect for variety × Zn dose × stage of Zn application on growth duration, plant height and ear length of wheat

| Variety                       | Zn <sub>og</sub>    |                     | Zn <sub>3g</sub>    |                     | Zn <sub>6g</sub>    |                     |
|-------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                               | VS                  | RS                  | VS                  | RS                  | VS                  | RS                  |
| <b>Growth duration (days)</b> |                     |                     |                     |                     |                     |                     |
| BARI Gom-25                   | 107 <sup>c</sup>    | 107 <sup>bc</sup>   | 108 <sup>bc</sup>   | 109 <sup>b</sup>    | 107 <sup>bc</sup>   | 107 <sup>bc</sup>   |
| BARI Gom-30                   | 102 <sup>c</sup>    | 104 <sup>c</sup>    | 104 <sup>c</sup>    | 104 <sup>c</sup>    | 103 <sup>c</sup>    | 103 <sup>c</sup>    |
| BARI Gom-33                   | 112 <sup>ab</sup>   | 113 <sup>ab</sup>   | 113 <sup>ab</sup>   | 113 <sup>ab</sup>   | 113 <sup>ab</sup>   | 114 <sup>a</sup>    |
| CV (%)                        | 11.5                |                     |                     |                     |                     |                     |
| <b>Plant height</b>           |                     |                     |                     |                     |                     |                     |
| BARI Gom-25                   | 87.34 <sup>bc</sup> | 89.67 <sup>b</sup>  | 89.34 <sup>b</sup>  | 87.67 <sup>bc</sup> | 89.00 <sup>bc</sup> | 88.00 <sup>bc</sup> |
| BARI Gom-30                   | 83.04 <sup>cd</sup> | 86.34 <sup>c</sup>  | 83.67 <sup>cd</sup> | 83.67 <sup>cd</sup> | 85.34 <sup>cd</sup> | 83.34 <sup>d</sup>  |
| BARI Gom-33                   | 92.67 <sup>a</sup>  | 93.34 <sup>a</sup>  | 93.00 <sup>a</sup>  | 91.00 <sup>ab</sup> | 93.00 <sup>a</sup>  | 93.67 <sup>a</sup>  |
| CV (%)                        | 12.0                |                     |                     |                     |                     |                     |
| <b>Ear length (cm)</b>        |                     |                     |                     |                     |                     |                     |
| BARI Gom-25                   | 11.37 <sup>bc</sup> | 11.14 <sup>c</sup>  | 11.30 <sup>c</sup>  | 11.30 <sup>c</sup>  | 11.30 <sup>c</sup>  | 11.17 <sup>c</sup>  |
| BARI Gom-30                   | 10.17 <sup>d</sup>  | 11.34 <sup>bc</sup> | 11.27 <sup>c</sup>  | 11.10 <sup>c</sup>  | 11.44 <sup>bc</sup> | 11.24 <sup>c</sup>  |
| BARI Gom-33                   | 11.90 <sup>ab</sup> | 11.94 <sup>ab</sup> | 11.64 <sup>b</sup>  | 12.00 <sup>ab</sup> | 11.80 <sup>ab</sup> | 12.24 <sup>a</sup>  |
| CV (%)                        | 13.0                |                     |                     |                     |                     |                     |

VS = Vegetative (jointing) stage; RS = Reproductive (after anthesis) stage. CV = Coefficient of variation. BARI = Bangladesh Agricultural Research Institute. Mean values with dissimilar letters differ significantly ( $P < 0.05$ ), while values with same letter did not differ significantly.

(2018), however, reported that different levels of Zn affected morphological characters of wheat.

#### Yield components of wheat

The number of spikelets and grains spike<sup>-1</sup> responded positively to Zn application, particularly at the RS. Among

the Zn treatments, the number of spikelets spike<sup>-1</sup> ranged from 17.67 to 19.00 in the case of BARI Gom-25, 16.67 to 18.67 for BARI Gom-30 and 17.00 to 18.67 for BARI Gom-33 (Table 3). BARI Gom-25 produced the highest number of spikelets spike<sup>-1</sup> under both Zn<sub>3g</sub> and Zn<sub>6g</sub> treatments (19.0 spikelets spike<sup>-1</sup>) when applied at RS. On the other hand, BARI Gom-30 produced the lowest number

**Table 3.** Interaction effect for variety × Zn dose × stage of Zn application on number of spikes, number of grains and 1000-grain weight of wheat

| Variety                                       | Zn <sub>og</sub>     |                      | Zn <sub>3g</sub>     |                      | Zn <sub>6g</sub>     |                      |
|---|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|   | VS                   | RS                   | VS                   | RS                   | VS                   | RS                   |
| <b>Number of spikelets spike<sup>-1</sup></b> |                      |                      |                      |                      |                      |                      |
| BARI Gom-25                                   | 18.34 <sup>ab</sup>  | 18.67 <sup>ab</sup>  | 18.34 <sup>ab</sup>  | 19.00 <sup>a</sup>   | 17.67 <sup>b</sup>   | 19.00 <sup>a</sup>   |
| BARI Gom-30                                   | 16.67 <sup>b</sup>   | 18.00 <sup>ab</sup>  | 18.67 <sup>ab</sup>  | 18.67 <sup>ab</sup>  | 17.67 <sup>b</sup>   | 18.00 <sup>ab</sup>  |
| BARI Gom-33                                   | 17.00 <sup>b</sup>   | 18.34 <sup>ab</sup>  | 18.00 <sup>ab</sup>  | 17.34 <sup>b</sup>   | 18.67 <sup>ab</sup>  | 18.00 <sup>ab</sup>  |
| CV (%)  | 13.7                 |                      |                      |                      |                      |                      |
| <b>Number of grain spike<sup>-1</sup></b>     |                      |                      |                      |                      |                      |                      |
| BARI Gom-25                                   | 40.34 <sup>cd</sup>  | 39.67 <sup>cd</sup>  | 41.67 <sup>c</sup>   | 39.34 <sup>cd</sup>  | 39.67 <sup>cd</sup>  | 40.34 <sup>cd</sup>  |
| BARI Gom-30                                   | 37.67 <sup>d</sup>   | 41.67 <sup>c</sup>   | 38.34 <sup>d</sup>   | 43.00 <sup>bc</sup>  | 38.34 <sup>d</sup>   | 42.34 <sup>bc</sup>  |
| BARI Gom-33                                   | 46.00 <sup>b</sup>   | 45.00 <sup>b</sup>   | 47.34 <sup>b</sup>   | 50.67 <sup>a</sup>   | 50.00 <sup>ab</sup>  | 52.00 <sup>a</sup>   |
| CV (%)  | 13.7                 |                      |                      |                      |                      |                      |
| <b>1000-seed weight (g)</b>                   |                      |                      |                      |                      |                      |                      |
| BARI Gom-25                                   | 42.36 <sup>d</sup>   | 44.29 <sup>a-d</sup> | 43.45 <sup>bcd</sup> | 43.17 <sup>cd</sup>  | 47.68 <sup>a</sup>   | 46.02 <sup>abc</sup> |
| BARI Gom-30                                   | 46.78 <sup>ab</sup>  | 44.48 <sup>a-d</sup> | 45.45 <sup>a-d</sup> | 43.29 <sup>bcd</sup> | 45.52 <sup>a-d</sup> | 46.79 <sup>ab</sup>  |
| BARI Gom-33                                   | 46.49 <sup>abc</sup> | 43.95 <sup>bcd</sup> | 41.91 <sup>d</sup>   | 45.56 <sup>a-d</sup> | 44.08 <sup>bcd</sup> | 45.29 <sup>a-d</sup> |
| CV (%)  | 4.8                  |                      |                      |                      |                      |                      |

VS = Vegetative (jointing) stage; RS = Reproductive (after anthesis) stage. CV = Coefficient of variation. BARI = Bangladesh Agricultural Research Institute. Mean values with dissimilar letters differ significantly ( $P < 0.05$ ), while values with same letter did not differ significantly.

**Table 4.** Interaction effect for variety × Zn dose × stage of Zn application on yield and harvest index of wheat

| Variety                                | Zn <sub>0g</sub>    |                      | Zn <sub>3g</sub>     |                      | Zn <sub>6g</sub>     |                      |
|--|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|  | VS                  | RS                   | VS                   | RS                   | VS                   | RS                   |
| <b>Grain yield (t ha<sup>-1</sup>)</b> |                     |                      |                      |                      |                      |                      |
| BARI Gom-25                            | 3.99 <sup>bc</sup>  | 4.37 <sup>abc</sup>  | 4.48 <sup>ab</sup>   | 4.29 <sup>abc</sup>  | 4.48 <sup>abc</sup>  | 5.07 <sup>a</sup>    |
| BARI Gom-30                            | 3.85 <sup>c</sup>   | 3.89 <sup>c</sup>    | 4.03 <sup>bc</sup>   | 3.99 <sup>bc</sup>   | 3.96 <sup>c</sup>    | 3.97 <sup>c</sup>    |
| BARI Gom-33                            | 3.85 <sup>c</sup>   | 4.25 <sup>abc</sup>  | 4.42 <sup>abc</sup>  | 4.93 <sup>ab</sup>   | 3.78 <sup>c</sup>    | 4.06 <sup>bc</sup>   |
| CV (%)                                 |                     |                      | 13.6                 |                      |                      |                      |
| <b>Straw yield (t ha<sup>-1</sup>)</b> |                     |                      |                      |                      |                      |                      |
| BARI Gom-25                            | 5.74 <sup>bcd</sup> | 6.17 <sup>abc</sup>  | 6.23 <sup>abc</sup>  | 6.08 <sup>a-d</sup>  | 6.25 <sup>abc</sup>  | 6.81 <sup>a</sup>    |
| BARI Gom-30                            | 5.65 <sup>cd</sup>  | 5.65 <sup>cd</sup>   | 5.79 <sup>bcd</sup>  | 5.76 <sup>bcd</sup>  | 5.66 <sup>cd</sup>   | 5.74 <sup>bcd</sup>  |
| BARI Gom-33                            | 5.61 <sup>cd</sup>  | 6.00 <sup>a-d</sup>  | 5.16 <sup>d</sup>    | 6.63 <sup>ab</sup>   | 5.51 <sup>cd</sup>   | 5.83 <sup>bcd</sup>  |
| CV (%)                                 |                     |                      | 19.5                 |                      |                      |                      |
| <b>Harvest index (%)</b>               |                     |                      |                      |                      |                      |                      |
| BARI Gom-25                            | 40.89 <sup>bc</sup> | 41.33 <sup>abc</sup> | 41.79 <sup>abc</sup> | 41.12 <sup>abc</sup> | 41.71 <sup>abc</sup> | 42.64 <sup>abc</sup> |
| BARI Gom-30                            | 40.54 <sup>c</sup>  | 40.74 <sup>bc</sup>  | 40.99 <sup>c</sup>   | 40.93 <sup>bc</sup>  | 40.88 <sup>bc</sup>  | 40.75 <sup>c</sup>   |
| BARI Gom-33                            | 40.70 <sup>c</sup>  | 41.35 <sup>c</sup>   | 41.71 <sup>abc</sup> | 42.58 <sup>abc</sup> | 40.55 <sup>c</sup>   | 40.97 <sup>abc</sup> |
| CV (%)                                 |                     |                      | 12.5                 |                      |                      |                      |

VS = Vegetative (jointing) stage; RS = Reproductive (after anthesis) stage. CV = Coefficient of variation. BARI = Bangladesh Agricultural Research Institute. Mean values with dissimilar letters differ significantly ( $P < 0.05$ ), while values with same letter did not differ significantly.

of spikelets spike<sup>-1</sup> under Zn<sub>0g</sub> treatments (16.67 spikelets spike<sup>-1</sup>) at VS. These results are in accordance with findings of Ali et al. (2013) who concluded that growth and yield were not improved by adding appropriate amounts of Zn fertilizers.

BARI Gom-33 recorded the highest grains number spike<sup>-1</sup>, reaching 52 grains spike<sup>-1</sup> with Zn<sub>6g</sub> applied at the RS. The number of grains spike<sup>-1</sup> ranged from 45 to 52 in case of BARI Gom-33 and 37.67 to 43 for BARI Gom-30 and 39.34 to 41.67 for BARI Gom-25 (Table 3). BARI Gom-33 produced the highest number of grains spike<sup>-1</sup> under Zn<sub>6g</sub> treatments (52.0 grains spike<sup>-1</sup>) when applied at RS. On the other hand, BARI Gom-30 produced the lowest number of grains spike<sup>-1</sup> under Zn<sub>0g</sub> treatments (37.67 grains spike<sup>-1</sup>) at VS. Zinc plays a critical role in pollen viability and fertilization, which explains the improved grain set observed under Zn supplementation (Cakmak et al. 2010). Ziaeyan et al. (2009) reported that either foliar or soil application of Zn could increase total number of grains spike<sup>-1</sup>. The 1000-grain weight increased significantly with Zn application, with the highest values observed under Zn<sub>6g</sub> treatments. BARI Gom-25 produced the highest 1000-seed weight of 47.68 g when Zn applied at the rate of Zn<sub>6g</sub> at VS, while it was 46.02 g when Zn applied at RS. BARI Gom-33 produced lowest amount of 1000-seed weight of 41.91 g when Zn applied at the rate of Zn<sub>3g</sub> at VS, while it was 45.56 g when Zn applied at RS (Table 3). This improvement reflects enhanced photosynthate translocation and starch synthesis during grain filling. Similar improvements in grain weight

under Zn fertilization have been documented by Ram et al. (2016). However, Boorboori et al. (2012) and Moghadam et al. (2012) reported that foliar spraying of Zn on wheat, have no marked effect on 1000-grain weight. Thus, the role of Zn on increasing grain weight might be related to growing environment, growth stage of Zn application and wheat variety as well.

#### Wheat yield

Grain yield was significantly affected by the interaction of variety × Zn dose × stage of Zn application. Grain yield ranged from 3.99 g to 5.07g in case of BARI Gom-25, 3.85 g to 4.03 g for BARI Gom-30 and 3.78 g to 4.93 g for BARI Gom-33 (Table 4). The highest grain yield (5.07 t ha<sup>-1</sup>) was recorded in BARI Gom-25 with Zn<sub>6g</sub> applied at the RS. In contrast, BARI Gom-30 showed comparatively lower yield (3.85 t ha<sup>-1</sup>) in response to Zn, indicating varietal differences in Zn uptake and utilization efficiency. Singh et al. (2012) and Boorboori et al. (2012) reported that increasing levels of Zn increased wheat yield. Grewal et al. (1997) also reported increased grain yield in (oilseed rape) due to Zn application. The increase in the grain yield was attributable to the improved physiology of plants with the added Zn that presumably connected to the efficiency of different enzymes, chlorophyll content, IAA hormone and improvement in nitrate conversion to ammonia in plant (Hacisalihoglu et al. 2003, Abbas et al. 2010).

The straw yield ranged from 5.74 to 6.81 t ha<sup>-1</sup> in case of BARI Gom-25, 5.65 to 5.76 t ha<sup>-1</sup> for BARI Gom-30 and 5.51 to 6.63 t ha<sup>-1</sup> in case of BARI Gom-33 (Table 4). BARI

**Table 5.** Interaction effect for variety × Zn dose × stage of Zn application on electrical conductivity, Zn content in seeds and germination characteristics of wheat seed

| Variety  | Zn <sub>0g</sub>    |                      | Zn <sub>3g</sub>    |                     | Zn <sub>6g</sub>    |                      |
|--|---------------------|----------------------|---------------------|---------------------|---------------------|----------------------|
|  | VS                  | RS                   | VS                  | RS                  | VS                  | RS                   |
| <b>Electrical conductivity (<math>\mu\text{S cm}^{-1} \text{g}^{-1}</math> seed)</b> |                     |                      |                     |                     |                     |                      |
| BARI Gom-25  | 0.25                | 0.29                 | 0.25                | 0.28                | 0.27                | 0.25                 |
| BARI Gom-30  | 0.27                | 0.26                 | 0.27                | 0.27                | 0.25                | 0.27                 |
| BARI Gom-33  | 0.26                | 0.26                 | 0.24                | 0.21                | 0.25                | 0.27                 |
| CV (%)   | 12.3                |                      |                     |                     |                     |                      |
| <b>Zn content (ppm)</b>  |                     |                      |                     |                     |                     |                      |
| BARI Gom-25  | 48.85 <sup>f</sup>  | 44.98 <sup>g</sup>   | 52.73 <sup>de</sup> | 60.09 <sup>bc</sup> | 59.65 <sup>c</sup>  | 60.05 <sup>bc</sup>  |
| BARI Gom-30  | 35.48 <sup>i</sup>  | 43.49 <sup>gh</sup>  | 44.94 <sup>g</sup>  | 44.39 <sup>gh</sup> | 41.65 <sup>g</sup>  | 46.99 <sup>fg</sup>  |
| BARI Gom-33  | 49.42 <sup>f</sup>  | 50.85 <sup>e</sup>   | 62.43 <sup>b</sup>  | 54.7 <sup>d</sup>   | 57.56 <sup>c</sup>  | 65.69 <sup>a</sup>   |
| CV (%)   | 13.3                |                      |                     |                     |                     |                      |
| <b>Germination index</b>   |                     |                      |                     |                     |                     |                      |
| BARI Gom-25  | 51.21 <sup>b</sup>  | 53.33 <sup>ab</sup>  | 54.79 <sup>ab</sup> | 48.94 <sup>b</sup>  | 54.64 <sup>ab</sup> | 55.14 <sup>2ab</sup> |
| BARI Gom-30  | 49.99 <sup>b</sup>  | 52.30 <sup>ab</sup>  | 54.23 <sup>ab</sup> | 54.43 <sup>ab</sup> | 54.75 <sup>ab</sup> | 58.28 <sup>ab</sup>  |
| BARI Gom-33  | 52.63 <sup>ab</sup> | 58.11 <sup>a</sup>   | 50.81 <sup>b</sup>  | 50.09 <sup>b</sup>  | 50.74 <sup>b</sup>  | 50.33 <sup>b</sup>   |
| CV (%)   | 13.3                |                      |                     |                     |                     |                      |
| <b>Seed vigor index</b>  |                     |                      |                     |                     |                     |                      |
| BARI Gom-25  | 1526                | 1594                 | 1570                | 1527                | 1588                | 1538                 |
| BARI Gom-30  | 1456                | 1494                 | 1592                | 1516                | 1467                | 1531                 |
| BARI Gom-33  | 1532                | 1477                 | 1502                | 1530                | 1478                | 1496                 |
| CV (%)   | 12.3                |                      |                     |                     |                     |                      |
| <b>Germination (%)</b>   |                     |                      |                     |                     |                     |                      |
| BARI Gom-25  | 94.34 <sup>ab</sup> | 95.166 <sup>ab</sup> | 97.40 <sup>a</sup>  | 97.34 <sup>a</sup>  | 94.67 <sup>ab</sup> | 96.67 <sup>ab</sup>  |
| BARI Gom-30  | 97.34 <sup>a</sup>  | 95.16 <sup>ab</sup>  | 96.33 <sup>a</sup>  | 94.67 <sup>ab</sup> | 93.67 <sup>b</sup>  | 97.00 <sup>a</sup>   |
| BARI Gom-33  | 96.33 <sup>a</sup>  | 92.16 <sup>b</sup>   | 94.00 <sup>ab</sup> | 96.56 <sup>ab</sup> | 95.25 <sup>ab</sup> | 94.89 <sup>abc</sup> |
| CV (%)   | 12.4                |                      |                     |                     |                     |                      |

VS = Vegetative (jointing) stage; RS = Reproductive (after anthesis) stage. CV = Coefficient of variation. BARI = Bangladesh Agricultural Research Institute. Mean values with dissimilar letters differ significantly ( $P < 0.05$ ), while values with same letter did not differ significantly.

Gom-25 produced higher straw yield followed by BARI Gom-33 (6.81 t ha<sup>-1</sup>) under Zn<sub>6g</sub> treatment when applied at RS. On the other hand, BARI Gom-30 gave the lowest straw yield (5.65 t ha<sup>-1</sup>) under Zn<sub>0g</sub>. Keram et al. (2013) also reported that the straw yield of wheat significantly increased with the application of Zn. The highest harvest index (HI) was observed from BARI Gom-25 under Zn<sub>6g</sub> at RS. BARI Gom-25 gave HI of 42.64 % when Zn<sub>6g</sub> applied at RS, while it was 40.89 % when no Zn applied VS. Almost similar results observed in case of other two varieties (Table 4).

The harvest index (HI) reflects the physiological ability of a plant to convert photosynthates into grain yield, and HI data were non-significant across Zn fertilizer levels. Khan et al. (2008) has reported that Zn applications decreased HI.

### Seed quality of wheat

Interaction effect for variety × Zn dose × stage of Zn application exerted a significant effect on grain Zn content, germination index and germination percent of wheat. On the contrary, interaction effect for variety × Zn dose × stage of Zn application did not have significant effect on electrical conductivity (EC) and seed vigor index of wheat (Table 5). The EC ranged from 0.25 to 0.29  $\mu\text{S cm}^{-1} \text{g}^{-1}$  in case of BARI Gom-25 and 0.21 to 0.27  $\mu\text{S cm}^{-1} \text{g}^{-1}$  for BARI Gom-33. In the same way, about 0.25 to 0.27  $\mu\text{S cm}^{-1} \text{g}^{-1}$  of EC was observed for BARI Gom-25 in all the Zn applied treatments (Table 5). Wheat variety BARI Gom-25 showed numerically higher EC under Zn<sub>0g</sub> at RS (0.29  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) followed by Zn<sub>3g</sub> at same stage in same wheat variety. Regarding EC of wheat seed, BARI Gom-33 gave the lower results at RS with Zn<sub>3g</sub>.

The amount of Zn content ranged from 49.42 to 65.69 ppm in case of BARI Gom-33 and 35.48 to 46.99 ppm for

BARI Gom-30. In the same way, about 48.98 to 60.09 ppm of Zn content was measured for BARI Gom-25 in all Zn applied treatments (Table 5). BARI Gom-33 showed the highest amount of Zn (65.69 ppm) concentration in their grain under  $Zn_{6g}$  during application at RS, which was followed by VS with the application of  $Zn_{3g}$ . The Zn concentrations of grain of BARI Gom-25 were statistically identical under both  $Zn_{3g}$  and  $Zn_{6g}$  when applied at RS. Wheat variety BARI Gom-30 gave the lower results under no Zn application. Ranjbar and Bahmaniar (2007) reported that the lower effectiveness of soil and foliar applications in comparison with soil plus foliar applications of Zn fertilizer may be credited to lower levels of Zn in wheat shoot. Zeidan (2001) indicated that Zn application increased grain protein and enhanced grain Zn concentration. Cakmak (2008) and Rengel (1995) also found that higher Zn content in wheat grains gave plants with better root and shoot, and accumulated more dry matter compared to those from the low Zn content in seeds. Improvements in grains quality parameters may be attributed to the role of microelements to enhancing accumulation of assimilate in the grains (during grain filling stage) and thus the resultant seeds had greater individual mass (Baskin and Baskin 1998). Similar results of improving grain quality with Zn application were also reported earlier (Roach and Wulff 1987; Fenner 1992).

Germination index (GI) indicates the estimated time required to reach a given germination percentage. The number of seedlings observed or counted that day is divided by the number of days after planting (Fakorede and Agbana 1983). BARI Gom-30 along with higher dose of Zn produced highest GI (58.28) when Zn was applied at RS, while it was (49.99) with  $Zn_{0g}$  at early stage of Zn application (Table 5). Seed with 0.05%  $ZnSO_4$  solution increased germination and field emergence by 38 and 41%, respectively (Babaeva et al. 1999). Seed priming with Zn improved germination and seedling development (Ajouri et al. 2004). Seed Zn content stimulates the activities of enzymes like  $\alpha$ -amylase which then accelerate the breakdown of food reserves and supply energy to growing embryos (Kaur et al. 2002; Farooq et al. 2006).

Seed vigor index (SVI) is calculated by multiplying germination and seedling length. The seed lot showing higher SVI is considered to be more vigorous (Abdul-Baki and Anderson, 1973). Interaction effect of variety and Zn concentration showed a numerical effect on SVI of wheat but there was no statistical difference (Table 5). BARI Gom-25 gave the highest SVI (1594) at VS of wheat, even though BARI Gom-33 gave the lowest SVI (1477) at VS. The Zn-biofortified seeds assure better seed vigor and seedling growth, particularly when Zn and water were limited in the growth medium. Germination percent indicates a measure of how many seeds are alive and

can develop into normal plants. BARI Gom-25 gave the highest germination (97.34%) under  $Zn_{6g}$ , whereas BARI Gom-33 gave the lowest germination (92.16%) values under  $Zn_{3g}$ . Similarly, BARI Gom-25 and BARI Gom-30 gave statistically identical germination under  $Zn_{3g}$ .

## Conclusion

Zinc fertilization significantly improved growth performance, yield components, seed quality, and grain Zn concentration in wheat regarding varieties and application at different stages. Application of 6 g Zn at the reproductive stage was more effective than vegetative application, particularly for enhancing grain yield and Zn biofortification. Among the tested varieties, BARI Gom-33 showed superior grain Zn accumulation, while BARI Gom-25 produced the highest grain yield. These findings suggest that reproductive-stage Zn application can be an effective strategy for improving both productivity and nutritional quality of wheat.

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