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Zinc biofortification of wheat grain through agronomic approaches in Bangladesh

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ABSTRACT

Zinc (Zn) scarcity in human wellness affects about two billion people worldwide, is a ubiquitous all-embracing health headache especially in emergent nations like Bangladesh. Geographic regions with dearth of Zn in human wellbeing are mostly where zinc shortfall occurs in soil, bespeaking their built-in linkage. Unfortunately, the capability of agricultural set-up to come up with nutritive foods to conquer micronutrient malnourishment (i.e. hidden hunger) for human well-being has gained limited consideration than the usual malnutrition and related issues such as food demand, calorie intake, crop yield and environmental sustainability. Various breaking-in could be used to overcome malnutrition, but biofortification is most impressive, favourable, viable and justifiable. Wheat is one of the major crops grown and consumed worldwide and Bangladesh as well which could prevalent Zn malnutrition; therefore, this is the ultimate sitting duck for Zn biofortification. Zn biofortification of wheat grain could be achieved through agronomic approaches like fertilizer application as soils in Bangladesh are deficit to Zn. The wheat varieties BINA Gom-1 and BARI Gom-30 were grown during 2020-2021 and BARI Gom-32 and BARI Gom-33 wheat varieties during 2021-2022 winter seasons extended from mid November to mid March. The Zn treatments in each year's of experiment were nil Zn (control), root Zn application, foliar Zn application and root Zn + foliar Zn application and laid-out following a Randomized Complete Block Design (RCBD) with three replications. The root Zn application treatment consisted of 50 kg $ZnSO4\cdot7H2O$ ha^{-1}, incorporated into soil before seed sowing. The foliar Zn application treatment consisted of two times of Zn spray on leaves at the heading and milk stages. At each time of foliar Zn application, 0.5 % (w/v) of aqueous solution of $ZnSO4\cdot7H2O$ with 800 liter per hectare was sprayed at the very late afternoon until most of the leaves were wet. The root Zn + foliar Zn application is the combination of root Zn application together with foliar Zn spray. Zn application in root (i.e. in soil) or in foliage or apply in root plus foliar had no significant effect on the plant growth and yield of wheat crops. But Zn treatments increased zinc concentration in wheat grain as compared to nil Zn application in the order of: Root Zn + foliar Zn>foliar Zn>root Zn>nil Zn. Thus people's malnutrition can be reduced with providing wheat grain biofortified through foliar Zn apply in alone or combine with Zn apply in root.

Keywords: Agronomic approaches, Biofortification, Harvest Plus Programme, Malnutrition, Root and foliar applications, Wheat, Zinc

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1. INTRODUCTION

Zinc (Zn) deficiency is a grave micronutrient shortfall in human health, which affects more than one-third of the world population estimated to about 2 billion (mostly pregnant women and children under five years old) (Hotz and Brown, 2004; Stein, 2010). There is a direct geographical intersecting between the global disposition of Zn deficiency in soil and in human being (Alloway, 2008; Cakmak, 2008) which highlights the core linkage among agriculture, food crops and human health (Welch, 2008). It's also known that the most common human Zn deficiency occurs particularly in areas with high cereal production but low animal products consumption (Bouis et al., 2011) as like in Bangladesh.

It is reported that Zn deficiency in human is widespread mainly in areas where cereal-based foods are dominant in the diet (Bouis, 1996; Gibson, 2006). Cereal grains are inherently low Zn both in concentration and bioavailability perspectives, particularly when grown in potentially Zn-deficient soils (Welch and Graham, 2004; Cakmak et al., 2010a). Release of high-yielding cereal cultivars also contribute to the high incidence of Zn deficiency in human (Cakmak, 2008). In most cases, there is an inverse relationship between grain yield and grain Zn concentration (Garvin et al., 2006; McDonald et al., 2008). For breaking this trade-off breeding, transgenic technology or agronomic approaches can be explored to increase the Zn concentration in the edible parts of high yielding crop varieties (Bouis and Welch, 2010; Waters and Sankaran, 2011; Zhao and McGrath, 2009).

Several crush staple food fortification programmes have been taken globally to increase the nutrient content in staples to pull out the endanger people from malnutrition. Currently one of the most cost-effective strategies to address global malnutrition is biofortification. Boosting the bioavailability of micronutrient contents like zinc and other essential nutrient elements like Fe, provitamin A etc in the edible part of staple crops is the major goal of the biofortification programme. Wheat is a principal cereal (i.e. number one) and represents the main dietary source of calories, proteins and micronutrients for the majority of world's population (Shewry, 2009). It is responsible up to 70 percent of daily calorie consumption of the global people and also an important source of Zn for human beings (Cakmak, 2008). Therefore, wheat is considered as the suitable target for Zn biofortification programme.

There is a large genotypic variation for grain Zn concentration among the genomes of modern wheat and its wild relatives (Cakmak et al., 2010a; Gomez-Becerra et al., 2010). This genetic variation for Zn is now being exploited under different schemes, especially under the Harvest Plus Programme (www.harvestplus.org) to provide a long-term solution to the problem. Plant breeding and/or transgenic technologies are promising to overcome human malnutrition by releasing new genotypes with high levels of Zn to the target regions (Bouis et al., 2011; Welch and Graham, 2004; White and Broadley, 2005). The combination of high grain Zn content together with superior agronomic parameters (i.e. the traits for high yield) under diverse environmental conditions is an important task of the on-going breeding programmes. As breeding approach takes a longer time for achieving a success at mark, agronomic biofortification approach (e.g. application of Zn fertilizers) represents a short-term solution to the problem (Cakmak, 2008). But pertinent research work is hardly conducted in Bangladesh context.

Wheat ranks second after rice in Bangladesh and provides the important food source to the people through consuming a variety of food stuffs or meals. It has enormous potentials to feed the huge malnourished people of Bangladesh through providing Zn-biofortified wheat grains. As the Zn supply to Bangladesh people is found inadequate (Arsenault et al., 2015), so this nutrition can be provided with wheat grain where biofortification in terms of fertilization approach might be considered as the best and immediate solution. Besides the Zn biofortification in grain, plant growth and yield of wheat crop is also influenced by the zinc fertilization by its rate, timing and method of application (Khattab et al., 2016; Dawar et al., 2022; Jalal et al., 2022).

Therefore, the aim of the study is to find out the effect of zinc nutrition through applying zinc fertilizer in roots (i.e. in soil) as basal dose and leaves through foliar spray and combination of these twos on the Zn enrichment in wheat grain and growth and yield of wheat crop as well.

2. MATERIALS AND METHODS

Experimental work

The study was conducted in the Field Laboratory of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh. Geographically the experimental site is located at 24°25''N latitude and 90°50''E longitude with an elevation of about 18 m above the sea level. It is non-calcareous dark grey flood plain soil under the Sonatola Series of Old Brahmaputra Alluvial Soil which included in Agro-Ecological Zone 9. The experimental field is a medium low land, fairly leveled and silt loam in texture having a soil pH 6.32 and available zinc concentration was 1.04ppm. The experimental site can be described with minimum rainfall along with dry climate during November to April and much rainfall with humid weather during the remaining period of the year. Major weather parameters during the experimental period were recorded at the weather yard and shown in Figures 1-2.

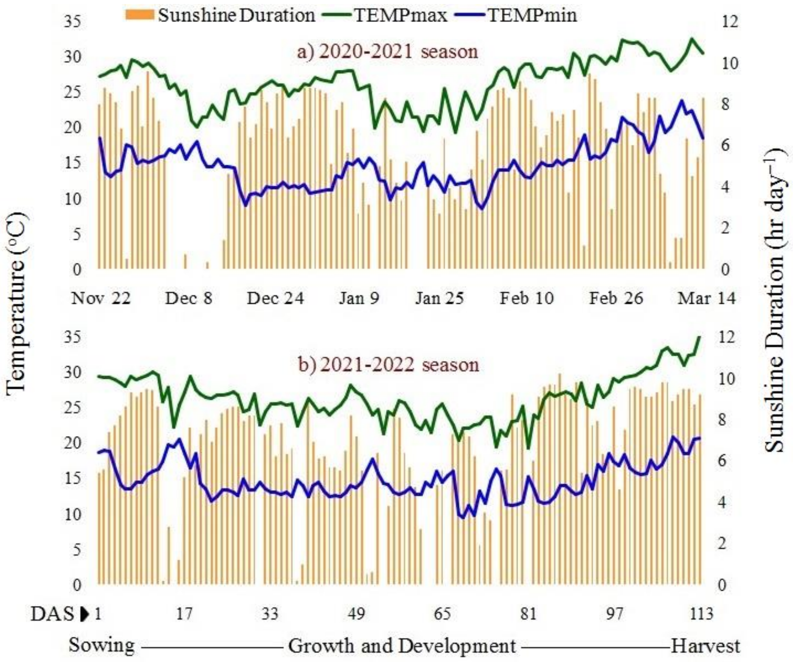

Figure 1a-b Seasonal course of daily mean maximum and minimum temperatures and sunshine duration at the experimental site. DAS indicates the days after sowing.

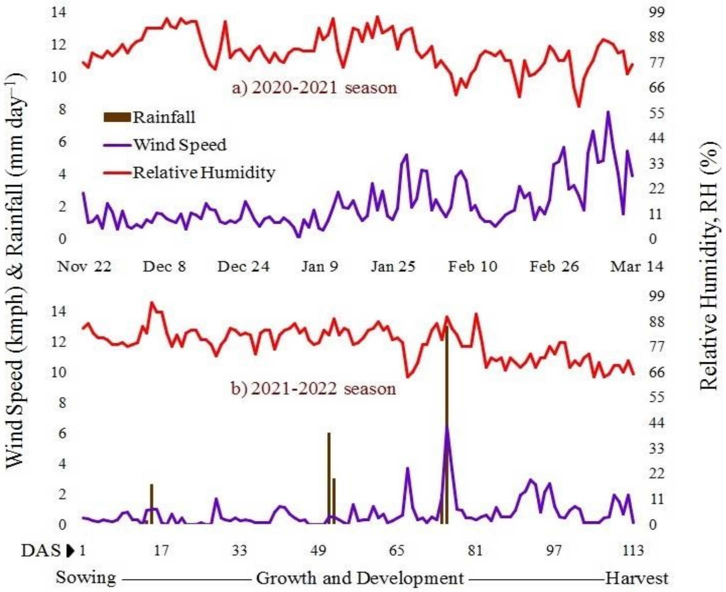

Figure 2a-b Seasonal course of daily mean wind speed and relative humidity and daily total rainfall at the experimental site. DAS indicates the days after sowing.

Four zinc treatments made up of nil or zero Zn i.e. control, root Zn, foliar Zn and root Zn + foliar Zn treatments. The root Zn treatment amounted to 50 kg ZnSO4·7H2O ha^{-1}, which was sprayed to the soil surface and then mixed well into soil before seed sowing (Zou et al., 2012). The foliar Zn treatment composed of two times of foliar Zn use at the heading (emergence of spike) (Plate 1c) and milk (initial phase of grain ripening) stages (Zou et al., 2012). At each time of application of foliar Zn, 0.5% (w/v) of liquid (aqueous) solution of Zinc Sulphate Heptahydrate (ZnSO4·7H_2O) with 800 L ha^{-1} (litre/hectare) was applied (sprayed) at late afternoon as far as most of the bulk of canopies were moistened (i.e. wet) as expressed by Cakmak et al., (2010b). Root Zn + foliar Zn treatment is the combination of root Zn use together with spray of foliar Zn. Two field experiments were conducted where four wheat varieties namely BINA Gom-1, BARI Gom-30, BARI Gom-32 and BARI Gom-33 were used. The first two varieties were used during 2020-2021 winter season (first year) while the later two wheat varieties used during 2021-2022 winter season (second year)'s experiment. In each year's of experiment, there was a total 8 (four Zn treatments × two wheat varieties) treatments which were laid-out with a Randomized Complete Block Design (RCBD) with three replications. It is mentioned that the BARI Gom-33 is Zn-enriched i.e. genetically biofortified with Zn.

Mean values of daily maximum and minimum temperatures, relative humidity and sunshine duration are almost similar in both experimental seasons (Table 1). The 2020-2021 cropping season extended from 22 November to 14 March received only a trace amount of rain while the 2021-2022 season during the same time period received a total of 27 mm rain while mean wind speed quite lower than the previous season.

Table 1 Mean major microclimatic parameters during experimental periods

Cropping season (from 22 November to 14 March)	Mean microclimatic parameters					Total rainfall (mm)
	Maximum air temperature (^{o}C)	Minimum air temperature (^{o}C)	Sunshine duration (hr day^{-1})	Relative humidity (%)	Wind speed (kmph)	
2020–2021	26.4±3.3	14.7±3.2	5.6±3.0	81.9±7.8	2.1±1.5	Trace
2021–2022	26.3±3.1	14.8±2.6	6.5±2.9	78.5±6.9	0.7±0.9	26.8

Figure followed by ± indicates the Standard Deviation (SD) over the cropping season (*n* = 113 days).

a) Top dressing of nitrogen fertilizer after weeding and thinning during seedling stage

b) Supply of irrigation water after completion of tillering stage

c) Foliar spraying of Zn during spike emergence/heading stage d) Physiological maturity stage prior harvesting

Plate 1a-d some important developmental stages and management practices of experimental wheat crop.

To make sure that no other mineral nutrient elements were limiting 60 kg K as muriate of potash, 30 kg P as triple super phosphate, 10 kg S as gypsum and 1.5 kg B as boric acid per hectare were applied as basal dose at the last land preparation. The rate of nitrogen was 120 kg/ha as urea, applied twice, the first half as basal dose mixed with the other fertilizers prior sowing and the second half at the tillering stage (Plate 1a). The plot size for each treatment was 3 m×2 m. The plots were separated by 0.5-m bunds, and the treatment's block-to-block distance was 1 m.

The wheat seeds of BINA Gom-1 variety were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and those of BARI Gom 30, BARI Gom 32 and BARI Gom 33 varieties were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. The seeds were sown in the plots in a line in 40 cm apart as per experimental design on 22 November 2020 as of first year's experiment and 22 November 2021 as of second year's experiment. Weeding was conducted two times, the first at 3 weeks after sowing and the second weeding before the heading. To avoid water stress, a single irrigation was applied after completion of tillering (Plate 1b). Agronomic practices with plant protection measures including control of insect pests and diseases were done as and when necessary. All management practices were performed in similar way for two successive years of experiments except wheat varieties.

Data collection

The experimented wheat crops were harvested at physiologically maturity with 113 days life span (Plate 1d) on 14 March 2021 and 14 March 2022, respectively for first and second years study. Mean data were recorded on plant height, culm girth or circumference as diameter, number of tiller per plant and per square meter as well, spike length, number of grain per spike and thousand seed weight from the sampled plants collected from 1×1 m^2 area of each plot. Plant height was measured with a Graduated Meter Scale from the ground level to the top of the spike. Culm diameter was measured with a Digital Slide Caliper from the middle of internode above the 3rd node from ground level. Spike length was measured from the basal node of the rachis to the apex of spike. All components of sampled plant parts were dried at 80±2°C in the fan-forced oven until the constant weight is reached.

Grain yield and straw yield were recorded as whole plot harvest basis. Harvested crops were dried under the sun. Grains were threshed, winnowed, cleaned, and processed and the weight of both grain and wheat straw was recorded. Grain and straw yields per plot were converted to ton per hectare (t ha^{-1}) basis and adjusted for 13 percent moisture.

Analysis of Zn concentration of wheat grain

Zn concentration from the experimented wheat grain samples was estimated with standard chemical/laboratory technique from Regional Laboratory of the Soil Resources Development Institute (SRDI), Jamalpur. Grain samples of wheat were dried in an air forced oven at 60°C for 48 h (Liu et al., 2006). Finely-ground 1.0 g samples of wheat flour were digested in a Di-acid (HNO3:HClO4 ratio of 2:1) mixture (Jones and Case, 1990). The Zn concentration in the digest was estimated by Atomic Absorption Spectrophotometer (AAS).

Statistical analysis

All collected data were subjected to one-way analyses of variance (ANOVA) and mean differences were compared with lowest standard deviations (±). Least Significance Difference (LSD) was employed to determine if any significant (at $P < 0.01$ and 0.05 levels) difference occurred between the Zn treatments or between the varieties used (Gomez and Gomez, 1984).

3. RESULTS

Plant growth traits

Plant height

Zinc application in soil or root somewhat increased the plant height in all the four varieties of wheat but the difference between the treatments was statistically insignificant at $P<0.05$ (Figure 3a). Plant height in all the varieties of wheat crop grown with foliar Zn application showed similar plant height that grown with control treatment (i.e. nil or zero Zn). Although the BINA Gom-1 variety produced taller statured canopy to some degree than that found from the other three BARI Gom varieties studied but the variation that found in height between the varieties studied was not significant at $P<0.05$ (Table 2).

Table 2 Plant growth, yield traits and yield and Zn concentration of wheat grains as influenced by varieties (pooled over the Zn treatments)

Parameter or trait	Wheat variety				LSD with Level of Significance
	BINA Gom-1	BARI Gom-30	BARI Gom-32	BARI Gom-33	
Plant stature or height (cm)	92.7±2.6	86.9±2.0	89.4±3.0	87.5±2.9	NS
Culm girth (mm)	3.33±0.06	3.72±0.01	3.67±0.04	4.29±0.06	0.01 (0.01)
No. of effective tiller plant^{-1}	4.07±0.03	3.50±0.13	3.52±0.04	3.40±0.12	0.02 (0.01)
No. of effective spike m^{-2}	304.0±5.2	284.0±7.6	311.5±8.8	300.1±8.9	28.7 (0.01)
Spike length (cm)	7.71±0.13	8.72±0.25	8.52±0.19	9.60±0.13	0.09 (0.01)
No. of grain spike^{-1}	33.4±1.6	34.9±0.3	38.6±1.7	41.2±0.6	2.8 (0.01)
Thousand seed weight (g)	33.4±1.6	34.9±0.3	43.4±1.5	45.7±0.9	3.8 (0.01)
Grain yield (t ha^{-1})	3.52±0.10	4.41±0.08	4.62±0.15	4.98±0.06	0.02 (0.01)
Straw yield (t ha^{-1})	4.12±0.07	5.06±0.12	6.62±0.14	6.78±0.15	0.03 (0.01)
Grain Zn conc. (μg g^{-1})	29.7±1.4	30.9±1.8	30.9±2.2	36.5±2.1	5.6 (0.05)

Figure followed by ± indicate the Standard Deviation (SD) of the treatment mean (*n* = 3). LSD = Least Significance Difference, NS = Not Significant. Figure in the parenthesis i.e. 0.01 and 0.05 indicate the $P<0.01$ and $P<0.05$ levels, respectively.

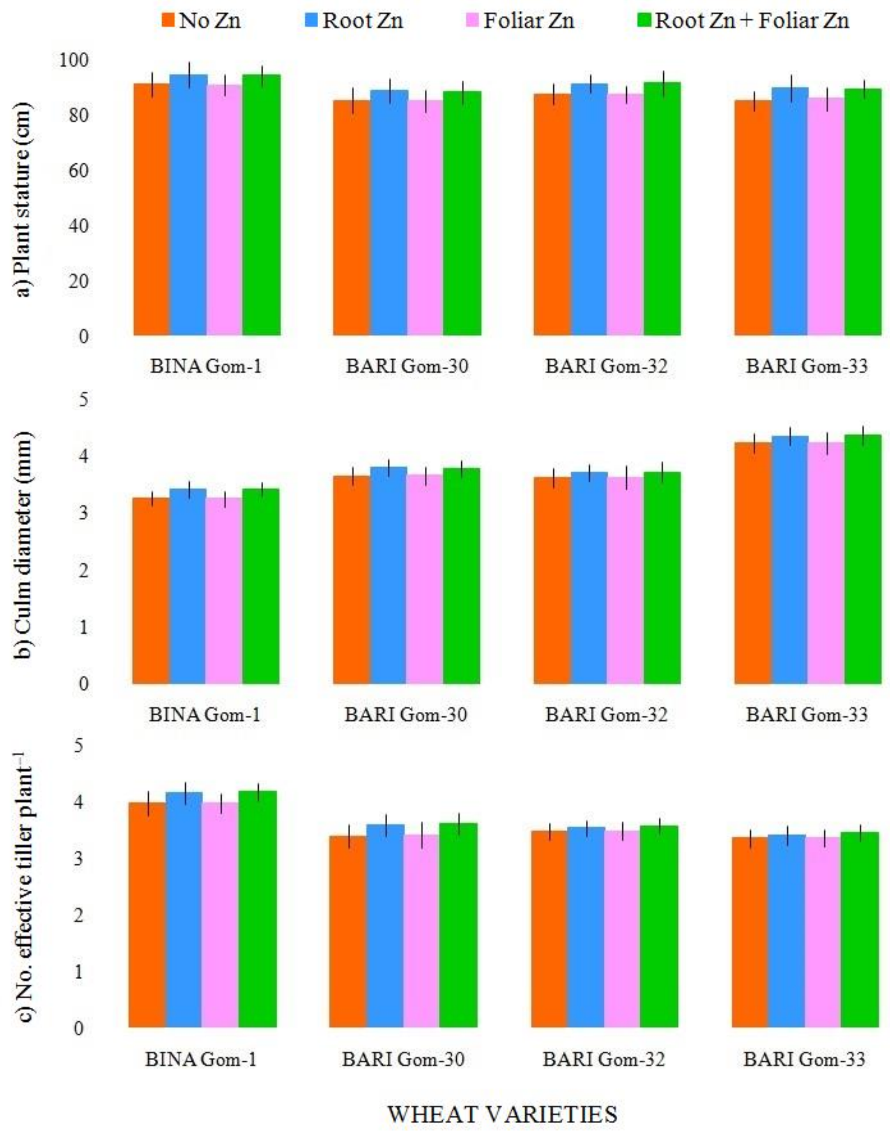

Figure 3 Plant height or stature (a), culm diameter (b) and number of effective spike per plant or hill (c) of four varieties of wheat crops as affected by root and foliar application of zinc. Vertical bar represents the standard deviation (SD, ±) of treatment mean (*n* = 3). The effect of Zn treatments is insignificant (at $P<0.05$) for any variety or trait.

Culm girth

The wheat plant grown with root (soil) Zn application produced thicker culm in all the varieties of wheat but the difference between the treatments was found statistically insignificant at $P<0.05$ (Figure 3b). Culm diameter in all four varieties of wheat crop grown with foliar Zn application showed similar cum girth than that grown with control treatment (nil Zn application). The wheat crop grown with the BARI Gom-33 variety produced thicker culm while thinner culm produced from the BINA Gom-1 variety while the remaining two varieties ranked intermediate ($P<0.01$; Table 2).

Number of effective tiller per plant or hill

Zinc application in root increased the number of effective tillers (those bear the spike with healthy grain) per plant in all the varieties of wheat but the difference between the Zn treatments was statistically insignificant at $P<0.05$ (Figure 3c). The number of effective tiller per plant or hill in all the varieties of wheat crop grown with foliar Zn application showed similar tiller number per plant than that grown with control treatment (i.e. nil Zn). The BINA Gom-1 variety produced more number of tillers in each hill and minimum tillers per plant was produced from BARI Gom-33 variety while the other wheat varieties ranked in middle (at $P<0.01$; Table 2).

Yield components

Number of effective spike per square meter

Zinc application in root increased the number of effective spikes (those bear healthy grain) per square meter in all four varieties of wheat but the difference between the treatments was statistically insignificant at $P<0.05$ (Figure 4a). The number of effective spike/m^2 in all varieties of wheat crop grown with foliar Zn application showed similar tiller number per plant than that grown with control treatment (i.e. zero Zn). The BARI Gom-32 variety produced more tiller per unit area of land followed by the BINA Gom-1 and minimum number of spikes/m^2 was produced from the BARI Gom-30 variety ($P<0.01$; Table 2).

Spike length

Zinc application in root faintly increased the spike length in all four varieties of wheat but the difference between the treatments was found statistically insignificant at $P<0.05$ (Figure 4b). Spike length in the said four varieties of wheat crop grown with foliar Zn application showed similar spike length than that grown with no zinc treatment. The BARI Gom-33 variety produced significantly (at $P<0.01$) longer spike followed by BARI Gom-30 while the BINA Gom-1 variety produced the shorter spike (Table 2).

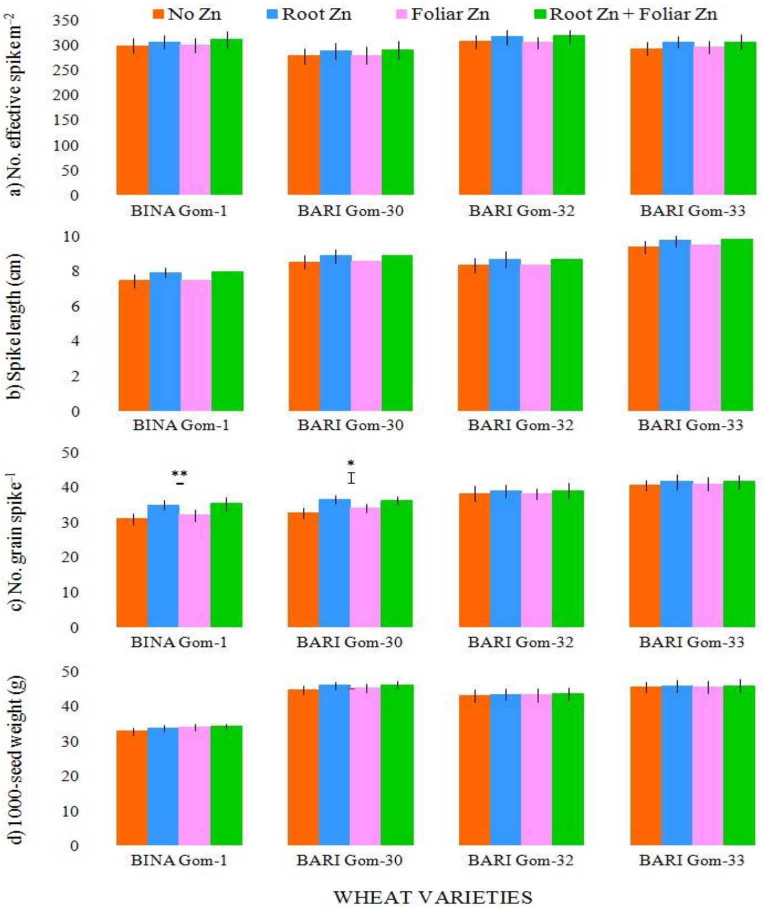

Figure 4 Number of effective spike per square meter (a), spike length (b), number of grain per spike (c) and thousand seed weight (d) of four varieties of wheat as affected by root and foliar application of zinc. Attached vertical bar (without cap) represents the standard deviation (SD, ±) of treatment mean (n = 3) whereas detached vertical bar (with cap) indicates the Least Significant Difference (LSD) at P<0.01 (**) or P<0.05 (*). The effect of Zn treatments for any variety or trait without asterisk is insignificant (at P<0.05).

Number of grain per spike

Zinc application in root marginally increased the number of grain per spike in the all four varieties of wheat but the difference between the treatments is statistically insignificant at P<0.05 (Figure 4c). The number of grain per spike in the said four varieties of wheat crop grown with foliar Zn application showed similar number of grain per spike as compared to that grown with no Zn application. The varietal effect of the number of grain per spike was found statistically significant at P<0.01 in the order of BARI Gom-33>BARI Gom-32 >BARI Gom-30>BINA Gom-1 (Table 2).

Thousand seed weight

All the zinc treatments or applications slightly increased the grain size in the said four varieties of wheat but the difference between the treatments was statistically insignificant at P<0.05 (Figure 4d). The varietal effect of the number of grain per spike was found statistically significant at P<0.01 in the order of BARI Gom-33>BARI Gom-32 >BARI Gom-30>BINA Gom-1 (Table 2).

Yield

Grain yield

Zinc application in root either alone or in combine with foliar spray increased the grain yield a bit in the said four varieties of wheat but the difference between the treatments was statistically insignificant at P<0.05 (Figure 5a). Pooled over the varieties, root plus foliar Zn, root Zn and foliar Zn applications increased the 9.5, 7.7 and 2.4 percent grain yield, respectively. The BARI Gom-33 variety respectively produced 7.8, 12.9 and 41.5 percent higher (significant at P<0.01) grain yield than that found from the crop grown with BARI Gom-32, BARI Gom-30 and BINA Gom-1 varieties (Table 2).

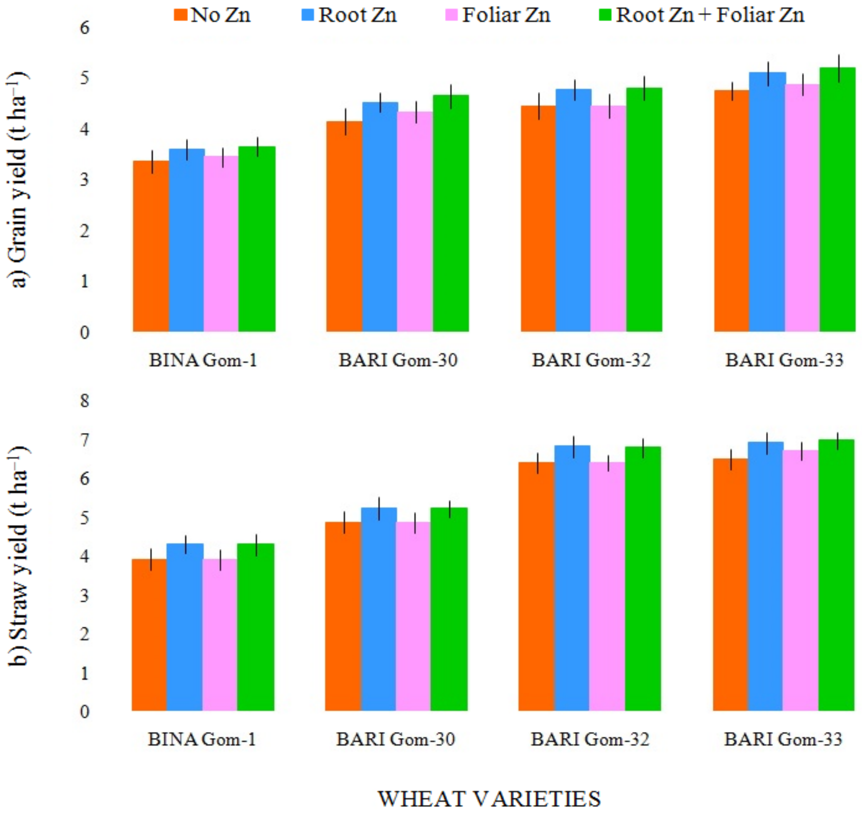

Figure 5 Grain yield (a) and straw yield (b) of four varieties of wheat as affected by root and foliar application of zinc. Vertical bar represents the standard deviation (SD, ±) of treatment mean ($n = 3$). The effect of Zn treatments is insignificant (at $P<0.05$) for any variety or trait (i.e. grain yield or straw yield).

Straw yield

Zinc application in root (soil) or Zn application in root plus foliage increased the straw yield in all the said varieties of wheat but the difference between the treatments was not statistically significant at $P<0.05$ (Figure 5b). Irrespective of the Zn treatments however, the varietal effect of straw yield was highly significant at $P<0.01$. The BARI Gom-33 variety produced 2.4, 34.0 and 64.6 percent higher straw yield than that obtained from the crop grown with BARI Gom-32, BARI Gom-30 and BINA Gom-1 varieties, respectively (Table 2).

Zn enrichment in wheat grain

The effect of Zn treatment on the zinc concentration in grain in the said four varieties of wheat is significant at $P<0.01$ (Figure 6a). However, higher enrichment of Zn in wheat grain was noticed for the foliar zinc application than that from the root (soil) application. Combine application of Zn in root and foliar didn't ensure higher Zn content in grain of BINA Gom-1 and BARI Gom-32 varieties where foliar zinc application increased higher Zn enrichment in wheat grain. But in BARI Gom-30 and BARI Gom-33 varieties, a combine application of Zn through root and foliage increased the higher zinc enrichment in wheat grain than that their individual applications. Irrespective of the Zn treatments, the grain from BARI Gom-33 variety enriched about 18, 18 and 23 percent higher zinc content than that from the BARI Gom-32, BARI Gom-30 and BINA Gom-1 varieties, respectively (at $P<0.05$) (Table 2).

In all four wheat varieties, crop grown with control treatment (i.e. no Zn application) attracted minimum amount of Zn in grain. Pooled over the varieties, root Zn + foliar Zn, foliar Zn and root Zn treated wheat crop's grain respectively attracted 28, 23 and 12 percent higher Zn accumulation as compared to no zinc application (at $P<0.01$) (Figure 6b).

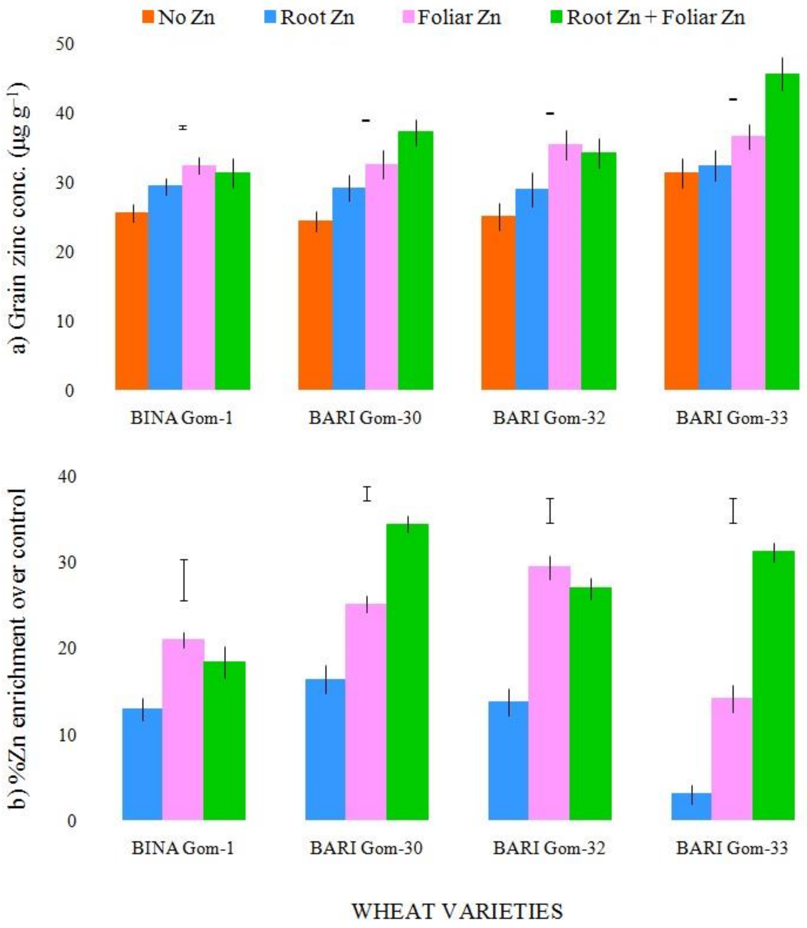

Figure 6 Accumulation of Zn concentration in grain (a) and percent Zn enrichment over no zinc application (i.e. control) of four varieties of wheat. Attached vertical bar (without cap) represents the standard deviation (SD, ±) of treatment mean (*n* = 3) whereas detached vertical bar (with cap) indicates the Least Significant Difference (LSD, at *P*<0.01) for a variety.

4. DISCUSSION

Zinc is one of the eight trace micronutrients or elements such as manganese, copper, boron, iron, zinc, chlorine, molybdenum and nickel, indispensable for sound vegetative and reproductive growth and development of plants. Although plants require Zn in smaller amounts than the macronutrients like nitrogen, phosphorus, potassium and sulfur but it is prerequisite for numerous key reactions in tissues or cells to synthesize hormones like indole acetic acid (IAA), enzyme and protein which are liable for operating many metabolic responses in plants (Escudero-Almanza et al., 2012; Hafeez et al., 2013; Castillo-González et al., 2018; Gomes et al., 2021). Zn also provides necessary support and stability to many enzymes by triggering them up. The trades brought about by Zn insufficiency on the proper growth and progress or development in plants are mostly multiplex (Hong et al., 2007). Alterations in plant metabolism pressed by Zn inadequacy consist of baneful consequences on the synthesis of carbohydrates, proteins, auxins and also impairment to membrane solidarity. As a part and parcel of proteins, Zn takes action as an architectural or structural, operational or functional and controlling or regulatory co-factor of a great number of enzymes.

Many Zn dependent enzymes are call for carbohydrate metabolism, especially in plant leaves (Xing et al., 2016). Zn governs plant growth and development through its secondary action on auxins (Castillo-González et al., 2018). Zn also plays immune or resistance role in plants against the activities of pest and diseases (Cabot et al., 2019). So, if a sufficient amount of Zn is not available, the plants would suffer from physiological sufferings due to the non-fulfillment of metabolic tasks. Thus, crop growth and development would stop if specific enzymes are not present in the plant tissues. Zinc is critically required for pollen function and fertilization processes and thus it is linked to seed formation as well (Pandey et al., 2006). Therefore, a constant and continuous flow of supply of Zn is required for optimum growth and development and maximum yield of crops. Phenotypically, Zn inadequacy or deficiency in plants main springs a type of leaf discoloration or disfigurement called greensickness or chlorosis, which undertakes the tissue between the veins to turn yellowish while the veins last greenish (Carroll, 2021). Chlorosis due to Zn paucity generally affects the plinth or base of the leaf connecting to the stem. Zinc shortfall has become more usual in seedling or young wheat plants growing in dry soil but this shortage is generally short-lived and vanished or disappeared i.e. resumed the original colour when the crop land is irrigated or rainfall re-wets the upper soil (Zinc Fact Sheet, 2013).

The preeminent characteristic symptoms or responses of wheat plants to Zn inadequacy are limiting in plant stature and size of leaves. These expressions are followed by the development of whitish-brown necrotic patches or spots on middle-aged leaves of wheat plants. As the intensity of Zn-deficiency extremes, the necrotic patches advanced on the leaves, and the middle parts of the leaves are often subsided and discoloured, exhibiting a "scorched" or drought-stress like appearance (Zinc Fact Sheet, 2013). Zn deficiency in wheat crop occurs in soils where Zn is inconvenient or unavailable to uptake by plants or in soils where available Zn to plant has dwindled and in soils generally inherently low content in Zn. Consequently, entire crop fields may be damaged or affected but, more generally, there are chlorotic patches within the crop (http://wheatdoctor.org/zinc-deficiency; accessed on 2 May 2022). In contrast, wheat crop is also susceptible to Zn toxicity when access amount of zinc is present in the soil. Zinc concentration is about 7ppm or more in soil and 60ppm or more in plant cells being reported as lethal for wheat plant (Takkar and Mann, 1978).

In the present experiment, neither any deficiency symptoms nor any toxic effects due to access Zn on the crop growth of wheat were observed. So, the experimental soil even the control treatment (i.e. the no or nil Zn) was not critically limited to Zn for the normal growth of wheat crop in one hand, and applied Zn treatments either through root or foliar application or in combine of these were not as much as responsible for toxic on the other. Hence the growth and yield traits and yield of experimental wheat crops were not significantly differed between the treatments of nil Zn and Zn applied either through soil mix (i.e. root) or foliar spray or combine of these two. Nevertheless, wheat crops may show significant decreases in growth and grain yield under Zn-deficient conditions in the field else (Graham et al., 1992; Ma et al., 2017; Sadeghi et al., 2021) where Zn can be fertilized to prevent the yield penalty.

Although the Zn application treatments did not significantly increase the crop growth and grain yield but it remarkably enhanced the Zn concentration in wheat seed. Lu et al., (2012), Wang et al., (2020), Lan et al., (2021) and other workers also found the significant increase of Zn concentration in wheat grain through agronomic approaches of Zn use or zinc fertilization treatments. Zn insufficiency below critical threshold in soils was reported to bring down the Zn content in the edible slabs of foremost food crops (Welch and Graham, 2004). Although the present experimental soil was not critically limited to Zn content to the normal growth and development of wheat crop but supplemental use of Zn positively enhanced the Zn concentration in wheat grain. So it can be said that the soil was secretly (hidden) hunger to Zn to zinc enrichment target in wheat grain. In order to bifortification of Zn in wheat grain, therefore, zinc fertilization programme can be used whatever the soil is Zn-deficient or remained optimum Zn for crop growth. In the sites where soil is acutely Zn-deficient, the zinc fertilization programmes could not only increase the grain yield remarkably and but also enhance the Zn content in the seeds (Zou et al., 2012).

The various methods of Zn application may differentially influence the grain yield and grain Zn enrichment in wheat crop. In the present result, the application of Zn in root (soil) exhibited less effective in increasing grain Zn, while foliar Zn application or combine of root and foliar applications of Zn result in remarkable increases in grain Zn enrichment and the results are supported by Yilmaz et al. (1997) and Cakmak et al. (2010a, b). By revitalizing the timing and the Zn concentration during foliar application, wheat grain Zn concentration could further be increased in whole grain and endosperm as well (Cakmak et al., 2010b; Zhang et al., 2010). Foliarly-applied Zn in the time of grain development stage is also reported to contribute to increase seed Zn content (Zhang et al., 2010) as aerial (foliar) spraying of Zn is absorbed by the leaf epidermis and then transported to other plant parts via veins and vein-lets i.e. vascular systems (Haslett et al., 2001).

Biofortification through genetic engineering that lead to develop mineral-dense crop variety offers a worthwhile solution to malnutrition problems by scrutinizing natural genomic variation (Pfeiffer and McClafferty, 2007). It engages traditional or classical breeding techniques to characterize and exploit genetic diversity for mineral content like Zn, as well as new perspectives involving gene devising and marker fostered breeding (Grusak, 2002). Therefore, it needs a long-lasting time to desire the target to find a befitting cultivar. So, agronomic means of biofortification through root and foliar applications of Zn as like as the present experiment could complement the existing breeding approach towards a short-term solution to the problem (Cakmak, 2008; Velu et al., 2014).

Increasing Zn content in cereal seeds for shrinking Zn malnutrition in two billion people stands for a supreme global humanitarian challenge. Seed Zn concentration in the field-grown wheat crop at the universal scale ranges from 20.4 to 30.5 $\mu g\ g^{-1}$, exhibiting a clear and concrete gap to the biofortification target (40 $\mu g\ g^{-1}$) for human health (Chen et al., 2017). From a 320-pair plot field trial of wheat, Chen et al. (2017) found a mean zinc increase of 10.5 $\mu g\ g^{-1}$ in grain through foliar zinc fertilization process. Hussain et al. (2012) found from a nutrient management study that root zinc fertilization increased the Zn bioavailability to humans. Increasing grain Zn by root and/or foliar applications of zinc nutrient also provides additional positive effects in terms of seed vitality and seedling vigour which may come up with the beneficial influences on seed germination, seedling emergence, plant growth and grain yield of the subsequent wheat crop (Linde, 2018; Shandu, 2021).

Nutriment and pesticide overseeing can provide a sudden and effective option for increasing grain Zn concentration and productivity of wheat crop, particularly in soils with severe Zn deficiency prevails. Nutrient applications can also be combined with other agrochemicals, for example, using Zn-containing N fertilizers for root application. The foliar Zn application will also become feasible while combining with herbicides, insecticides and fungicides, to reduce the time and cost of money. Raising awareness in the midst of meager-resourced farmers may additionally ramp up to take on the worthwhile approaches of Zn application.

5. CONCLUDING REMARKS

Growth and most of the yield traits and grain and straw yield of wheat crop increased a little (insignificant at $P<0.05$) when zinc applied at root and combine Zn application in root plus foliar but there was no effect on these traits when Zn was foliarly applied. Irrespective of the Zn treatments, the grain from BARI Gom-33 variety enriched about 18, 18 and 23 percent higher zinc content than that from the BARI Gom-32, BARI Gom-30 and BINA Gom-1 varieties, respectively. However, root Zn plus foliar Zn, foliar Zn and root Zn treated wheat crops respectively contributed 28, 23 and 12 percent higher Zn accumulation in wheat grain as compared to no Zn treatment. Root Zn application contributes to plant growth and yield while foliar Zn application contributes to accumulate zinc content in grain. Therefore, combine Zn application like Zn apply in root (soil) and in foliage can be recommended for higher grain yield along with higher zinc content in grain of wheat crop.

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Ethical approval

Not applicable.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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