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Research Article

Zinc Bio-fortification of Wheat through Zinc Application Methods and Nitrogen Rates

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Abstract

Deficiency of zinc (Zn) is a common issue in both crop plants as well as human beings. Wheat (*Triticum aestivum* L.) is staple of most of world population; however, it has low Zn concentration. Agronomic biofortification is an emerging promising approach to combat Zn malnutrition. Aim of this experiment was to study the impact of various Zn applications methods (seed priming, soil application, and foliar application) and nitrogen (N) levels (120 kg ha^{-1} and 240 kg ha^{-1}) on growth, yield and Zn concentration in the wheat grains. Results of this study reveal that application of Zn significantly increased plant height, spike length, grains per spike, spikelets per spike, test weight and yield. Similarly, higher N level exhibited significant higher plant height, spike length, spikelets per spike, grains per spike, test weight, and yield than the low N level. Seed priming with Zn displayed the highest yield (3.09 t ha^{-1}) under high level of nitrogen which is about 9% higher than lower N level. Foliar application was the most effective for grain Zn enrichment and about 38% higher Zn was observed in foliar treatment than control). It is suggested that grain Zn concentration may be increased by Zn foliar application. In addition, N application may enhance Zn uptake.

Keywords: Zinc; Biofortification; Wheat; Nitrogen

1. Introduction

Zinc (Zn) is an essential micronutrient for plants as well as animals. It has many significant roles in plants including growth regulation, dry matter accumulation, fertilization, viability (Kaya and Higgs, 2002), genes expression, protein synthesis, and stress tolerance (Alloway, 2004). Zinc is also a part of several plant enzymes including DNA replicating proteins, peroxidases, oxidases, carbonic anhydrase and alcohol dehydrogenase (Storey, 2007). Zinc also has many important roles in animal and human health. It plays significant role in the maintenance of immune system functioning (Beck *et al*., 1997). Zinc deficiency is a major health issue and about thirty percent of the world's population is Zn deficient (Stein, 2010). In Pakistan, about 12 million children including 18.6% pre-school children are Zn deficient (Ministry of National Health Services Nutrition Wing, 2018).

Wheat is a major cereal crop globally and is important source of essential micronutrients, vitamins and amino acids for majority of world's population particularly in the developing countries (Shewry, 2009). According to an estimate, about 19% of daily human calorie intake is fulfilled by wheat globally. About 60% of wheat is produced by the developing countries which meets more than 50% of everyday nutrition in these countries (Cakmak, 2008).

Although wheat is an excellent source of calorie, it is inherently low in essential micronutrients especially Zn (Newell-McGloughlin, 2008). Therefore, wheat based diet may lead to hidden hunger and contributes to diseases (Nestel *et al*., 2006). The options for minimizing Zn deficiency in humans include Zn supplementation, diet diversification, food fortification, and biofortification (WHO, 2009). Biofortification is a better option because it has capacity to overcome this deficiency by enhancing the mineral element of the crops and plant availability in the edible parts (Rehman *et al.,* 2020). It is of two types: agronomic biofortification and genetic biofortification (Rengel *et al*., 1999). Agronomic biofortification is a safe approach that can raise the uptake/bioavailability of mineral elements to plants (Rashid *et al*., 2018). It can be achieved through seed treatment, soil and foliar application of fertilizers (Rehman and Farooq 2016). Various studies have reported that Zn concentration of grains increased through these approaches (Harris *et al*., 2008). Application of nitrogen (N) also has a significant role in the re-translocation of Zn in grains (Kruger *et al*., 2002) because N increases the grain protein which correlate with the grains Zn concentration (Asif *et al*., 2019). Many studies have documented interaction of Zn uptake and N application; however, the interaction of N and

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various Zn application methods is unclear, especially the method of seed priming. Therefore, the present study was conducted to examine the impact of various Zn application methods (soil application, foliar feeding and seed priming) and nitrogen levels on the growth, yield, and grain Zn concentrations of wheat.

2. Materials and Methods

2.1. Experimental details

The experiment was conducted at field research area of University of Agriculture, Faisalabad following randomized complete block design (RCBD) in factorial arrangement with four replications. Seed of wheat cultivar Punjab 2011 was obtained from Wheat Research Institute, Ayub agricultural research institute (AARI), Faisalabad, Pakistan. Seedbed was prepared by tractor mounted cultivator and planker to a depth of 12 cm. Wheat was sown on 25th November 2014 by hand drill using seed rate of 125 kg ha^{-1} on a plot with net plot size of 5 × 1.8 m in 22.5 cm spaced rows. Altogether five irrigations were applied keeping in view of crop requirement. First irrigation was applied at 22 days after sowing and subsequent irrigations were applied according to the need of the crop. Fertilizer was applied at the rate of 120 or 240 nitrogen and 100 Kg P kg ha^{-1}. Whole of the phosphorus was applied at sowing while N was applied in two splits with first and second irrigation. There were three Zn application methods along with control selected from the already published literature. These were foliar application of Zn (0.5%), seed priming with Zn (0.4%), and soil application of Zn (5 kg ha^{-1}). In the control, Zn was not applied except the soil indigenous Zn (≤ 0.8 ppm). In this study, $ZnSO_4$ was used as source of Zn. For priming, seed was soaked in aerated solution of 0.4% Zn for 24 h keeping seed to solution ratio of 1:5 (w/v). After three surface washings the primed seed was dried under forced air to original moisture level.

2.2. Observations

Data regarding crop growth rate were taken after 45 days of sowing with the interval of 15 days till 105 days after sowing (DAS). Height of five plants was taken at maturity and averaged. At the time of maturity five spikes were selected at random from each plot and their length, number of spikelet's and grains per spike were calculated and averaged.

Crop growth rate (CGR) was taken with an interval of 15 days after 45 DAS and using following equation (Hunt, 1978).

$$CGR = \frac{W2 - W1}{T2 - T1}$$

Where W_1 is total dry matter at the first harvest, W_2 is total dry matter at the second harvest, T1 is date of observation of first dry matter and T2 is date of observation of second dry matter.

The crop was harvested, tied into bundles, and then total wheat biomass was recorded. The crop was threshed using mini thresher and the weight of the grains was measured with the help of electric balance. Harvest index was calculated as the ratio of grain yield to total biological yield. A sub sample of thousand grains was taken from each plot and was weighed. Grains were grinded using grinding mill. Zinc concentrations in grains were determined with the wet digestion method using HNO_3-$HClO_4$. For digestion, 1g of grinded grain was taken in 100 mL digestion flask and 10 mL of bi-acid mixture (HNO_3-$HClO_4$) with the ratio of 2:1 was added. The samples were digested on hot plate till a colourless endpoint. Then, the mixture was allowed to cool and the desired volume was achieved by adding distilled water. After the digestion, aliquots were analyzed for Zn concentrations on the Atomic Absorption Spectrophotometer, (AAS) (Hitachi Polarized Zeeman AAS, Z-8200, Japan).

2.3. Statistical analysis

Data were analyzed statistically using two-way analysis of variance, and least significant difference (LSD) test at 5% probability level was used to compare means of treatments (Steel *et al.*, 1997.

3. Results and Discussion

3.1. Growth related traits

The data relating crop growth rate is given in the Figure 1 A&B. Zinc application methods affected the crop growth rate under both N levels. During earlier growing period, maximum crop growth rate was observed when Zn was applied in soil, however, in mid-season, growth rate was maximum in Zn foliar-application. Minimum crop growth rate was recorded in the control throughout the growing season. Almost similar trend was found at higher nitrogen level i.e. 240 kg ha^{-1} N. Further, the results also show that various N levels and Zn application methods significantly affected plant height (Table 1). However, their interaction was non-significant. Highest plant height was observed in treatments where Zn was applied as soil amendment, however, minimum height was observed in the control.

Results also show that significantly higher plant height was observed in treatment when 240 kg ha^{-1} N was applied. These findings are also in alliance with the finding of Nadim *et al.* (2012). They reported that N and Zn application significantly increased crop growth rate of wheat. Nitrogen is involved in the processes of cell division, elongation, and differentiation that results in as an increase in the vegetative growth and ultimately, high biomass production (Nasser, 2002). Zinc is also involved in the metabolism of plant growth regulators such as auxin (IAA) and tryptophan. Tryptophan synthesis is affected by the Zn that is essential for the formation of IAA. Crop growth rate is generally affected by these growth regulators (Zeidan *et al.*, 2010).

3.2. Yield and Yield Components

Results of this study show that Zn application improved spike length, spikelet's per spike, grains per spike, thousand grains weight, grain yield, and harvest index. Higher values of yield and yield contributing traits were recorded in treatment of seed priming with Zn. In

Table 1. Table 1: Impact of nitrogen rate and Zn application methods on plant height (cm), productive tillers, spike length (cm), spikelets per spike, grains per spike, thousand grain weight (g), grain yield (t ha^{-1}), harvest index and grain zinc concentration (mg kg^{-1}) of wheat

	Plant Height (cm)			***Productive tillers (m^{-2})***			***Spike Length (cm)***		
Treatments	**N$_1$**	**N$_2$**	**Mean**	**N$_1$**	**N$_2$**	**Mean**	**N$_1$**	**N$_2$**	**Mean**
Control	83.8	86.1	84.9 D	296.0	296.2	296.1	7.1	7.4	7.2 D
Foliar application	86.9	88.8	87.9 C	301.7	325.0	313.3	7.5	7.7	7.6 C
Seed priming	90.4	92.0	91.2 B	297.5	297.5	297.5	8.4	9.1	8.7 A
Soil application	92.8	93.2	93.0 A	302.5	301.2	301.8	8.0	8.3	8.1 B
Mean	88.5 B	90.0 A		299.4	305.0		7.8 B	8.1 A	
LSD(P≤0.05)	*AM=1.7, NR=1.2*						*AM=0.2, NR=0.1*		
	Spikelet's per spike			***Grains per spike***			***Thousand grain weight (g)***		
Treatments	**N$_1$**	**N$_2$**	**Mean**	**N$_1$**	**N$_2$**	**Mean**	**N$_1$**	**N$_2$**	**Mean**
Control	10.9	12.6	11.7 C	36.9	37.7	37.3 B	35.9	37.1	36.5 C
Foliar application	13.2	13.6	13.4 B	39.9	39.3	39.6 AB	38.0	40.7	39.4 B
Seed priming	14.8	15.3	15.0 A	41.6	41.7	41.6 A	39.1	44.4	41.8 A
Soil application	14.0	14.0	14.0 B	38.2	38.5	38.3 B	36.2	38.1	37.2 BC
Mean	13.2 B	13.8 A		39.1	39.3		37.3 B	40.1 A	
LSD(P≤0.05)	*AM=0.6, NR=0.4*			*AM=2.5*			*AM=2.4, NR=1.7*		
	Grain yield (t ha^{-1})			***Harvest index (%)***			***Grain Zn concentration (mg kg^{-1})***		
Treatments	N$_1$	N$_2$	Mean	N$_1$	N$_2$	Mean	N$_1$	N$_2$	Mean
Control	2.0	2.4	2.2 D	33.7	33.8	33.8	30.9	33.5	32.2 C
Foliar application	2.4	2.6	2.5C	33.7	33.7	33.7	39.5	49.6	44.6 A
Seed priming	3.3	3.6	3.4A	33.7	33.7	33.7	34.0	40.2	37.1 BC
Soil application	3.0	3.1	3.0B	33.7	33.7	33.7	41.6	46.2	43.9 AB
Mean	2.7B	2.9A		33.7	33.7		36.5 B	42.4 A	
LSD(P≤0.05)	*AM=0.2, NR=0.16,*						*AM=7.35, NR=5.20*		

addition, application N at higher rate i.e. 240 kg ha^{-1} significantly increased spike length, spikelet's per spike, grains per spike, thousand grains weight, and grain yield (Table 1). In addition, a non-significant interaction was observed between Zn application methods and N levels in all parameters. The grain yield was the highest in the treatment of seed priming at higher N level which is about 9% higher than lower N level. Zinc is known to play a role in many physiological processes of plants including cell elongation, enlargement and division (Gomez-Coronado *et al.*, 2017). It is also involved in the synthesis of chlorophyll which indirectly has a key role in determining spike length, number of spikelet's spike^{-1}, number of grains spike^{-1}, thousand grain weight, grain yield, and harvest index (Kaya and Heggs, 2002). Similar findings were reported by Slaton *et al*. (2001) who reported that priming with Zn improved the rice growth and yield compared to soil application and control.

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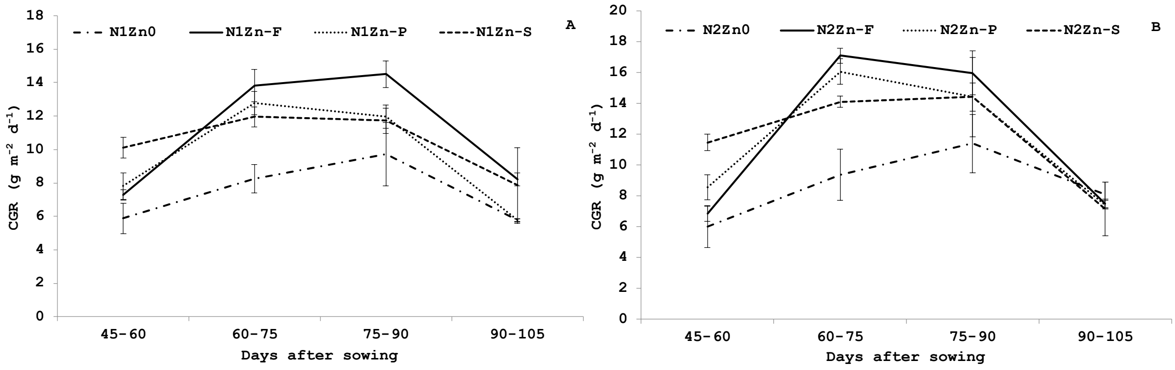

Figure 1: Impact of Zn application methods on crop growth rate of wheat at (A) low nitrogen rate (N1=120 kg ha^{-1}) and (B) high nitrogen rate (240 kg ha^{-1}), Where Zn0 = no zinc; Zn-F= Foliar application of zinc; Zn-P=Seed priming with zinc; Zn-S= Soil application of zinc; CGR= Crop growth rate

Rehman *et al.* (2014) reported that primed seed gave good stand establishment, grew energetically, produce early flowering, and give high yields.

In another study, it was reported that seed priming with $ZnSO_4$ enhanced grain weight and seed yield compared to control (Masuthi *et al.*, 2009). Aboutalebian *et al.* (2012) reported that seed treatment with $ZnSO_4$ increased the stand establishment of crops and enhanced economic yield. The increase in yield under seed priming at high N level may be attributed to the synergistic interaction of N and Zn (Grujcic *et al.*, 2021).

3.3. Grain Zn Concentration

The results show that grain Zn concentration were improved by Zn application. The highest grain Zn concentration (about 38% higher than control) were found in the foliar application treatment, followed by soil application, however, seed priming had no impact on grain Zn concentration. This study confirms that foliar application of nutrients is a viable method for improvement of nutritional condition in plant (Erenoglu *et al.*, 2011). It is promising short-term strategy to increase Zn concentration in edible parts and may be helpful in alleviation of Zn deficiency in the populations of developing world (Ahmed *et al.*, 2011). Application of of Zn is known to inhibit the conversion of inorganic P in grain to phytic acid and thus, reduces phytic acid concentrations in grain. Phytic acid to Zn molar ratio which result in increased grain ZN concentration because phytic acid is the main compound that reduce Zn bioavailability to plants (Lonnerdal, 2000). Our results also show that high N application improved about 16% grain Zn concentration (Table 1). Kutman *et al.*, (2010) found that N supply to plants was effective in enhancing grain Zn concentration because the application of N postpones senescence and prolongs the period of grain-filling. Therefore, accumulation of Zn throughout the prolonged period of grain-filling may add to Zn buildup in grain (Yang and Zhang, 2006) and also enhanced plant growth which increases the surface area of roots and root volume. This increases the uptake of Zn by the roots through the expression of transport protein in the root cell membrane (Nie *et al.*, 2019). In high N environments, continuous absorption of Zn by the roots and its movement to seeds throughout the prolonged period of grain filling could be an additional pathway that facilitates the buildup of Zn in grains. Nie *et al.* (2019) suggested that higher N supply enhance Zn concentration in grains though increasing uptake of Zn and translocation of Zn from root to shoot in wheat especially under Zn sufficient conditions.

4. Conclusions

Zinc application improved the growth and grain Zn concentration in wheat. Similarly, high N application also resulted in high yield and Zn concentration. The highest yield was recorded in seed priming under high N dose. The foliar application of Zn exhibited the highest grain Zn concentration followed by the soil application.

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Author Contributions

“Conceptualization, S.A.C. and M.I.K..; methodology, S.A.C. and Z.M.; writing—original draft preparation, Z.M.; writing—review and editing, S.A.C., M.I.K., B.S. and I.A supervision, S.A.C; All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare

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