

# Agronomic zinc biofortification of wheat to improve accumulation, bioavailability, productivity and use efficiency

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## Abstract

Zinc (Zn) deficiency causes low crop production and malnutrition in human. Agronomic biofortification of food crops can resolve the issues of global food security and human nutrition on sustainable basis. Field experiments were conducted to improve Zn bioavailability, growth and yield of wheat in response to varying Zn application rates for two consecutive years (2016-17 & 2017-18). Significant increase in grain yield was recorded with the application of Zn. Highest grain yield (5.41 t ha<sup>-1</sup>) was recorded with the application of 5.00 kg Zn ha<sup>-1</sup>. Human available Zn fraction was also improved in response to Zn application. Zn application resulted in lowering phytate/Zn molar ration in wheat grains. Higher Zn accumulation (338.72 g ha<sup>-1</sup>) was observed by applying 7.5 kg Zn ha<sup>-1</sup>. Zinc application was found critical to meet internal (36.53 μg g<sup>-1</sup>) and external (4.48 kg Zn ha<sup>-1</sup>) Zn requirements to achieve near maximum yield of wheat. The results reinforced the concept of Zn fertilization to achieve better productivity and quality.

## Article Info

Received : 18.07.2019

Accepted : 11.11.2019

**Keywords:** Biofortification, cereals, food security, nutrition.

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## Introduction

Wheat is important crop for our food security and human nutrition. It is staple food for 35% of world population (Poursarebani et al., 2014). It is inherently low in Zn and consuming large proportion of wheat based food products can lead to the Zn deficiency in human (Bouis and Welch, 2010). Its deficiency can affect human health by impairing growth, immune functions, mental health and sexual maturation (Andreini et al., 2006; Kręzel and Maret, 2016).

In plants Zn deficiency causes disruption in enzymatic functions, protein synthesis and hampers plant growth (Akram et al., 2017). Moreover, Crops cultivated on the Zn deficient medium is more prone to biotic and abiotic stresses (Cakmak and Kutman, 2018). Breeding wheat varieties with high Zn accumulation potential can resolve the prevalent Zn deficiencies in human and crop plants on sustainable basis (Bouis and Saltzman, 2017). However, this is a long and tedious process. Therefore, scientists are more inclined to agronomic Zn biofortification of food crops to enhance Zn bioavailability to human and sustaining crop yield because this type of biofortification have more adaptability and access to rural population (Cakmak, 2008).

To achieve the goal of biofortification plant should be able to translocate Zn from vegetative parts to grains (Gibson, 2012; Gupta et al., 2016). Increasing Zn accumulation in edible parts of the crop is not the only challenge for biofortification efforts but the availability of bio-accumulated Zn to the consumer is another challenge. Cereals constitute major fraction of daily human diet, yet they do not provide sufficient concentrations of essential metals to fulfill their daily body requirements due to high phytic acid contents (Juliano, 1993). Phytic acid is indigestible inositol phosphorus stored in grains (Raboy, 2000). It has net negative charge and chelates positively charged mineral ions and render them insoluble in human digestive system due to absence of phytase enzymes (Marounek et al., 2010). Nonetheless, protein absorption in

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e-ISSN: 2147-4249

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DOI: [10.18393/ejss.647585](https://doi.org/10.18393/ejss.647585)

human stomach and small intestine is also affected by phytate. Therefore, development of high yielding food crops with greater mineral bioavailability have become prime research goal of scientific community to achieve global food security in both quantitative and qualitative terms (Bouis and Saltzman, 2017).

Crop genotypes with better yield and high micronutrient concentrations in grains are suitable to address food security and human micronutrient deficiencies (Cakmak and Kutman, 2018). However, high grain yield and high nutrient concentrations are usually achieved by consuming costly inputs at high doses, which often affects nutrient use efficiency (Farooq et al., 2018). Therefore, determination of Critical internal and external Zn requirements of crops is important to sustain their yield and quality of crop (Kanwal et al., 2009). The critical internal Zn concentration delineates the quantity or intensity fraction of nutrient in the plant, associated with the near to maximum yield of the crop. Whereas, the intensity/quantity of the nutrient in the soil equilibrated with the soil solution concentration, associated with the near maximum crop yield is called as external requirement (Sarfrasz et al., 2008). The present study was conducted to evaluate wheat grain yield, Zn concentration, accumulation, bio-availability, Zn use efficiency and critical internal and external Zn requirement in response to Zn application.

## Material and Methods

A field experiment was conducted at Research Farms of Nuclear Institute of Agriculture (NIA), Tando Jam, Sindh-Pakistan for the two consecutive years 2016-17 and 2017-18. The crop was sown on fixed layout in the months of November and was harvested in the end of April in during both growing seasons. The site was located at latitude of 25°41'30.22" North and longitude of 68°51'78.09" East. The experimental site has arid climate (Figure 1) and average high and low temperatures in the growing seasons were 26.5.1°C and 14.7°C, respectively (NAMC, 2018). Before sowing soil samples were collected from the field at depth of 0-15cm and 15-30cm and were analyzed for various physio-chemical properties of soil (Table 1). Five treatments of Zn (0, 1.25, 2.5, 5.0 and 7.5 kg Zn ha<sup>-1</sup> from ZnSO<sub>4</sub>.H<sub>2</sub>O) were randomly allotted to fifteen experimental units each of dimension 4m x 4m so that each treatment was repeated thrice. Wheat genotype "SD-1013" was sown by using manual hand drill and row to row distance of 30 cm was maintained.

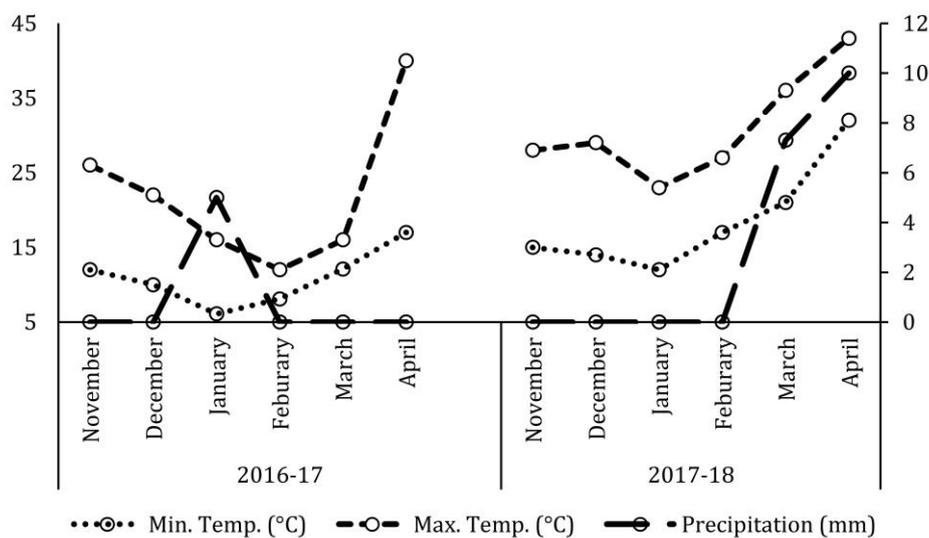


Figure 1. Agro-meteorological conditions during Rabi 2016-17 and 2017-18

Table 1. Physico-chemical properties of soil at experimental site

| Soil properties                | Unit                | Value     |           | Reference/method              |
|--------------------------------|---------------------|-----------|-----------|-------------------------------|
|                                |                     | 2016-17   | 2017-18   |                               |
| Textural class                 | --                  | Silt loam | Silt loam | Bouyoucos (1962)              |
| EC <sub>(1:2.5)</sub>          | dS m <sup>-1</sup>  | 0.32      | 0.35      | Anderson and Ingram (1993)    |
| pH <sub>(1:2.5)</sub>          | --                  | 8.1       | 8.0       | Anderson and Ingram (1993)    |
| Organic matter                 | %                   | 0.53      | 0.59      | Nelson and Sommers (1982)     |
| Kjeldhal nitrogen              | %                   | 0.03      | 0.03      | Jackson (1962)                |
| AB-DTPA extractable phosphorus | mg kg <sup>-1</sup> | 5.86      | 6.01      | Soltanpour and Workman (1979) |
| AB-DTPA extractable zinc       | mg kg <sup>-1</sup> | 0.51      | 0.57      | Soltanpour and Workman (1979) |
| AB-DTPA extractable potassium  | mg kg <sup>-1</sup> | 160       | 150       | Soltanpour and Workman (1979) |

AB-DTPA = Ammonium bicarbonate-diethylene triamine penta acetic acid

Di-Ammonium Phosphate (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and Sulfate of Potash (60 kg K<sub>2</sub>O ha<sup>-1</sup>) were applied at the time of sowing to supplement Phosphorus (P) and Potassium (K) to the crop. Whereas, Nitrogen (150 kg N ha<sup>-1</sup>) was supplemented in the form of urea in three equal splits *viz.*, at the time of sowing, tillering, and panicle initiation. Zn was applied at tillering stage alongwith nitrogen. Four irrigations of canal water were applied to the crop at critical growth stages. Chemical and manual eradication of weeds was performed as per requirement. At maturity attributes related to plant growth, yield and Zn concentrations and Zn accumulation were recorded.

Grain and straw samples were collected from each treatment replication and were dried at 70°C in oven. Dried plant samples were then grinded and digested in 5:1 di-acid mixture of HNO<sub>3</sub> and HClO<sub>4</sub> (Rashid, 1986). Digested samples were analyzed for Zn concentration using Atomic Absorption Spectrophotometer (Analytik Jena Nova 400, Germany). Zinc uptake and use efficiency related attributes were calculated using following equations (Farooq et al., 2018).

$$\begin{aligned} \text{Zinc accumulation (g ha}^{-1}\text{)} &= \text{Zn concentration} \times \text{yield} \\ \text{Agronomic efficiency (kg kg}^{-1}\text{)} &= \left( \frac{\text{GY}_f - \text{GY}_c}{\text{N}_{\text{ap}}} \right) \\ \text{Physiological efficiency (kg g}^{-1}\text{)} &= \left( \frac{\text{BY}_f - \text{BY}_c}{\text{TAC}_f - \text{TAC}_c} \right) \\ \text{Apparent recovery efficiency (\%)} &= \left( \frac{\text{TAC}_f - \text{TAC}_c}{\text{N}_{\text{ap}}} \right) \times 100 \end{aligned}$$

Where:

- GY<sub>f</sub> = Grain yield of fertilized plots
- GY<sub>c</sub> = Grain yield of control (unfertilized) plots
- N<sub>ap</sub> = Nutrient applied
- BY<sub>f</sub> = Biomass yielded from fertilized plots
- BY<sub>c</sub> = Biomass yielded from control
- TAC<sub>f</sub> = Total Zn accumulation in fertilized plots
- TAC<sub>c</sub> = Total Zn accumulation in control

To assess the Zn complexation by phytate (PA) the total inositol Phosphorus/phytate was determined according to the method explained by Haug and Lantzsch (1983). Briefly, wheat grains were grinded to flour and extracted with dilute HCl. 0.5 mL extract was mixed with 0.4mM Iron (III) sulfate (dissolved in 0.2N HCl) solution in a capped glass tube. Afterwards, tubes were first heated for 30 minutes in a boiling water bath then cooled for 15 minutes in ice water. Samples were allowed to rest to attain room temperature. Then 2mL of 2, 2'-bi-pyridine solution (prepared by dissolving 5g of 2,2'-bi-pyridine of in 500mL of 1% (v/v) thioglycollic acid solution) were added to the mixture. Absorbance of pink Colour produced by the unreacted Fe(III) was measured at 519 nm using UV-Visible Spectrophotometer (U-2900, Hitachi, Japan). Phytate concentration was calculated using standards calibration curve established by running standards prepared from sodium phytate.

To calculate [phytate]: [Zn] in wheat grains their molar concentrations were used. Tivariate model of Zn absorption derived by Miller et al., (2007) was used to estimate the bioavailable Zn. Given below is the model based on Zn homeostasis in human digestive system. Total available Zn was calculated on reference adults consuming 300g wheat flour per day as the only daily Zn source (Rosado et al., 2009).

$$\text{TAZ} = 0.5 \cdot \left( A_{\text{max}} + \text{TDZ} + K_R \times \left( 1 + \frac{\text{TDP}}{K_P} \right) \right) - \sqrt{\left( A_{\text{max}} + \text{TDZ} + K_R \times \left( 1 + \frac{\text{TDP}}{K_P} \right) \right)^2 - 4 \times A_{\text{max}} \times \text{TDZ}}$$

Where:

- TAZ = Total daily absorbed Zn (mg Zn/day)
- A<sub>max</sub> = maximum Zn absorption
- TDZ = Total daily dietary Zn (mmol Zn/day)
- K<sub>R</sub> = Equilibrium dissociation constant of the Zn-receptor binding reaction
- TDP = Total daily dietary PA (mmol PA/day)
- K<sub>P</sub> = Equilibrium dissociation constant of the Zn-PA binding reaction

For Zn homeostasis in human intestine 0.091, 0.680 and 0.033 are the constant values for  $A_{max}$ ,  $K_p$  and  $K_R$  (Hambidge et al., 2010).

The Zn requirement of wheat crop was determined on the basis of near to maximum crop yield (95% of the maximum attainable crop yield) following Kanwal et al. (2009). The data were analyzed using Microsoft Excel 2010® (Microsoft Cooperation, USA) and Statistix 8.1® (Analytical Software, Tallahassee, USA). Significantly different means were separated using Tukey's HSD test (Steel et al., 1997).

## Results

Plant growth attributes viz., plant height, number of tillers and spike length are presented in Table 2. Plant height and number of tillers per plant increased significantly with the application of Zn. The increments recorded in the parameters with Zn, applied at the rate of 5 kg ha<sup>-1</sup> were statistically higher than all the treatments with exception to the increments recorded with application of 7.5 kg Zn ha<sup>-1</sup>. Highest plant height of 93.33 cm was observed where 5 kg Zn ha<sup>-1</sup> was applied. Minimum height of 87.42 cm was recorded in the control. Whereas, highest number of tillers (5.87) were recorded in response to 5 kg Zn ha<sup>-1</sup> which were statistically at par with the number of tiller recorded in the plants, applied with 7.5 kg Zn ha<sup>-1</sup>. In case of spike length, Zn applications affected the parameter positively but the increases in the spike length were recorded statistically similar to each other at all Zn application rates. Maximum spike length (11.43 cm) was recorded with the application of 2.5 kg Zn ha<sup>-1</sup>. Whereas, minimum (10.83 cm) was recorded in control.

Table 2. Growth attributes of wheat as influenced by Zn application rates

| Treatments,<br>kg Zn ha <sup>-1</sup> | Plant height, cm |           |         | Number of tillers, per plant |          |         | Spike length, cm |         |         |
|---------------------------------------|------------------|-----------|---------|------------------------------|----------|---------|------------------|---------|---------|
|                                       | 2016-17          | 2017-18   | Mean    | 2016-17                      | 2017-18  | Mean    | 2016-17          | 2017-18 | Mean    |
| 0.00                                  | 87.47 e          | 87.37 e   | 87.42 C | 4.40 f                       | 4.47 ef  | 4.43 D  | 11.20            | 10.47   | 10.83 A |
| 1.25                                  | 90.13 d          | 91.80 bc  | 90.97 B | 4.53 d-f                     | 5.33 b-d | 4.93 C  | 11.57            | 10.87   | 11.22 A |
| 2.50                                  | 91.47 cd         | 89.97 d   | 90.72 B | 4.87 c-f                     | 5.27 b-e | 5.07 BC | 11.93            | 10.93   | 11.43 A |
| 5.00                                  | 93.20 ab         | 93.47 a   | 93.33 A | 5.53 a-c                     | 6.20 a   | 5.87 A  | 11.43            | 11.20   | 11.32 A |
| 7.50                                  | 93.07 ab         | 92.10 a-c | 92.58 A | 5.87 ab                      | 5.20 b-f | 5.53 AB | 11.33            | 11.37   | 11.35 A |
| Mean                                  | 91.07 A          | 90.94 A   |         | 5.04 B                       | 5.29 A   |         | 11.49 A          | 10.97 B |         |
| Tuckey's HSD (0.05)                   |                  |           |         |                              |          |         |                  |         |         |
| Zn                                    | 0.929            |           |         | 0.479                        |          |         | 1.038            |         |         |
| Yr                                    | 0.408            |           |         | 0.211                        |          |         | 0.456            |         |         |
| Zn × Yr                               | 1.557            |           |         | 0.803                        |          |         | 1.739            |         |         |

Means sharing similar letters in a column are statistically similar to each other at  $p \leq 0.05$ . Data is average of 3 replicates.

The grain yield of wheat responded appreciably to the application of Zinc. Significant increase in the yield was observed with the increasing Zn application rates (Table 3). Average grain yields during 2016-17 and 2017-18 were 5.85 and 4.09 t ha<sup>-1</sup>. The yields obtained with the five Zn rates (0, 1.25, 2.5, 5.0 & 7.5 kg Zn ha<sup>-1</sup>) were 4.12, 5.02, 4.97, 5.41 and 5.34 t ha<sup>-1</sup>, respectively. Maximum increase in grain yield (31.31%) over the control was recorded with the application of 5 kg Zn ha<sup>-1</sup> but this increase was statistically similar with the grain yield (5.34 t ha<sup>-1</sup>) attained with the application of 7.5 kg Zn ha<sup>-1</sup>. Similarly, straw yield was also affected by the application of Zn. Highest mean straw yield of both years (5.95 t ha<sup>-1</sup>) was observed with the application of 7.5 kg Zn ha<sup>-1</sup>. Whereas, lowest (4.58 t ha<sup>-1</sup>) was in the control. Straw yield increased by 28.82% over the control with the application of 5 kg Zn ha<sup>-1</sup>. Likewise, total biomass of yielded with the application of Zn was appreciably higher than of control. Highest biomass (11.35 t ha<sup>-1</sup>) was yielded with the application of 5 kg Zn ha<sup>-1</sup> which was statistically comparable with the biomass yielded from experimental units applied with of 1.25, 2.50 and 7.5 kg Zn ha<sup>-1</sup> (Table 3).

Table 3. Yield attributes of wheat as influenced by Zn application rates

| Treatments<br>kg Zn ha <sup>-1</sup> | Grain yield, t/ha |         |        | Straw yield, t/ha |          |        | Total biomass yield, t/ha |         |         |
|--------------------------------------|-------------------|---------|--------|-------------------|----------|--------|---------------------------|---------|---------|
|                                      | 2016-17           | 2017-18 | Mean   | 2016-17           | 2017-18  | Mean   | 2016-17                   | 2017-18 | Mean    |
| 0.00                                 | 5.21 b            | 3.03 e  | 4.12 C | 5.94 a-c          | 3.22 d   | 4.58 B | 11.15 b                   | 6.25 d  | 8.70 B  |
| 1.25                                 | 5.79 a            | 4.24 cd | 5.02 B | 6.08 a-c          | 5.45 bc  | 5.77 A | 11.88 ab                  | 9.69 c  | 10.78 A |
| 2.50                                 | 5.88 a            | 4.06 d  | 4.97 B | 6.52 a            | 5.73 a-c | 6.13 A | 12.40 a                   | 9.79 c  | 11.09 A |
| 5.00                                 | 6.21 a            | 4.61 c  | 5.41 A | 6.31 ab           | 5.49 bc  | 5.90 A | 12.60 a                   | 10.10 c | 11.35 A |
| 7.50                                 | 6.19 a            | 4.49 cd | 5.34 A | 6.50 a            | 5.41 c   | 5.95 A | 12.60 a                   | 9.90 c  | 11.25 A |
| Mean                                 | 5.85 A            | 4.09 B  |        | 6.27 A            | 5.06 B   |        | 12.13 A                   | 9.15 B  |         |
| Tuckey's HSD (0.05)                  |                   |         |        |                   |          |        |                           |         |         |
| Zn                                   | 0.259             |         |        | 0.522             |          |        | 0.604                     |         |         |
| Yr                                   | 0.114             |         |        | 0.229             |          |        | 0.266                     |         |         |
| Zn × Yr                              | 0.434             |         |        | 0.875             |          |        | 1.013                     |         |         |

Means sharing similar letters in a column are statistically similar to each other at  $p \leq 0.05$ . Data is average of 3 replicates.

The Zn concentrations in grains and straw increased significantly with different application rates of Zn fertilizer (Table 4). Highest concentrations were recorded in grain ( $42.12 \mu\text{g g}^{-1}$ ) with the application of  $7.50 \text{ kg Zn ha}^{-1}$ . Minimum concentration of  $8.57 \mu\text{g g}^{-1}$  was depicted by the control. Highest input rate of Zn fertilizer depicted 4.91 times higher grain Zn concentration than of control. Straw Zn concentration was also increased in response to the applied Zn. Highest straw Zn  $19.54 \mu\text{g g}^{-1}$  was recorded with the application of  $5.00 \text{ kg Zn ha}^{-1}$ . However, this was statistically similar to the straw Zn concentration ( $19.43 \mu\text{g g}^{-1}$ ) found, with the application of  $7.50 \text{ kg Zn ha}^{-1}$ . The minimum straw Zn was ( $4.89 \mu\text{g g}^{-1}$ ) found in the plants applied with no Zn fertilizer.

Table 4. Zinc concentrations [Zn] in wheat as influenced by Zn application rates

| Treatments,<br>kg Zn ha <sup>-1</sup> | [Zn] in grains, $\mu\text{g/g}$ |          |         | [Zn] in straw, $\mu\text{g/g}$ |          |         |
|---------------------------------------|---------------------------------|----------|---------|--------------------------------|----------|---------|
|                                       | 2016-17                         | 2017-18  | Mean    | 2016-17                        | 2017-18  | Mean    |
| 0.00                                  | 9.81 f                          | 7.33 g   | 8.57 D  | 5.01 f                         | 4.77 f   | 4.89 D  |
| 1.25                                  | 36.51 d                         | 32.08 e  | 34.30 C | 11.18 e                        | 20.67 c  | 15.92 C |
| 2.50                                  | 37.66 d                         | 38.06 cd | 37.86 B | 11.40 e                        | 24.00 a  | 17.70 B |
| 5.00                                  | 39.84 bc                        | 37.06 d  | 38.45 B | 16.69 d                        | 22.39 b  | 19.54 A |
| 7.50                                  | 41.19 ab                        | 43.06 a  | 42.12 A | 17.59 d                        | 21.28 bc | 19.43 A |
| Mean                                  | 33.00 A                         | 31.52 B  |         | 12.37 B                        | 18.62 A  |         |
| Tuckey's HSD (0.05)                   |                                 |          |         |                                |          |         |
| Zn                                    |                                 | 1.162    |         |                                | 0.739    |         |
| Yr                                    |                                 | 0.511    |         |                                | 0.325    |         |
| Zn × Yr                               |                                 | 1.947    |         |                                | 1.239    |         |

Means sharing similar letters in a column are statistically similar to each other at  $p \leq 0.05$ . Data is average of 3 replicates.

Zn quantity factor (accumulation) was also calculated (Table 5). The results showed that highest Zn accumulation in grains ( $224.08 \text{ g ha}^{-1}$ ) was with the application of  $7.50 \text{ kg Zn ha}^{-1}$ . Application of  $5.00 \text{ kg Zn ha}^{-1}$  resulted in second highest Zn accumulation in grain ( $209.11 \text{ g ha}^{-1}$ ). Lowest grain Zn accumulation of  $36.64 \text{ g ha}^{-1}$  was recorded in control plots. Accumulation of Zn in straw was also highest ( $114.64 \text{ g ha}^{-1}$ ) with the application of  $7.50 \text{ kg Zn ha}^{-1}$  but statistically similar to  $114.09 \text{ g Zn ha}^{-1}$  accumulated in straw of wheat plants treated with  $5.00 \text{ kg Zn ha}^{-1}$ . Control showed lowest straw Zn accumulation of  $22.31 \text{ g ha}^{-1}$  among all the treatments. Total Zn accumulated in wheat plant was recorded as highest ( $338.72 \text{ g ha}^{-1}$ ) with the application of  $7.50 \text{ kg Zn ha}^{-1}$  while lowest ( $58.95 \text{ g ha}^{-1}$ ) was recorded in control. Overall, Zn application increased Zn accumulation in wheat plant.

Table 5. Zinc accumulation in wheat as influenced by Zn application rates

| Treatments,<br>kg Zn ha <sup>-1</sup> | Zn accumulation in grains, g/ha |          |          | Zn accumulation in straw, g/ha |          |          | Total Zn accumulation, g/ha |          |         |
|---------------------------------------|---------------------------------|----------|----------|--------------------------------|----------|----------|-----------------------------|----------|---------|
|                                       | 2016-17                         | 2017-18  | Mean     | 2016-17                        | 2017-18  | Mean     | 2016-17                     | 2017-18  | Mean    |
| 0.00                                  | 51.06 i                         | 22.23 j  | 36.64 E  | 29.24 h                        | 15.38 i  | 22.31 D  | 80.30 h                     | 37.61 i  | 58.95 E |
| 1.25                                  | 211.10d                         | 136.02h  | 173.56D  | 68.05 g                        | 112.62d  | 90.33 C  | 279.15f                     | 248.31 g | 263.73D |
| 2.50                                  | 220.89 c                        | 154.61 g | 187.75 C | 74.36 f                        | 137.66 a | 106.01B  | 295.25d                     | 292.27 e | 293.76C |
| 5.00                                  | 247.29 b                        | 170.94 f | 209.11 B | 105.31 e                       | 122.87 b | 114.09 A | 352.60 b                    | 293.81de | 323.20B |
| 7.50                                  | 254.85 a                        | 193.31 e | 224.08 A | 114.20cd                       | 115.08 c | 114.64 A | 369.05 a                    | 308.39 c | 338.72A |
| Mean                                  | 197.04 A                        | 135.42 B |          | 78.23 B                        | 100.72 A |          | 275.27 A                    | 236.08 B |         |
| Tuckey's HSD (0.05)                   |                                 |          |          |                                |          |          |                             |          |         |
| Zn                                    |                                 | 1.153    |          |                                | 1.165    |          |                             | 1.475    |         |
| Yr                                    |                                 | 0.507    |          |                                | 0.512    |          |                             | 0.649    |         |
| Zn × Yr                               |                                 | 1.933    |          |                                | 1.953    |          |                             | 2.473    |         |

Means sharing similar letters in a column are statistically similar to each other at  $p \leq 0.05$ . Data is average of 3 replicates.

The better quality of produce is as important as the quantity factor in order to ensure the availability of nutritious food to the masses. Therefore, the quality traits of the wheat genotype were also recorded (Table 6). The Zn fertilization had minimum effect on the grain PA concentration. Highest grain PA concentration ( $7.39 \text{ mg g}^{-1}$ ) was recorded where  $1.25 \text{ kg Zn ha}^{-1}$  was applied but it was statistically similar to the grain PA concentrations ( $7.454$  and  $7.06 \text{ mg g}^{-1}$ ) recorded where  $2.50$  and  $5.00 \text{ kg Zn ha}^{-1}$  were applied, respectively. Lowest grain PA concentration ( $6.83 \text{ mg g}^{-1}$ ) was observed in control where no Zn was applied and it was comparable with the concentrations ( $7.06$  and  $7.00 \text{ mg g}^{-1}$ ) observed in response to the application of  $5.00$  and  $7.50 \text{ kg Zn ha}^{-1}$ .

Higher levels of PA in wheat flour can affect Zn bioavailability of Zn to human. Therefore, PA/Zn ratios were also calculated (Table 6) on the molar concentration basis at each Zn input level. It was observed that the PA/Zn ratio decreased with increasing Zn fertilizer. Higher the input, greater was the grain Zn uptake therefore, lower the PA/Zn ratio was. Lowest PA/Zn ratio (16.51) was recorded where 7.50 kg Zn ha<sup>-1</sup> was applied. Application of 5 kg Zn ha<sup>-1</sup> resulted in second lowest PA/Zn ratio (18.23) which was statistically similar to PA/Zn ratio (18.82) recorded in the grain supplied with 2.50 kg Zn ha<sup>-1</sup>. Although, control had lowest PA concentration yet it depicted highest PA/Zn ratio of 81.96.

Table 6. Grain phytate, PA/Zn ratio and estimated Zn bioavailability from wheat as influenced by Zn application rates

| Treatments,<br>kg Zn ha <sup>-1</sup> | Grain phytate, mg/g |          |          | Phytate-Zn ratio |         |         | Estimated Zn bioavailability, mg/300g/day |         |        |
|---------------------------------------|---------------------|----------|----------|------------------|---------|---------|---|---------|--------|
|                                       | 2016-17             | 2017-18  | Mean     | 2016-17          | 2017-18 | Mean    | 2016-17                                   | 2017-18 | Mean   |
| 0.00                                  | 7.14 bc             | 6.53 d   | 6.83 C   | 75.55 b          | 88.37 a | 81.96 A | 0.33 e                                    | 0.27 e  | 0.30 D |
| 1.25                                  | 7.85 a              | 6.92 b-d | 7.39 A   | 21.38 c          | 21.41 c | 21.39 B | 0.97 d                                    | 0.98 d  | 0.97 C |
| 2.50                                  | 7.45 ab             | 6.90 b-d | 7.18 AB  | 19.64 d          | 18.00 e | 18.82 C | 1.04 cd                                   | 1.14 bc | 1.09 B |
| 5.00                                  | 7.31 a-c            | 6.81 cd  | 7.06 A-C | 18.23 e          | 18.23 e | 18.23 C | 1.11 bc                                   | 1.13 bc | 1.12 B |
| 7.50                                  | 7.17 bc             | 6.83 cd  | 7.00 BC  | 17.26 e          | 15.75 f | 16.51 D | 1.17 b                                    | 1.27 a  | 1.22 A |
| Mean                                  | 7.384 A             | 6.799 B  |          | 30.41 B          | 32.35 A |         | 0.924 B                                   | 0.957 A |        |
| Tuckey's HSD (0.05)                   |                     |          |          |                  |         |         |   |         |        |
| Zn                                    |                     | 0.333    |          |                  | 0.748   |         |   | 0.057   |        |
| Yr                                    |                     | 0.146    |          |                  | 0.329   |         |   | 0.025   |        |
| Zn × Yr                               |                     | 0.557    |          |                  | 1.254   |         |   | 0.096   |        |

Means sharing similar letters in a column are statistically similar to each other at  $p \leq 0.05$ . Data is average of 3 replicates.

Bioavailability of Zn to human on the basis of trivariate mathematical was also calculated (Table 6). The current study showed that application of had significant role in improving Zn bioavailability to human (Table 6). Highest bioavailable Zn (1.22 mg/300g/day) was recorded with the application of 7.50 kg Zn ha<sup>-1</sup>. Zn bioavailability observed with the application of 2.50 and 5.00 kg Zn ha<sup>-1</sup> were 1.09 and 1.12 mg/300g, respectively. Lowest bioavailability (0.30 mg/300g) was depicted by control where Zn was not applied.

Better nutrient use efficiency is added quality of contemporary wheat genotypes. Therefore, Zn use efficiency was calculated (Table 7). The results showed that higher input rates have inverse relation with nutrient use efficiency. Higher efficiencies were recorded at lower Zn application rates. Highest Zn recovery efficiency (16.40%) was recorded with the application of 1.25 kg Zn ha<sup>-1</sup>. Whereas, lowest recovery efficiency (4.23%) was found where 7.50 kg Zn ha<sup>-1</sup> was applied which was statistically comparable to the recovery efficiency (5.14%) found with application of 5.00 kg Zn ha<sup>-1</sup>. Likewise, in case of agronomic efficiency lowest efficiency (162.50 kg kg<sup>-1</sup>) was recorded in the plant treated with 7.50 kg Zn ha<sup>-1</sup> while highest (716.67 kg kg<sup>-1</sup>) was observed with the application of 1.25 kg Zn ha<sup>-1</sup>. Higher Zn inputs has non-significant effect on physiological efficiency. Lower efficiency (9.24 kg kg<sup>-1</sup>) was recorded with the application of 7.50 kg Zn ha<sup>-1</sup>. Whereas, highest (10.23 kg kg<sup>-1</sup>) was recorded with 5.00 kg Zn ha<sup>-1</sup>.

Table 7. Zinc use efficiency of wheat as influenced by Zn application rates

| Treatments<br>kg Zn ha <sup>-1</sup> | Recovery efficiency, % |         |         | Agronomic efficiency, kg kg <sup>-1</sup> |          |          | Physiological efficiency, kg g <sup>-1</sup> |         |         |
|--------------------------------------|------------------------|---------|---------|---|----------|----------|--|---------|---------|
|                                      | 2016-17                | 2017-18 | Mean    | 2016-17                                   | 2017-18  | Mean     | 2016-17                                      | 2017-18 | Mean    |
| 0.00                                 | 15.93 a                | 16.86 a | 16.40 A | 466.67 b                                  | 966.67 a | 716.67 A | 3.75 b                                       | 16.31 a | 10.03 A |
| 1.25                                 | 6.52 c                 | 10.19 b | 8.35 B  | 266.67 e                                  | 412.50 c | 339.58 B | 5.81 b                                       | 13.90 a | 9.85 A  |
| 2.50                                 | 5.16 cd                | 5.12 cd | 5.14 C  | 200.00 f                                  | 316.67 d | 258.33 C | 5.42 b                                       | 15.04 a | 10.23 A |
| 5.00                                 | 4.85 d                 | 3.61 d  | 4.23 C  | 130.56 g                                  | 194.44 f | 162.50 D | 5.02 b                                       | 13.46 a | 9.24 A  |
| 7.50                                 | 8.115 B                | 8.944 A |         | 262.97 B                                  | 472.49 A |          | 5.00 B                                       | 14.68 A |         |
| Mean                                 | 15.93 a                | 16.86 a | 16.40 A | 466.67 b                                  | 966.67 a | 716.67 A | 3.75 b                                       | 16.31 a | 10.03 A |
| Tuckey's HSD (0.05)                  |                        |         |         |   |          |          |  |         |         |
| Zn                                   |                        | 0.941   |         |   | 8.36     |          |  | 2.07    |         |
| Yr                                   |                        | 0.491   |         |   | 4.37     |          |  | 1.08    |         |
| Zn × Yr                              |                        | 1.614   |         |   | 14.35    |          |  | 3.55    |         |

The relative grain yield was plotted against grain Zn concentration and Zn application rates to calculate critical internal and external requirements of Zn to attain near maximum (95%) relative grain yield (Figure 2). The critical internal requirement of wheat for near maximum yield was calculated as 36.53  $\mu\text{g g}^{-1}$ . Similarly, critical external Zn requirement to attain near maximum grain yield of wheat was found to be 4.48 kg ha<sup>-1</sup>.

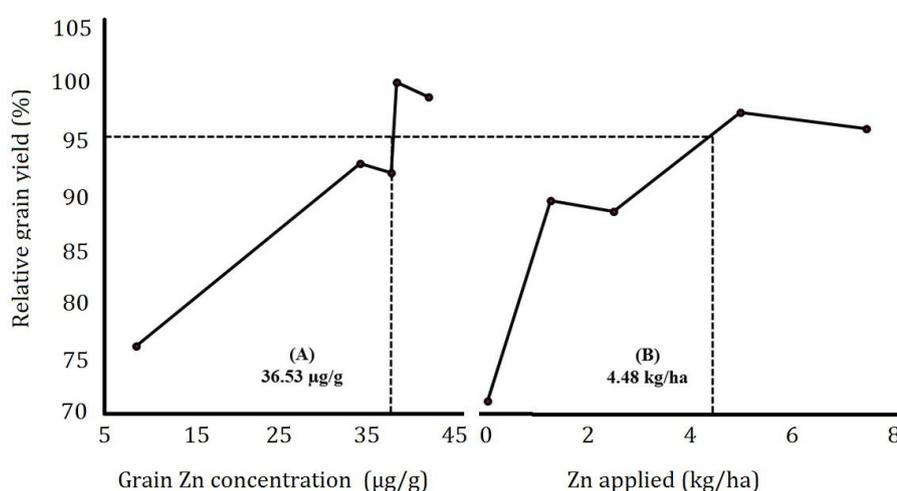


Figure 2. Critical internal (A) and external (B) Zn requirements of wheat to achieve near maximum grain yield

## Discussion

Application of Zn had significant effect of wheat growth and productivity. Zn application enhanced plant height, number of tiller per plant and spike length (Table 2). Likewise, Grain, straw and total biomass yields of wheat were also enhanced (Table 3). Lower average yield during 2017-18 was due to higher temperature (Figure 1) throughout the growing season as compared to 2016-17. [Kutman et al. \(2011\)](#) explained this stimulating effect of Zn to improve plant growth and development. Increased growth and development of plants is attributed to the role of Zn as co-factor of all the six classes of enzymes ([Imran et al., 2015](#)). Application of Zn increased the wheat growth and yield, this increment is due to the role of zinc in plant enzymatic functions, pollination and grain development ([Kaya and Higgs, 2002](#)). [Pedda Babu et al. \(2007\)](#) reported better grain yield due to increased translocation and synthesis of carbohydrates in grains due to Zn fertilization. [Muthukumararaja and Sriramachandrasekharan \(2012\)](#) also found similar results they reported 80-100% increase in the crop yield with the application of Zn in a pot experiment. While, with the 97% increase in grain yield has been reported by [Fageria et al. \(2011\)](#) in response to Zn application to the rice.

Zn application improved its concentration in wheat grains and straw appreciably (Table 4). In grains the increment was found almost 5 times higher than of control. Similarly, highest Zn concentration in straw was found four times higher than control. Zn accumulation in grains and straw was also improved with Zn application (Table 5). Accumulation in wheat grains supplied was almost eight times higher than control upon application of 7.50 kg Zn ha<sup>-1</sup>. Similar pattern of increase was observed in Zn accumulated in wheat straw where highest accumulation was 6 times higher than control. [Chen et al. \(2017\)](#) explained increased Zn bioavailability to plants through calcareous soils upon application of Zn fertilizer. [Cakmak et al. \(2010\)](#) explained role of improved protein synthesis and activity due to better enzymatic functions in plants, in increasing Zn concentration in vegetative parts and Zn translocation into grains. [Kutman et al. \(2012\)](#), [Barunawati et al. \(2013\)](#), [Sperotto et al. \(2013\)](#) and [Akram et al. \(2017\)](#) also found similar results when Zn was applied to different crops.

Phytic acid is indigestible in human yet, its concentration is important for grain vigor and plant functions. PA acts as cation source, precursor to the cell wall, energy and P storage in seeds/grains. Reduced availability of PA bound micronutrients to human is the only negative effect of high PA concentration in grains ([Harland and Morris, 1995](#)). The results showed slight decrease in the phytic acid concentration in grains with increasing Zn application rate (Table 6). Overall, this decrease was found non-significant. However, [Erdal et al. \(2002\)](#) found appreciable decrease in the PA concentrations in seeds of wheat genotypes with agronomic Zn biofortification. They explained this decrease was due to the dilution effect of increased grain yield of wheat. However, [Zhang et al. \(2012\)](#) reported that application of up to 50 kg ZnSO<sub>4</sub>·7H<sub>2</sub>O ha<sup>-1</sup> did not affect PA concentration in wheat products. In agreement with the [Zhang et al. \(2012\)](#) the present study showed overall insignificant changes in grain PA concentrations in response to different Zn input levels. Despite of the dilution effect due to increase in the grain yield the change in grain PA concentration was minimal. It suggests that PA concentration was independent of grain Zn concentration. The PA/Zn ratio decreased with increased Zn input (Table 6). This was indicator to attainment of biofortified wheat grains. These results were in agreement with [Ma et al. \(2005\)](#), [Morris and Ellis \(1989\)](#) and [Lim et al. \(2013\)](#). [Ryan et al. \(2008\)](#)

found the lower PA/Zn ratio with increased grain Zn uptake. Hussain et al. (2013) also advocated that lower PA/Zn ratio is more desirable for increased Zn bioavailability to human.

Ideally, 3mg Zn must come from 300g of wheat products to meet daily Zn requirement of an adult (Rosado et al., 2009). In the present study, the Zn bioavailability was lower irrespective of higher Zn concentration in grains. This was due to higher PA concentration in whole grain especially in wheat bran (Liu et al., 2017). According to Ryan et al. (2008) PA/Zn ratio and PA/Ca ratio were found lower in refined flour than whole grain. Moreover, Erdal et al. (2002) and Tang et al. (2008) have reported that among the wheat varieties the Zn and PA concentrations vary significantly.

Zn use efficiency (ZnUE) in wheat was decreased significantly with increasing Zn application levels (Table 7). Also the diminishing response of grain yield to higher input rates explains the low Zn use efficiency with high Zn rates due to poor distribution and formation of insoluble products of Zn applied at high rates (Genc et al., 2002; Figeria et al., 2011).

## Conclusion

The present study reinforces the concept of agronomic biofortification of wheat to achieve higher yield and quality of produce. We can conclude that Zn application affects wheat growth and yield appreciably. Zn fertilization increases Zn concentration in grains and subsequently improves Zn bioavailability to human. Increased Zn accumulation is a direct indicator to higher Zn bioavailability. Higher Zn accumulation lowers the PA/Zn ratio. The Zn use efficiency is also affected by the Zn application rate. Zinc application is very critical to attain near maximum yield potential of wheat.

## Acknowledgement

We are thankful to Mr. Muhammad Yousuf Memon, Director/Deputy Chief Scientist, and Dr. Mahboob Ali Sial, Deputy Chief Scientist/Wheat Breeder, Plant Breeding and Genetics Division, Nuclear Institute of Agriculture, Tando Jam for their coordination and facilitations to conduct this study.

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