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Full Length Article

Agronomic Biofortification to Improve Productivity and Grain Zn Concentration of Bread Wheat

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Abstract

Zinc (Zn) deficiency is one of the major problems in food crops, affecting humans and animals. Present study was carried out to determine the effect of Zn application through different methods for improving the productivity and grain bio-fortification of bread wheat. Zn was applied through different methods as soil application (10 kg $ZnSO_4$ ha^{-1}), seed priming (0.3 *M* $ZnSO_4$) and foliar application (0.5% $ZnSO_4$ at booting and milking stages). Zn application through either method increased the productivity and grain Zn concentration of wheat. The order of improvement in grain yield was soil application (6.60 Mg ha^{-1}), seed priming (6.09 Mg ha^{-1}) and foliar application (5.75 Mg ha^{-1}). Likewise, increase in grain Zn concentration was in order of foliar application (70%), soil application (39%) and seed priming (15%). Zn application through foliar spray had highest partial factor productivity, agronomic use efficiency and Zn mobilization efficiency index owing to low quantity of Zn applied. All Zn application methods remarkably enhanced the profitability; however, soil application remained more profitable method, followed by seed priming and foliar application. In conclusion, Zn application through soil was beneficial in improving the productivity of wheat, while in case of biofortification, Zn application through foliar spray was best.

Keywords: Zinc deficiency; Grain; Wheat; Yield; Biofortification

Introduction

Cereals are major source of food in developing countries and considered to be low in important micronutrients including zinc (Zn) (Cakmak, 2008). Therefore, frequent intake of cereal based products in daily diet is major reason for widespread Zn deficiency in developing countries (Erdal *et al.*, 2002). Wheat occupies a central position in the provision of calories, micronutrients and protein especially in developing countries (Shewry, 2009). Moreover, it also provides more than the 70% daily calories to rural inhabitant and also has major role in provision of Zn (Cakmak, 2008). Zn is an indispensable nutrient for biological systems in animals, humans and as well as in plants. In plants, Zn plays a crucial role in activation of enzymes, auxin metabolism and in integrity of biological membranes (Broadley *et al.*, 2007).

Similarly, in human beings, Zn also plays role in growth and reproduction. Many health impediments, like poor physical growth, cancer and damage to DNA may occur due to less intake of Zn (Prasad, 2008). Globally, >2 billion people, suffering from micronutrients deficiency (Kumssa *et al.*, 2015), however, more than 1.1 billion people are under the risk of Zn deficiency (Kumssa *et al.*, 2015). In countries, like Pakistan, Iran and Turkey, most of the soils are Zn deficient which contributes towards the Zn deficiency in crops and humans (Hotz and Brown, 2004). In Pakistan, more than 40% mothers and one third children are under Zn malnutrition, with higher rate in rural communities (MINH, 2009).

Zinc deficiency in soils is a well-known problem throughout the wheat growing countries. This problem is associated with poor availability and high adsorption of Zn on soil particles due to high pH and calcareous nature of soils (Alloway, 2009; Hussain *et al.*, 2011). In addition to a substantial reduction of yield, insufficient supply of Zn from soils also resulted in lower Zn concentration (Alloway, 2009) in grain as well. So, to produce optimum quantity and better grain Zn concentration, application of Zn is necessary (Rehman *et al.*, 2012). Grain Zn content in wheat and other cereals *i.e.*, rye and triticale decreased by more than 80% grown under Zn deficient soils (Cakmak *et al.*, 1997). Grain bio-fortification is viable strategy to increase the grain Zn concentration in order to reduce the wide spread Zn deficiency (Farooq *et al.*,

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2018). Different approaches are used for grain bio-fortification *i.e*., breeding approaches; the costly (Cakmak, 2008; Johnson-Beebout *et al.,* 2009) and micronutrient fertilization is latter being a cost-effective approach for increasing Zn in grains (Cakmak, 2008; Phattarakul *et al.,* 2012).

Application of Zn can be done by different methods like, seed priming, soil and foliar application (Rehman *et al*., 2018a, b, c). Soil application is mostly done for greater supply of nutrients required in large quantity, while foliar feeding provides the quick remediation to Zn deficient plants particularly in the situation of low Zn availability (Yilmaz *et al.,* 1997; Cakmak *et al.,* 2010). Seed treatment is another viable option for the delivery of micronutrients including Zn (Farooq *et al.,* 2012). It was hypothesized that application of Zn through various methods may increase the grain production and grain Zn concentration. Therefore, this study was conducted to assess the most effective method of Zn application for increasing the grain yield and grain Zn concentration of wheat.

Materials and Methods

Experimental site, Soil and Climatic Conditions

The present study was conducted at Student Research Farm, Department of Agronomy, University of Agriculture, Faisalabad (31°N, 73°E, 184.4 m asl), Pakistan during 2013–14 and 2014–15. The soil was sandy loam having 7.75 pH, 1.06 dS m^{-1} electrical conductivity, 0.85% organic matter, 0.033% available nitrogen, 21 mg kg^{-1} available phosphorus, 130 mg kg^{-1} potassium, 32 mg kg^{-1} DTPA extractable Zn and 5.89% calcium carbonate. Total nitrogen, extractable phosphorus, and potassium were determined by the standard procedures of Hanway and Heidel (1952), Olsen *et al.* (1954), Bremner and Mulvaney (1982) respectively. Regarding climatic conditions, maximum mean air temperature was recorded in April 32.2 and 33.2°C during 2013–2014 and 2014–2015, respectively, while minimum air temperature was noted in the month of January 6.1°C during 2013–2014 and 5.9°C during 2014–2015. Maximum monthly total rainfall was recorded in the month of March (41.7 mm) and (67.9 mm) during 2013–2014 and 2014–2015, respectively.

Experimental Design and Treatments

Seeds of wheat cultivar AAS-2011 were obtained from wheat research institute Faisalabad. The randomized complete block design with three replications was used for study. The study consisted of soil applied Zn (10 kg ha^{-1}), foliar application (0.5% Zn solution at booting and milking stages) and seed priming (0.3 *M* Zn). In seed priming, seeds were soaked in 0.3 *M* Zn by keeping seed and solution ratio of 1:5 for 12 h. Thereafter, seeds were washed with distilled water and dried until the original weight. Dried seeds were kept in plastic bags and stored in refrigerator at 8 ± 1°C. The $ZnSO_4.7H_2O$ was used as Zn source in this experiment.

Land Preparation and Crop Husbandry

The information, regarding soil preparation, time of sowing, seed rate, application of fertilizers, irrigation and harvesting time are given as the supplementary Table S1. In both years, nitrogen (N) was applied as urea (46% N) and phosphorus as single super phosphate (21% P) while potassium in the form of sulphate of potash (50% K). All P, K and half of N was applied at sowing while; reimagining N was applied in two splits at tillering and milking stage.

Observation and Measurements

Numbers of productive tillers were Recorded from an area of 1 m^{-2} from each plot at the time of maturity. Number of grains per spike, and spikelets were recorded by the random selection of ten spikes from each experimental treatment. A sub sample of 1000 grains was taken from the threshed grains in order to determine the 1000 grain weight. Moreover, the whole plots were harvested, sun dried, tied into bundles and weighed to determine the biological yield. The dried bundles were threshed with mechanical thresher and grain yield was measured and was converted into ton ha^{-1}. Harvest index was measured as ratio between grain and biological yield. The value of partial factor productivity was determined using the formula given by Fageria and Baligar (2003). Agronomic use efficiency was calculated using the formula of Fageria (2009) while, Zn mobilization efficiency index was calculated according to the equation of Srivastava *et al.* (2009).

For grain Zn concentration, grains were dried in oven at 60°C for 48 h (Liu *et al*., 2006), after which samples were grinded in mill fitted blades and stainless-steel chamber. The 1 g of ground wheat samples were digested in di-acid ($HClO_4$: HNO_3 3:10 ratio) (Prasad, 2006) on a digestion plate. The concentration of Zn was determined by the atomic absorption spectrophotometer (Perkin Elmer, CA, USA).

Data Analysis

Data were statistically analyzed by using Statistix 8.1. Least significant difference (LSD) test was employed to compare the treatment means at 5% probability (Steel *et al.,* 1997). The year effect was non-significant; thus, two-year data was pooled and averaged. To know the net benefit and benefit cost ratio of Zn application methods economic analysis was done following protocol of (CIMMYT, 1988).

Table S1: Detail of crop husbandry practices of wheat during 2013–2014 and 2014–2015

Preparation of Seedbed	Time of sowing	Seed rate (kg ha^{-1})	Weed control	Irrigations	Fertilizers (kg ha^{-1})	Crop harvesting
2013-2014						
Soaking irrigation + 4 cultivations + 2 plankings* + direct drilling	November 24, 2013	125	Topik 15WP (Clodinafop-propargyl 300 g a.i. ha^{-1}) at tillering	6 irrigations (Tube well + canal source)	N:P:K at 100:90:60	April 18, 2014
2014-2015						
Soaking irrigation + 4 cultivations + 2 plankings + direct drilling	November 26, 2014	125	Topik 15WP (Clodinafop-propargyl 300 g a.i. ha^{-1}) at tillering	6 irrigations (Tube well + canal source)	N:P:K at 100:90:60	April 21, 2015

***=** Planking is a regional term meaning leveling seedbed after plowing and cultivation; N= Nitrogen; P= Phosphorous; K; Potassium

Table 2: Analysis of variance for influence of zinc application methods and years on yield and yield attributes of wheat

Treatments	DF	Number of productive tillers (m^{-2})	Spikelet's per spike	Grains per spike	1000-grain weight (g)	Biological yield (Mg ha^{-1})	Grain yield (Mg ha^{-1})	Harvest index (%)
Zinc application methods	3	1390.15**	10.54**	95.93*	136.42*	1.461**	1.378**	729.54**
Year	1	260.04NS	0.081NS	22.04 NS	15.04NS	0.070NS	0.129NS	4.860NS
Zinc application methods × Year	3	0.37NS	0.019NS	0.708NS	0.38NS	0.028NS	0.012NS	0.321NS

DF= degree of freedom; ** at $P \leq 0.01$; * at $P \leq 0.05$; NS: Non-significant

Table 3: Analysis of variance for influence of zinc application methods on partial factor productivity, agronomic use efficiency and zinc mobilization efficiency index

Treatments	DF	Partial factor productivity	Agronomic use efficiency	Zn mobilization efficiency index
Zinc application methods	3	8381596**	2188.67**	0.57764**
Year	1	2112^{NS}	251.54^{NS}	0.00634^{NS}
Zinc application methods × Year	3	1737^{NS}	158.45^{NS}	0.00104^{NS}

DF= degree of freedom; ** at $P \leq 0.01$; * at $P \leq 0.05$; NS: Non-significant

Results

Analysis of Variance

The analysis of variance (ANOVA) indicated that Zn application methods had significant effect on all studied observations; however, effect of years (Y) and their interaction was non-significant for all the observations. Thereby, the data of both years were pooled and averaged.

Yield and Yield Components

The application of Zn had significant effect on yield and yield contributing traits while, the year's effect and interaction between years × Zn application methods was non-significant for all studied traits (Table 2). The application of Zn through different methods considerably influenced the yield traits (Table 4). Maximum productive tillers (343) was recorded with soil applied Zn followed by seed priming. Likewise, maximum spikelets and grains per spike were recorded with soil application followed by seed priming and foliar application and lowest was noted with no Zn control (Table 4).

Soil application of Zn was more effective in improving the 1000-grain weight than other Zn application methods. Maximum 1000-grain weight (51.67 g) was noticed with Zn soil application followed by seed priming and lowest 1000-grain weight (40.83 g) was recorded for no Zn (Table 4). The maximum biological and grain yields were recorded with Zn soil application followed by seed priming and foliar application while, lowest was observed in control (Table 4). Maximum biological and grain yields were recorded with soil applied Zn afterwards seed priming and lowest was in control (Table 4). Moreover, Zn application methods significantly affected the harvest index. Maximum harvest index (40.41%) was recorded with soil applied Zn followed by seed priming, whereas the lowest harvest index (36.24%) was observed for no Zn application (Table 4).

Grain Zn Concentration

The results indicated that maximum grain Zn concentration was noted with foliar applied Zn followed by soil application, whereas the lowest was recorded where no Zn was applied (Fig. 1).

Partial Factor Productivity (PFP), Agronomic use Efficiency (AUE) and Zinc Mobilization Efficiency Index (ZMEI)

The application of Zn had significant effect on PFP, AUE and ZMEI while, the year's effect and interaction between years × Zn application methods was non-significant for these traits (Table 3). The maximum PFP was recorded for foliar Zn application followed by seed priming

Table 4: Influence of zinc application methods on yield and yield attributes of wheat

Treatments	Number of productive tillers (m^{-2})	Spikelet's per spike	Grains per spike	1000-grain weight (g)	Biological yield (Mg ha^{-1})	Grain yield (Mg ha^{-1})	Harvest index (%)
Control	308 C	15.31 C	40.83 C	40.88 D	15.27 C	5.53 C	36.24 C
Seed priming	324 B	17.35 AB	45.66 B	48.67 B	16.03 AB	6.09 B	37.99 B
Soil application	343 A	18.41 A	50.33 A	51.67 A	16.42 A	6.63 A	40.41 A
Foliar application	314 BC	16.38 BC	43.67 BC	44.18 C	15.67 BC	5.75 BC	36.69 BC
LSD ($P \leq 0.05$)	14.49	1.41	3.27	2.26	0.45	0.37	1.62

Means have same letter not differed at $P \leq 0.05$

Table 5: Influence of zinc application methods on partial factor productivity, agronomic use efficiency and zinc mobilization efficiency index

Treatments	Partial factor productivity	Agronomic use efficiency	Zn mobilization efficiency index
Control	-	-	2.43 A
Seed priming	531.6 B	50.03 B	1.83 C
Soil application	132.8 C	22.00 C	2.02 B
Foliar application	2350.0 A	100.00 A	2.43 A
LSD ($P \leq 0.05$)	98.51	4.73	0.19

Means have same letter not differed at $P \leq 0.05$

Table 6: Economic analysis for the effect of Zn application methods

Zinc application	Grain yield (Mg ha^{-1})	Adjusted grain yield (Mg ha^{-1})	Straw yield (Mg ha^{-1})	Adjusted straw yield (Mg ha^{-1})	Grain value ($)	Straw value ($)	Gross Income($)	Permanent cost ($)	Variable cost ($)	Total cost ($)	Net benefit ($)	BCR
Control	5.53	4.98	9.74	8.77	1456.43	328.69	1785.12	456.89	---	456.89	999.53	3.91
Seed priming	6.09	5.48	9.94	8.95	1603.94	335.44	1939.36	456.99	21.76	478.65	1125.26	4.05
Soil application	6.63	5.97	9.79	8.81	1746.13	330.38	2076.52	456.89	22.25	479.15	1266.98	4.33
Foliar application	5.75	5.17	9.92	8.93	1514.37	334.77	1849.14	457.0	42.21	499.11	1015.26	3.70
					$11.70/40 kg	$ 1.52/40 kg						

BCR= benefit cost ratio

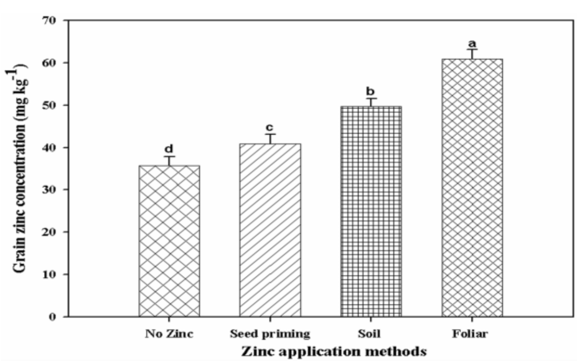

Fig. 1: Effect of Zn application methods on grain zinc concentration (mg kg^{-1})

while, lowest PFP was recorded with Zn soil application (Table 5). Likewise, maximum AUE and ZMEI was recorded for foliar applied Zn, whereas, the lowest AUE and ZMEI with soil applied Zn (Table 5).

Economic Analysis

The Zn application through different methods significantly increased the net benefit and benefit cost ratio in wheat. Zinc soil application gave maximum net returns ($ 1266.98) and benefit cost ratio (4.33) followed by seed priming and foliar application of Zn (Table 6).

Discussion

The application of Zn through different methods substantially improved the yield, its attributes and grain Zn concentration. Application of Zn substantially increased the number of productive tillers (Table 4) owing to increase in the translocation of photosynthates, enzymatic activation and improvement in auxin metabolism as, deficiency of Zn reduces enzymatic activities and auxin metabolism which decreases the number of tillers (Maqsood *et al.*, 1999; Khan *et al.*, 2006).

Zinc application increased the spikelets, grains per spike and 1000-grain weight (Table 4) as application of Zn increased physiological functions (Bodruzzaman *et al.*, 2002) which increases the translocation of photosynthates to affect the grains per spike and 1000-grain weight (Soleimani, 2006). Moreover, application of Zn increased the pollination in plants by affecting fertilization and development of pollen tube (Kaya and Higgs, 2002). Improvement in the yield and yield contributing traits might be due to involvement of Zn in carbohydrates metabolism, indole acetic acid, RNA and functions of ribosomes (Khalifa *et al.*, 2011). Continuous uptake of Zn during later stages *i.e.*, grain filling stages and its continuity in loading of endosperm from xylem improve plant growth, grain formation and seed setting (Yin *et al.*, 2016; Rehman *et al.,* 2018b) resulting in better crop performance in terms of yield.

The results indicated that Zn application methods significantly affected the grain Zn concentration; nonetheless foliage applied Zn performed appreciably better than other methods (Fig. 1). The desired quantity of Zn in wheat grain can be attained through adjusting rate and time of foliar applied Zn (Cakmak *et al.*, 2010). Foliar application of Zn significantly improved the grain Zn concentration compared to soil application, even though small quantity of Zn is applied through foliar application (Erdal *et al.,* 2002; Cakmak *et al.,* 2010).

Soil applied Zn has poor mobility in soil and it quickly gets fixed with soil particles in calcareous soils with high pH (Alloway, 2008). Moreover, wheat roots and soil applied Zn has different distributions in the soil profile, which reduced the Zn uptake by roots and therefore, the grain Zn concentration (Holloway *et al.*, 2010). Top soil is mostly dried at reproductive stages and the root activities usually declined owing to less allocation of assimilates (Zhang *et al.*, 2012).

The uptake of Zn from soil as well as the applied Zn sources considerably reduced at later stages which resulted in the less Zn accumulation in grain at reproductive stages (Zhang *et al.*, 2010). Foliar applied Zn maintained higher pool of Zn availability within plant tissues during reproductive stages of plant and thereby, leads to significant increase in the grain bio-fortification (Zhang *et al.*, 2012; Chattha *et al.*, 2017). Therefore, the agronomic biofortification appears to be highly impressive and short term solution to combat micronutrients deficiency (Cakmak *et al.,* 2010).

The foliar feeding during later growth stages maintain a significant pool of Zn in vegetative tissues and contributes remarkably towards the bio-fortification of wheat under field conditions (Zhang *et al.,* 2012; Chattha *et al.*, 2017). The maximum partial factor productivity, agronomic use efficiency and Zn mobilization efficiency index were recorded with foliage applied Zn (Table 5) because foliage applied Zn is easily taken up by plants and translocated into reproductive parts (Ghasal *et al.*, 2017) resulting in better Zn efficiency. The maximum benefit cost ratio and net returns were recorded with soil applied Zn followed by seed priming. Use of Zn to prime seeds is an attractive and cost-effective strategy that helps to improve the economic returns (Harris *et al.*, 2008).

Conclusion

Zn deficiency had negative effect on the yield, yields related attributes and grain quality of wheat, however, Zn application through either method (soil application, seed priming and foliage applied) improved the yield and yield components. In this study, soil applied Zn proved most effective and economical method for increasing the grain yield of wheat; however, foliar spray of Zn was most effective in enhancing the grain quality of wheat compared with other Zn application methods. Therefore, the foliar application of Zn is a better option to improve the grain Zn concentration for healthy human life.

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