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Zinc application enhances grain zinc density in genetically-zinc-biofortified wheat grown on a low-zinc calcareous soil

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ABSTRACT

Human zinc (Zn) deficiency is a worldwide problem, especially in developing countries due to the prevalence of cereals in the diet. Among different alleviation strategies, genetic Zn biofortification is considered a sustainable approach. However, it may depend on Zn availability from soils. We grew Zincol-16 (genetically-Zn-biofortified wheat) and Faisalabad-08 (widely grown standard wheat) in pots with (8 mg kg^{-1}) or without Zn application. The cultivars were grown in a low-Zn calcareous soil. The grain yield of both cultivars was significantly ($P \leq 0.05$) increased with that without Zn application. As compared to Faisalabad-08, Zincol-16 had 23 and 41% more grain Zn concentration respectively at control and applied rate of Zn. Faisalabad-08 accumulated about 18% more grain Zn concentration with Zn than Zincol-16 without Zn application. A near target level of grain Zn concentration (36 mg kg^{-1}) was achieved in Zincol-16 only with Zn fertilisation. Over all, the findings clearly signify the importance of agronomic Zn biofortification of genetically Zn-biofortified wheat grown on a low-Zn calcareous soil.

KEYWORDS: Agronomic or genetic, grain zinc, wheat grain, zinc biofortification

INTRODUCTION

About 20% of the world population on average and even greater than 50% of the population of many countries of Africa and Asia are Zn deficient due to inadequate dietary Zn intake [1]. Human immunity, central nervous system, gastrointestinal tract, skin, skeleton and reproductive system are severely affected by Zn deficiency [2]. Zinc deficiency is involved in health complications such as diarrhoea, pneumonia and cancer development [3,4]. There are many reasons behind Zn deficiency, including inadequate dietary Zn intake and low Zn bioavailability from cereal grains consumed as staple food [5,6].

In most Asian countries, wheat is consumed as a staple food. Wheat provides up to 50% of total energy intake in the Indian sub-continent. Cereals have inherently a low concentration of Zn in grains, especially if grown in low-Zn soils. Several soil (high pH, low organic matter, low moisture availability, poor aeration and declining fertility) and genotypic factors (high endosperm-to-bran ratio, grain phytate content, capacity to take up Zn and transport it into developing grains) influence accumulation and bioavailability of Zn in cereal grains [7–9].

Biofortification, which is aimed at increasing the concentration of desired micronutrients in plant-based foods through agronomic and genetic approaches, is a cost-effective way to solve the problem of Zn deficiency [10]. Genetic biofortification of cereal crops provides sustainable and long-term solution to micronutrient deficiencies. More than 150 biofortified cultivars of crops have been released in different countries worldwide, and more than 20 million people are growing and consuming biofortified crops [11]. In Pakistan, first Zn-biofortified wheat (Zincol-16) was released in 2016 by joint efforts of *HarvestPlus* and Pakistan Agricultural Research Council [12]. Three varieties of Zn-biofortified wheat (Zinc Shakti, WB02 and HPBW-01) have been released in India [13]. The capability of newly developed Zn-biofortified wheat cultivars to accumulate more grain Zn may depend on the Zn availability from the soil for plant uptake; therefore, agronomic strategy of Zn fertilisation could be complementary to genetic Zn biofortification [14].

Zinc-biofortified wheat Zincol-16 has the capacity to accumulate about 20% more Zn than standard cultivars [12]; but, the role of soil Zn application on that capacity remains obscure. The study compared individual and combined effects of agronomic and genetic biofortification on grain Zn accumulation in newly developed genetically-Zn-biofortified (Zincol-16) and widely grown standard (Faisalabad-08) wheats of Pakistan.

MATERIALS AND METHODS

Bulk soil sample was collected (0 to 15 cm depth) from Agricultural Research Farm (30.2601°N; 71.5126°E) of Bahauddin Zakariya University, Multan (Pakistan). The air-dried soil was crushed to pass through a 2 mm sieve. Representative subsample of the soil was analysed in laboratory for basic properties. Soil texture (sand, silt and clay contents of respectively 23, 45 and 32% w/w) was determined by Hydrometer Method [15] with textural class clay loam (USDA classification). Soil pH (8.2) and EC (1.6 dS m^{-1}) were determined in 1:1 soil to water mixture. Soil was low in organic matter (0.3% w/w) as determined by Walkley-Black Method [16]. Soil was calcareous having free $CaCO_3$ 4.9% w/w as determined by acid dissolution [17]. AB-DTPA-extractable [18] Zn concentration was 0.7 mg kg^{-1}.

A pot experiment was conducted in a glasshouse at Bahauddin Zakariya University, Multan (Pakistan). Each pot was lined with polythene sheet and filled with 6 kg soil. Soil in the pots was supplied with or without 8 mg Zn kg^{-1} as $ZnSO_4 \cdot 7H_2O$. Uniform basal rates (in mg kg^{-1}) of 20 N, 20 P and 25 K were applied in solution by adding urea ($(NH_2)_2CO$) and potassium di-hydrogen phosphate (KH_2PO_4). After this, soil was thoroughly mixed. Seven seeds, per pot, of Zn-biofortified (*Zincol-16*) or standard (*Faisalabad-08*) wheats were sown at a uniform soil depth of 2 cm. All possible combinations of two Zn levels and two cultivars were arranged factorial completely randomized design (CRD). Seedlings in a pot were thinned to three at four-leaf stage. Soil in pots was irrigated daily with tube-well water to maintain soil moisture at field capacity. The irrigation water had electrical conductivity of about 0.32 dS m^{-1} and pH 7.7, and Zn concentration below the detection limit. The pots were randomized weekly to avoid differential effects of microclimate. The second and the third splits of N, each at 20 mg N kg^{-1}, were applied as urea at, respectively, 30 and 60 days after sowing.

At full maturity, spikes and the remaining above-ground parts were harvested. Spikes were manually threshed to separate grains. Harvested straw samples were washed with distilled water and blotted dry with tissue papers. Straw and grain yield, and thousand-grain weight were recorded after oven drying at 65°C for a period of 72 h.

Grain and straw samples were ground in a metal-free grinder, and homogenised subsamples were wet ashed in a di-acid mixture (2:1 ratio) of concentrated nitric and perchloric acids [19]. After calibration with the known Zn standards, Zn in the digests was determined on ASS (Spectr AA 220, Varian Inc., Palo Alto, California, USA).

All statistical analyses were carried out on SAS University Edition (SAS/STAT®, SAS Institute Inc., NC, USA). The data were statistically analysed by two-factorial ANOVA followed by post-hoc Tukey's HSD test at $P \leq 0.05$ [20].

RESULTS

Biomass Yield

Straw and grain yield were significantly influenced by interactive effect of Zn rate and cultivar (Table 1). Zinc application, as compared to control, increased straw and grain yield more in Zincol-16 than Faisalabad-08 (Figure 1). As compared with control level of Zn, Zn application increased straw and grain yield of Zincol-16 by 41 and 20%, respectively. A lower albeit similar percent increase of 12% was observed in straw and grain yield of Faisalabad-08.

Only main effects of Zn rate and cultivar significantly influenced thousand-grain weight (Table 1). Averaged across the Zn rates, thousand-grain weight of Zincol-16 was 8% lower than Faisalabad-08 (Table 2). On average, Zn application increased thousand-gain weight by only 3% than control.

Zinc in Grains

Similar to yield, grain Zn concentration and contents were significantly influenced by interactive effect of Zn rate and cultivar (Table 1). In two wheat cultivars grown at two Zn rates, grain Zn concentration ranged from 18 to 36 mg kg^{-1} (Figure 2). Zincol-16 had 23 and 41% more grain Zn concentration than Faisalabad-08 respectively at 0 and 8 mg Zn kg^{-1} (Figure 2). Zinc application than without Zn application increased grain Zn concentration of Zincol-16 and Faisalabad-08 respectively by 66 and 45%.

Grain Zn contents were also significantly increased with than without Zn fertilisation (Figure 2). The increase in Zincol-16 and Faisalabad-08 was respectively 101 and 63%. Zincol-16 had more grain Zn contents than Faisalabad-08 at 8 Zn mg kg^{-1}.

Table 1: Calculated *F values* for various parameters based on two-way analysis of variance (ANOVA) test

Parameters	Source of variation		
	Cultivar	Zn rate	Cultivar × Zn rate
Grain yield	2	266*	18*
Straw yield	24*	97*	28*
Thousand-grain weight	66*	16*	2
Grain Zn concentration	184*	441*	36*
Grain Zn contents	171*	616*	62*

*significance at $P \leq 0.05$

Table 2: Thousand-grain weight of wheat cultivars (*Zincol-16* zinc-biofortified wheat and *Faisalababd-2008* standard wheat) grown in pots

Treatment effects	Levels of treatments	Thousand-grain weight (g)
Main effect of cultivar	Zincol-16	36.5±1b
	Faisalabad-08	39.2±1a
Main effect of Zn rate	0 mg Zn kg^{-1}	37.2±2b
	8 mg Zn kg^{-1}	38.5±1a

Means±standard deviations; Lettering is based on Tukey's HSD test at $P \leq 0.05$

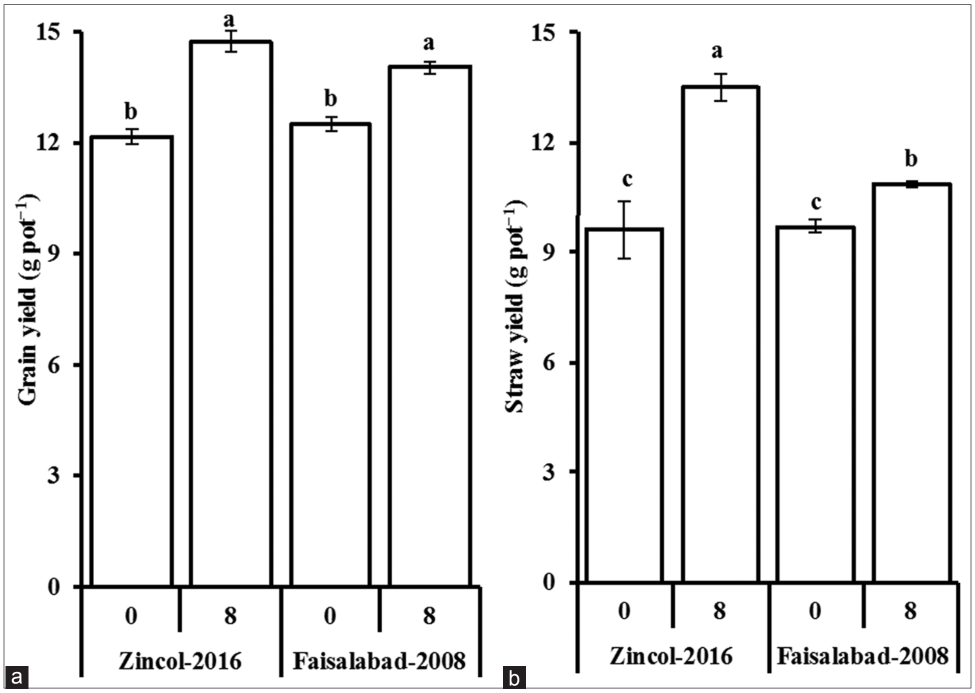

Figure 1: Grain (a) and straw (b) yield of two wheat cultivars grown in pots and supplied 0 or 8 mg Zn kg^{-1}. Error bars are of standard deviation of means. Lettering is based on Tukey's HSD test at $P \leq 0.05$

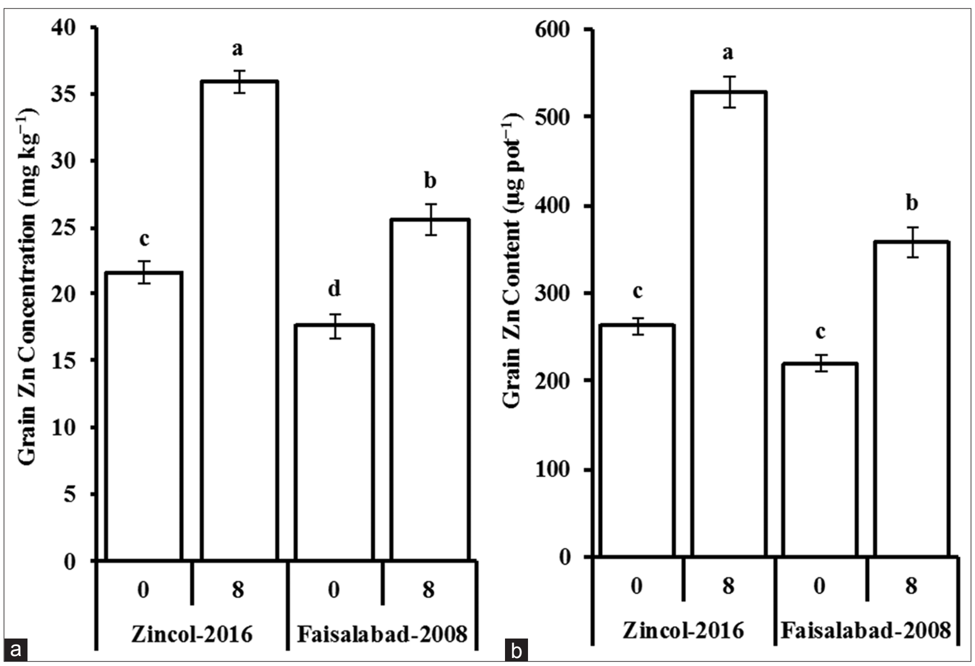

Figure 2: Concentration (a) and contents (b) of two wheat cultivars grown in pots and supplied 0 or 8 mg Zn kg^{-1}. Error bars are of standard deviation of means. Lettering is based on Tukey's HSD test at $P \leq 0.05$

DISCUSSION

Zinc plays important physiological and biochemical roles in plants [21]; its application to low-Zn soils increases grain and straw yield of cereals [22–24]. The soil used for present study was also low in Zn (0.7 mg AB-DTPA-extractable Zn kg^{-1}). Zinc application increased straw and grain yield of both cultivars (Figure 1). The relatively more increase in yield of Zincol-16 than Faisalabad-08 with Zn application might be due to a higher Zn demand of Zincol-16.

At both Zn rates, grain concentration and contents of Zn were more in Zincol-16 than Faisalabad-08 (Figures 2). Lower thousand-grain weight of Zincol-16 than Faisalabad-08 (Table 3) possibly relate with a higher micronutrient density in grains by decreasing the relative proportion of endosperm in total grain components [6]. Therefore, whole grains must be consumed to harvest the full benefits of Zn biofortification.

Zinc fertilisation also increased grain Zn concentration and contents in both cultivars (Figures 2). Even with Zn fertilisation, Faisalabad-08 accumulated below the desired concentration level of Zn in grains. However, it accumulated 18% more Zn in grains with Zn application (8 mg Zn kg^{-1}) than Zincol-16 without Zn application (control). Furthermore, with Zn fertilisation, Zincol-16 had 41% more grain Zn concentration than Faisalabad-08. Along with genotypic variations [25], the levels of grain Zn concentration in Zn-biofortified cultivars of cereals may also depend on soil available Zn [26]. The target Zn level (≥38 mg Zn kg^{-1}) for biofortification [27] was nearly attained in Zincol-16 only with Zn application (Figure 2). This indicates a complementary role of genetic and agronomic biofortification for each other in increasing grain Zn concentration of wheat to desired levels. For low-Zn soils, agronomic Zn biofortification seems to be comparatively more important than genetic Zn biofortification for increasing grain Zn concentration in wheat.

We repeated the experiment with another soil sampled from a farmer field (30.1126°N; 71.3942°E) in Multan (Pakistan) having an initial AB-DTPA-extractable Zn level of 1.3 mg kg^{-1}. The response of wheat cultivars for the studied parameters partly depended on the initial levels of Zn in two soils (data not shown). However, the trend was almost similar in the soils and data of the only experimental farm's soil were presented here.

CONCLUSIONS

Zinc fertilisation to low-Zn calcareous soil increased grain yield of both Zincol-16 and Faisalabad-08. Zincol-16 had more grain Zn concentration at two Zn rates. However, Faisalabad-08 accumulated about 18% more Zn in grains at 8 mg Zn kg^{-1} than Zincol-16 at control (0 mg Zn kg^{-1}). Zincol-16 had 23 and 41% more grain Zn concentration than Faisalabad-08 respectively at soil application of 0 and 8 mg Zn kg^{-1}. Therefore, Zn fertilisation is important for Zn biofortification of both standard and Zn-biofortified cultivars of cereals grown on low-Zn soils. Field investigations, however, are required to test the behaviour of Zincol-16 under different fertilisation approaches.

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