



## Improvement of Wheat Grain Zinc and Zinc Daily Intake by Biofortification with Zinc

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author IS conducted the experiment in the field, performed all the analysis and write the first draft of the article. Author SJ planned the work and review the article. Author SE helped in all field and laboratory work. Authors AN and FB managed the literature searches. Author ZAA supervised all the experimental work in the field and laboratory analysis of the study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Billions of people all over the world are being affected by zinc deficiency that causes stunted growth and poorly developed immune system. The approach to enrich the grains of food crops with nutrients is biofortification. To overcome the human zinc deficiency, improving wheat grain in zinc status by fertilization is an effective tool and main objective of the work. The wheat grain should have 40-60 mg kg<sup>-1</sup> zinc to meet up the recommended dietary allowance (RDA) of zinc. A field experiment was conducted on wheat crop to increase the zinc concentration in its grain. Three levels of zinc were applied 2.5, 5.0 and 7.5 kg ha<sup>-1</sup> as soil application at the time of sowing using ZnSO<sub>4</sub> as a source and foliar spray of 0.1% Zn solution was applied at booting and grain filling stage. A control plot was also kept where no zinc fertilization was done to the wheat. The wheat crop gave maximum grain yield (4.62 t ha<sup>-1</sup>) where Zn was applied as 7.5 kg ha<sup>-1</sup> as soil application

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than the treatment where no Zn supplementation was done ( $4.23 \text{ kg ha}^{-1}$ ). The grain analysis indicates that zinc concentration improved significantly where zinc was applied as compared to control (no Zn) plots. It increased in wheat grain up to  $39.80 \text{ mg kg}^{-1}$  in treatment where Zn was applied as foliar spray of 0.1% zinc solution as compared to NPK treatment ( $21.5 \text{ mg kg}^{-1}$ ). This increase was 84.8% more than control where no zinc supplementation was done. It is concluded from this study that Zn may be applied to wheat not only to increase the yield but also to improve the grain quality. The daily intake of Zn increased up to  $14.3 \text{ mg day}^{-1}$  by consuming the bio-fortified wheat which is near to the recommended daily intake of Zn for an adult person.

*Keywords: Biofortification; grain quality; recommended dietary allowance; wheat; zinc enrichment.*

## 1. INTRODUCTION

Zinc is a micronutrient which is deficient in crops as well as in human beings [1,2]. The Asian population (60-70%) is at the greatest risk of their health due to low feeding of Zinc. The health of humans all over the world is worsening due to low representation of micro-nutrients in diet. The children below the age of five and woman are facing this undernourishment severely [3]. This is a worldwide challenge as half of the population is under the influence of this malnutrition due to micronutrients [4]. Zinc is responsible for immune system development, increasing the learning ability and for proper gene expression in the progeny. Many health complication, DNA damage, cancer development and improper physical health may occur due to less intake of Zn [1,5,6]. The developing countries people have minimum diversification in their food and rely on cereal crop to fulfill their dietary needs [7,8,5]. The developing countries are facing more Zn deficiencies as their major food is cereal grains which have genetically low potential of Zn bioavailability and also low in zinc concentration [9].

Among the three major cereal crop (wheat, rice and maize), wheat is the one that not only is the dietary source but also cover the protein and micronutrients requirements of developing countries population [10]. For the population living in the rural areas of the developing countries wheat is responsible for the daily caloric intake up to 70%. In the developing world the main source of Zn is also wheat [8].

Recommended dietary allowance (RDA) of Zn for the adults is about  $15 \text{ mg day}^{-1}$  [11]. To meet this allowance, wheat grains should contain  $40\text{-}60 \text{ mg kg}^{-1}$  Zn. The wheat grown on soils with low content of Zn will produce grain containing between  $5\text{-}12 \text{ mg kg}^{-1}$  [12,13]. The deficiency of

Zn in plants is not due to low amount of total Zinc in soil but its solubility and availability to plants is a major factor [8].

Nowadays the requirement is the high wheat yield together with high Zn content in wheat grain [14]. Agronomic bio-fortification that is the application of Zn as a fertilizer is a solution to increase the Zn concentration in wheat grains [9]. This study was planned with the objective to improve the Zinc content of wheat grains through soil and foliar fertilizer application and ultimately to prevent the Zn- deficiency hazards in humans.

## 2. MATERIALS AND METHODS

The study was conducted at the research area of Institute of Soil Chemistry and Environmental Sciences, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The wheat was sown in November 2012 and harvested in April 2013 to evaluate the response of Zn application towards the wheat grain Zn concentration.

The experiment was arranged using statistical design Randomized complete block, with three replications and a plot size of  $5 \text{ m} \times 7 \text{ m}$ . Recommended doses of Nitrogen, Phosphorus and Potassium by Agriculture Department in Government of Punjab, Pakistan for wheat crop were applied to all the experimental plots ( $120 \text{ kg ha}^{-1}$  N,  $90 \text{ kg ha}^{-1}$   $\text{P}_2\text{O}_5$  and  $60 \text{ kg ha}^{-1}$   $\text{K}_2\text{O}$ ). On the wheat crop following treatments were applied.

- i. No zinc
- ii.  $2.5 \text{ kg ha}^{-1}$  of Zn (soil application)
- iii.  $5.0 \text{ kg ha}^{-1}$  of Zn (soil application)
- iv.  $7.5 \text{ kg ha}^{-1}$  of Zn (soil application)
- v. foliar spray of 0.1% Zn solution

The sources of fertilizers used were urea, super phosphate (SSP), potassium sulphate (SOP) and

**Table 1. Fertility status of field used for study**

Soil depth (cm)	pH <sub>s</sub>	EC <sub>e</sub> (dSm <sup>-1</sup> )	O.M (%)	Available P (mg kg <sup>-1</sup> )	Extractable K (mg kg <sup>-1</sup> )	DTPA Zn (mg kg <sup>-1</sup> )	Textural class
0-15	8.18	1.73	0.86	8.16	220	0.95	Sandy clay loam
15-30	8.04	1.36	0.66	7.43	190	0.84	Sandy clay loam

ZnSO<sub>4</sub>. All the phosphorus and potassium and ½ nitrogen was applied at the time of sowing and ½ N at first irrigation. All the treatments of Zn in soil were applied at the sowing. The foliar Zn application was done to the respective plots according to the treatment plan at booting and grain filling stage. All the recommended management practices (hoeing, pesticide and herbicides application when needed) for the wheat crop were performed throughout the growing season. At maturity, the yield data of complete plot was taken and converted into internationally used unit i.e. tons per hectare (t ha<sup>-1</sup>).

### 2.1 Soil Sampling and Analysis

Composite soil samples were collected from two depths (0-15 and 15-30 cm) and analyzed for fertility status before fertilizer application and sowing of wheat crop. The soil pH was measured in saturated paste in H<sub>2</sub>O and electrical conductivity (EC) from the soil extract [15]. Soil organic matter was determined using method described by [16]. Available phosphorus was determined by Olsen's method [17] and for potassium soil extraction was made with Ammonium acetate using [18] method. The soil zinc status was determined using DTPA extraction [19]. The pre-sowing soil analysis data of the field is given in above Table 1.

The soil analysis showed that the soil texture was sandy clay loam with pH 8.18. The field was free from salinity and sodicity problem. The organic matter content was low in soil. The soil was moderate in phosphorus and adequate in available potassium [20]. The results showed that field used for this study was deficient in DTPA Extractable Zn [21].

### 2.2 Plant Sampling and Analysis

Wheat was harvested at maturity for yield data, and samples of grain were collected for Zn analysis. The grain samples were dried at 70°C till constant weight in an air circulation oven and ground in a Wiley micro mill. The dried ground material (0.5 g) was digested in a mixture of sulphuric acid, nitric acid and perchloric acid [22]. The digested samples were run on Atomic

Absorption Spectrophotometer (Shemadzu 7000) for zinc determination. A graded series of standards (ranging from 0.5-2.0 mg kg<sup>-1</sup>) of Zn were prepared and Zn value was computed. The uptake of Zn; and daily dietary intake value of Zn by the use of fortified wheat was also calculated by using formula:

$$\text{Daily dietary intake} = [\text{Zn in wheat (mg)} \times \text{Total daily wheat intake (kg)}] \times 100$$

### 2.3 Data Analyses

Statistical analysis of data in this experiment was performed using Analysis of Variance (ANOVA) using the computerized system Statistix® 8.1. Difference among the treatment means were compared using least significant difference at 5% probability level [23].

## 3. RESULTS AND DISCUSSION

### 3.1 Wheat Grain Yield

The yield of wheat grain is presented in Fig. 1. The results showed that grain yield increased by application of zinc sulphate as zinc fertilizer over the no Zn treatment. The Zn application of 7.5 kg ha<sup>-1</sup> gave maximum grain yield (4.62 t ha<sup>-1</sup>) followed by the 5.0 kg ha<sup>-1</sup> Zn application (4.54 t ha<sup>-1</sup>). However both treatments had a non significance differences were remained non-significant with each another. The grain yield data showed that the effect of Zn application was leveled off after 5.0 kg ha<sup>-1</sup> Zn application. It was further noted that soil application of 2.5 kg ha<sup>-1</sup> was remained at par with two sprays of 0.1 % Zn. The minimum grain yield of 3.93 t ha<sup>-1</sup> was recorded where no zinc fertilizer was applied. [14] showed similar results that by applying Zn there was significant increase in grain yield. The increase in grain yield might be related to higher DTPA-Zn levels in soils that probably reduce the photo-oxidative damage in leaves [24].

### 3.2 Zinc Concentration and Uptake in Wheat Grain

The wheat grain at harvesting was collected to monitor the zinc concentration and uptake. The results are presented in Fig. 2.

The results revealed that Zn content in grains increased significantly according to levels of Zn application in soil. The control treatment, i.e. no Zn application had lower Zn content in grain. The treatment receiving foliar spray of 0.1% Zn showed maximum increase in Zn grain content (39.8 mg kg<sup>-1</sup>). This increase in grain Zn was 84.8 % over the control (no Zn application). Many studies by [25,26] on wheat crop indicated that movement of Zn from vegetative parts to the developing grains presents the phloem involvement in Zn transport. [27] reported that foliar applied Zn showed considerably swift movement in wheat. [28] reported that in field situations the Zn content in grain increased up to many folds with the application of Zn fertilizers. It was reported that there is significant increase in Zn content of wheat grain and also in its yield by

applying Zn fertilizers or Zn coated fertilizers to the soil [29].

Similarly, in case of uptake, the foliar application of 0.1% zinc gave maximum zinc uptake by wheat grain. Due to alkaline nature of soil there is inhibition in absorption of Zn<sup>+2</sup> as well as other alkaline earth cations by plants [30]. It is doable to supply adequate Zn to the wheat crop by foliar application [27].

### 3.3 Daily Dietary Intake of Zinc from the Fortified Wheat

The data regarding daily Zinc intake from the wheat flour is given in Table 2. The average national diet of Pakistani adult includes almost 360 g of whole wheat flour [31] that contributes

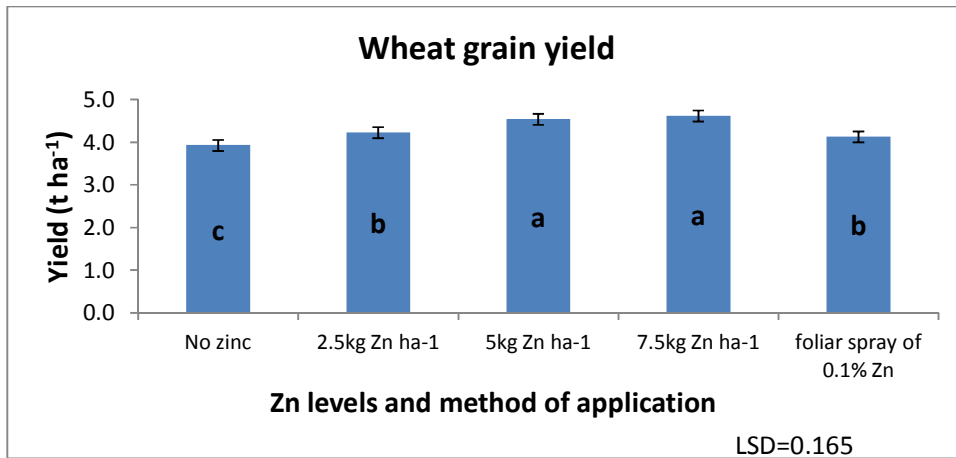


Fig. 1. Effect of Zinc application on grain yield of wheat (Means followed by the same letter in the bars do not differ significantly (p<0.05))

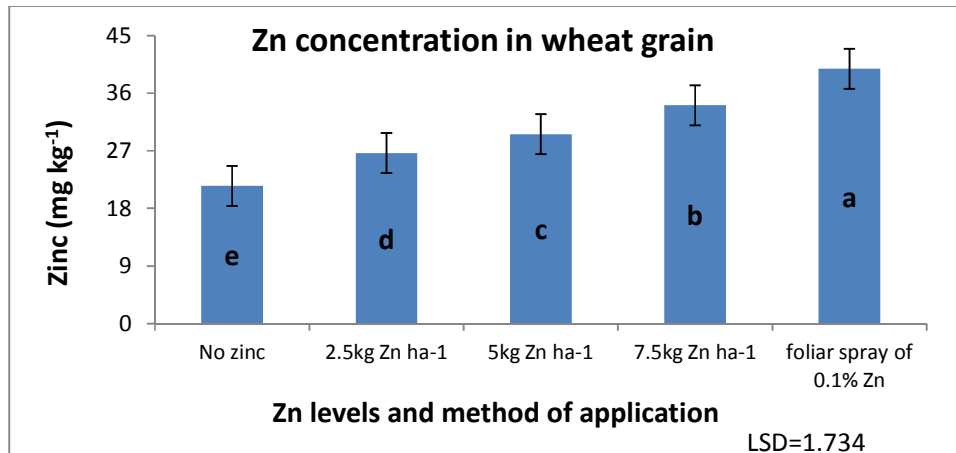


Fig. 2. Effect of Zinc application on zinc concentration in wheat grain (Means followed by the same letter in the bars do not differ significantly (p<0.05))

**Table 2. Daily dietary intake of zinc from the fortified wheat**

Rate of Zn application (kg ha <sup>-1</sup> )	No Zinc	2.5	5.0	7.5	Foliar spray of 0.1% Zn
Zinc in whole wheat grain (mg kg <sup>-1</sup> )	21.1	26.7	30.6	34.2	39.8
Daily Dietary Intake of Zinc (mg day <sup>-1</sup> )	7.0	9.3	10.3	11.9	14.3

more than 70% of the daily caloric intake. The average concentration of zinc in traditionally grown wheat where there is no Zn application is 21.5 mg kg<sup>-1</sup>. This showed that an adult taking 360 g wheat flour intake only 7.0 mg Zn day<sup>-1</sup> which is less than half of the RDA (15 mg Zn day<sup>-1</sup>) for an adult by [32]. As the wheat crop is biofortified by adding zinc fertilizer, the daily intake of Zn day<sup>-1</sup> increases by taking the same amount of wheat flour. The treatment where zinc was applied as foliar spray of 0.1% the zinc concentration increases up to 39.8 mg kg<sup>-1</sup> and resulted in the increase of daily Zn intake up to 14.3 mg day<sup>-1</sup> which is very near to recommended daily need of an adult person (15 mg day<sup>-1</sup>).

#### 4. CONCLUSION

Zn fertilization not only increases the grain yield but also improved the grain quality. The maximum increase in Zn content was recorded by foliar application of 0.1% Zn solution at booting and grain filling stage. By using the Zn-biofortified wheat, the daily dietary intake of Zn will be increased and health of people will be improved.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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