

Optimizing Zinc Content Through Agronomic Bio-Fortification To Enhance Wheat Productivity

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Abstract

Zinc (Zn) is required for maintenance of different functions in plants and humans. Its deficiency leads to abnormal functioning and certain diseases. So, zinc should be taken in appropriate amount as part of daily diet. Majorly, countryside based poor populations consume cereals as major part of their diet which usually have poor nutritional quality due to less availability of micronutrients such as zinc (Zn), iron (Fe) and vitamins. Therefore, most people are suffering from hidden hunger in Pakistan. This experiment was planned to study the interaction of different Zn application methods with two contrasting genotypes of wheat with respect to growth, yield and Zn accumulation. The experiment was performed with two factors: first factor was two different wheat genotypes having different zinc uptake potential (Akbar-19 and Faisalabad-2008) and second factor was different application methods of zinc fertilizer. No zinc fertilizer was applied in control. Zinc applications was done via soil application (10 kg Zn ha⁻¹), foliar application (1% w/v ZnSO₄), seed coating (1.25 g Zn kg⁻¹) and seed priming (0.5M ZnSO₄). Results show that Akbar-19 had significantly higher plant height (93.74 cm) and spike length (11.30 cm) than Faisalabad-2008. However, Faisalabad-2008 exhibited higher 1000-grain weight (43.22 g), the number of tillers m⁻² (524.50) and biological yield (15.47 t ha⁻¹). Among Zn application methods, soil application method was better in terms of¹ number of tillers m⁻² (555.31), 1000 grain weight (46.43 g), number of grains per spike (63.16), biological yield (16.40 t ha⁻¹), and harvest index (33.67%). In terms of yield, soil application method provided the greatest grain yield (5.06 t ha⁻¹), 16.85% higher than control method and Faisalabad-2008 provided the greatest grain yield (4.59 t ha⁻¹), 7.24% higher than Akbar-19. While foliar application method provided the highest grain zinc contents (43.33 mg kg⁻¹) which is 40.49% higher than control method and Akbar-19 provided highest grain zinc contents (35.34 mg kg⁻¹) which is 3% higher than Faisalabad-2008. Soil application and Faisalabad-2008 can provide the optimal yield and productivity while foliar application in Akbar-19 can provide optimal grain zinc contents.

Keywords: Biofortification, Zinc Application, Zinc Deficiency, Wheat genotypes, Grain Zinc Contents, Crop quality, food security.

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Introduction

Wheat is one of the earliest domesticated crops and belongs to Poaceae family. It is adapted to a wide range of environmental conditions across the whole world and it contributes around 35% in total grain production of the world and feeds the largest population of the world (Miransari and Smith, 2019). Pakistan is rated at eighth position in the world for wheat production, with Punjab accounting for 70% of the country's total wheat producing area (Ahmad et al., 2021). Wheat satisfies the 60% of daily dietary requirement of population in Pakistan (Ramzan et al., 2020). It is grown on an area of 9.18 million hectares and its production is 27.29 million tons. Its average yield is 2.97 tons ha⁻¹ and it contributes 1.8% to the GDP of Pakistan (Economic Survey of Pakistan, 2020-2021). Wheat is a key source of nourishment for both people and animals. It is used to produce breads, biscuits, noodles, pizza, snacks, semolina, and desserts (Ramzan et al., 2020).

Plants and humans both require zinc for optimal development. It aids in chemical reactions by stimulating enzymes. It is an important part of superoxide dismutase (antioxidant). Zinc aids in the regulation of functions of Rubisco, which helps the entrance of carbon dioxide (CO₂) into the photosynthetic dark cycle (Zaman et al., 2018). Zinc affects the photosynthetic activity, auxin synthesis, and it also affects on the yield and quality of food (Rakesh and Jitendra, 2014). Zinc helps in the formation of neutrophils and killer cells (Maares and Haase, 2016). Zinc deficiency increases the risk of infections and lowers the body's ability to produce antibodies. Ratio of infertility, Diarrhea and mortality are all increased by zinc deficiency (Khalid et al., 2014). Zinc application increases the grain Zn contents as well as grain yield (Zou et al., 2012).

An estimated 800,000 individuals and 1.5 million children die every year as a result of zinc deficiency (Ackland et al., 2016). Around the world, almost 2 billion people do not have access to sufficient amount of zinc (WHO, 2002). In Asia and Africa, almost 50% population is prone to zinc deficiency (Hussain et al., 2010). In Pakistan, Zinc deficiency affects almost 22% of population, primarily women and children (Rehman et al., 2020). Major reason of zinc deficiency is the excessive consumption of low quality wheat which has limited zinc concentration and its bioavailability (Cakmak et al., 2010; Welch and Graham, 2004). Growing wheat in zinc deficient soils is also another reason for low zinc wheat. It is estimated that almost 50% soils are deficient in zinc (Alloway, 2004; Cakmak, 2008).

Zinc daily requirement depends upon age group and gender. Zinc consumption for those aged 19 years and above, is recommended on daily basis at 11 mg and 8 mg per day for men and women respectively. Zn consumption for pregnant women should be 11 mg and 12 mg for breastfeeding mothers on a daily basis (National Institute of Health, 2021). Zinc requirement of body can be satisfied by using multiple methods, i.e. fortification and supplementation. However, these methods are expensive and poor people cannot afford them. The ideal option is biofortification (improvement in nutritional content of plants by agronomic approaches, selective breeding, and genetic approaches). This procedure is less costly than others (Saltzman et al., 2013). Agronomic biofortification (involving fertilizer approach), is a better and quick option for quality improvement (Cakmak, 2008). The use of appropriate fertilizer to boost the nutrient content and to improve the crop quality is referred to as agronomic biofortification. Agronomic biofortification enhances the zinc content of the crop's edible part (Frossard et al., 2000; Wang et al., 2017). ZnSO₄ is used as inorganic fertilizer to improve the crop and soil productivity and it is available in crystalline and granular forms. Zinc sulphate is useful and affordable to increase the fertility of crops. Due to the fact that Zn is an essential but a non renewable metal, further studies are needed to optimize the use of Zn application methods on crops or plants (Mortvedt and Gilkes, 1993).

Different wheat cultivars would have different interaction with different application method. Literature is sketchy about the interaction of cultivars with different zinc application methods especially seed coating and seed priming. For this study, we have selected two contrasting genotypes having different Zn uptake potential. This experiment is planned to study the interaction of different Zn application methods with two contrasting genotypes of wheat with respect to Zn accumulation.

Materials and Methods

Experimental Site and Planting Material

This study was performed at Agronomic research area, University of Agriculture, Faisalabad. This experiment conducted to study the effect of different zinc treatments methods in different wheat cultivars having different genetic potential for zinc in order to check the Zn accumulation and best Zn application method. Temperature range of this area was in between of -1°C to 48°C from Jan to June. Prevailing weather conditions are shown in below table 1. Seeds for two wheat varieties, Faisalabad-2008 and Akbar-19 were taken from Ayub Agriculture Research institute, Faisalabad and Agronomic Research Farm, University of Agriculture, Faisalabad.

Table 1: Weather figures of full wheat crop period 2021-2022

Months	Temperature			RH (%)	Wind Speed (Km/h)	Rainfall (mm)	Pan Evaporation (mm)	Sunshine radiation (hours)
	Max.	Mini.	Avg.					
	November-2021	28.7	11.2					
December-2021	22.0	6.0	14.0	81.6	2.9	0.4	01.0	6.7
January-2022	17.8	08.1	13.0	88.5	3.9	48.4	00.7	3.2
February-2022	23.9	08.6	16.3	82.9	4.7	28.2	02.0	7.6
March-2022	32.7	17.7	25.2	66.4	5.6	30.1	04.2	9.3
April-2022	41.0	22.0	31.5	42.5	15	5.9	07.3	10.3

Experiments and Treatments

The experiment was performed with two factors: first factor was two wheat genotypes (Akbar-2019 and Faisalabad-2008) and second factor was different zinc application methods. The experiment included two factors, Factor A was two wheat varieties $V_1 = \text{Akbar-2019}$, and $V_2 = \text{Faisalabad-2008}$ Factor B: Zinc Sulphate application $Z_0 = \text{Control}$, $Z_1 = \text{Soil application}$, $Z_2 = \text{Foliar application}$, $Z_3 = \text{Seed coating}$, $Z_4 = \text{Seed priming}$. Source of Zinc was zinc Sulphate fertilizer $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. "Naya Zinc," was used as the source of the zinc which contains 21% Zn as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

Control method Z_0 was performed with no zinc while in Soil application method Z_1 , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ Fertilizer was applied rate of 10 kg Zn ha^{-1} , for Foliar Z_2 , 1% Zinc Sulphate fertilizer was applied at two important growth stages i.e. booting and milking. For coating Z_3 , Seeds were coated with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at the rate of $1.25 \text{ g Zn kg}^{-1}$, and for priming Z_4 , seeds were primed with 0.5M $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution. Furthermore, for Z_1 , zinc Sulphate fertilizer was first applied at surface and then incorporated in soil before sowing of crop. For Z_2 , 1% Zinc sulphate solution was applied first at booting and then at milking stge. For Z_4 , Seeds were soaked in 0.5M Zinc sulphate solution and washed with distill water and then after drying, primed seeds were kept inside refrigerator in air tight bags.

Crop Husbandry

The land was prepared before sowing by applying two ploughing operations on soil followed by planking, in order to make the soil smooth level for sowing. Then soil was supplied with rauni irrigation. Wheat seeds of both varieties were sown on 08-12-2021 at Agronomic Research Farm, University of Agriculture Faisalabad. Seeds were sown when the soil moisture was at field capacity after rauni irrigation, by using hand drill and seed rate was 100 kg ha⁻¹ as per recommendation. For N: P: K fertilizer application, urea 46% nitrogen, di ammonium phosphate (DAP) with 46% phosphorus, sulphate of potash SOP was used at the rate of 120:100:60 kg ha⁻¹. One third nitrogen, complete phosphorus and potassium dose applied at the time of sowing while remaining two third nitrogen doses applied at two stages tillering and booting.

To avoid all kind of pests, Standard methods of weeds, diseases and pest management were used in order to eradicate them. Water requirement of wheat crop is 5-6 irrigation application. First watering was applied 22 days after sowing and farther watering was applied to crop as per requirement. Flood irrigation was done for watering the experimental plots. Irrigation requirement of wheat crop depends on multiple different factors like rainfall, soil, temperature and crop varieties. Wheat crop was harvested at full maturity on 20 April, 2022. Both crop varieties were manually threshed 30 April, 2022.

Physico-Chemical Properties of Soil

Soil samples were collected from 30 cm depth using augur at experimental sight and then dried, grinded, passed through 2mm sieve. Physico-chemical properties of collected samples were checked in lab and results are shown in table 2.

Table 2: Physico-chemical properties of soil

Characteristics	Unit	Values
Nitrogen (N)	%	0.46
Phosphorus (P)	Ppm	6.7
Potassium (K)	Ppm	130
SAR	-	7
Zinc (Zn)	Ppm	0.48
ESP	-	8
EC	dSm ⁻¹	0.98
Organic matter	%	1.2
Texture	-	Loam
Sand	%	43
Silt	%	41
Clay	%	17
pH	-	7.9
Saturation	%	33

Sampling and Measurements

Numbers of productive tillers were counted from one square meter of each plot. Thousand grain weight of each plot was recorded. In order to measure it, 100 grains were counted and taken randomly from subplot and their weight was measured. Recorded weight of grains was

multiplied with 10 and resultant value was 1000-grain weight. When wheat crop reached maturity, each plot was harvested and piled. Then weight of piles from each treatment was measured using weighing balance separately in order to record biological yield. Piles which were taken from each subplot, threshed manually and grains obtained from them was weighed to calculate the grain yield. Grain yield is also called economical yield. Harvest index determines about the total output production of crop or plant and it is calculated by dividing the grain yield (economical yield) with biological yield.

$$\text{Harvest Index \%} = \frac{\text{Grain Yield}}{\text{Biological yield}} \times 100$$

Sample Preparation and Analysis

Grains samples were taken from each plot and grounded in mill having fine sharp blade fitted inside a steel chamber. Then those samples were digested. For digestion, 1g grounded samples were mixed with two acid solutions. HNO₃ and HClO₄ were added with ratio of 2:1 (Jones and Case, 1990). The mixture left overnight. After keeping the digestion mixture for 24 hours, Hot plate was used for further digestion of grounded samples at 150°C and samples were digested until mixture became colorless. Then digested mixture was cooled and distilled water was added to attain the desired 50ml volume of sample. Digested mixture was filtered with the help of whatman filter paper and stored in air tight containers. Zinc contents were measured with help of atomic absorption spectrophotometer (PerkinElmer, 100 AAnalyst, Waltham, MA, USA).

Experimental Design

Experiment was conducted with randomized complete block design (RCBD) with factorial arrangement and three replications. Row to row distance for sowing of wheat was 22.5 cm.

Statistical Analysis

Evaluation of recorded observations and data analyzation was performed with the help of Statistix 8.1 (Analytical, Tallahassee, FL, USA) and mean comparison test of all treatments was done with LSD at 5% level of probability.

Results

Results of this study revealed that both genotypes had significant affects on crop growth parameters depending on their genetic potential such as Faisalabad-2008 performed better in terms of yield parameters while Akbar-19 showed optimal potential for physiological growth. Soil zinc treatment had appeared as the optimal method for growth and yield parameters while foliar treatment was recorded for optimal grain zinc content with Akbar-19

Plant Height (cm) and Spike Length (cm)

Table 3 showed that Akbar genotype affected the plant height and spike length significantly. Akbar showed greater plant height (95.47 cm) under the soil application of zinc sulphate and highest spike length (11.39 cm) was obtained from Akbar-19 under foliar zinc application. While lowest plant height and spike length (87.60, 9.89 cm respectively) was recorded in Faisalabad-2018 under seed coating treatment.

Table 3: Plant height (cm) and Spike length (cm) in different wheat genotypes under different Zinc treatments

Wheat Genotypes				
Zn Treatments	Plant Height (cm)		Spike Length (cm)	
	Akbar-19	Faisalabad-2018	Akbar-19	Faisalabad-2018
Control	93.33±3.16	89.40±3.03	11.29±0.51	10.56±0.49
Soil Application	92.33±4.10	92.07±4.82	11.63±0.50	10.61±1.08
Foliar Application	95.47±4.24	87.60±1.44	11.39±0.40	10.25±0.36
Seed Coating	92.20±4.30	89.47±2.54	11.18±0.79	9.89±0.66
Seed Priming	95.40±2.09	90.07±2.50	11.03±0.56	10.68±0.12

Number of Productive Tillers (m⁻²)

Number of productive tillers was significantly affected by genotypes and zinc treatments. Faisalabad-2008 was observed with maximum (565.81) number of productive tillers under the soil zinc treatment while the minimum number of tillers (477.67) was noted from seed coating shown in table 4.

Table 4: Number of productive tillers in different wheat genotypes under different Zinc treatments

Zn Treatments	Wheat Genotypes	
	Akbar-19	Faisalabad-2018
Control	501.78±5.97	513.33±2.52
Soil Application	544.80±6.58	565.81±13.86
Foliar Application	521.67± 31.13	534.83±5.56
Seed Coating	477.67±11.50	495.53±5.82
Seed Priming	461.69± 5.94	513.00±3.61

No of Grains per Spike and 1000-Grain Weight (g)

Figure 1 show that no of grains per spike and 1000 grain weight was substantially affected by wheat genotypes and zinc treatments. Faisalabad-2008 provided the maximum (63.16, 50.70 g respectively) for number of grains spike⁻¹ and 1000 grain weight under the soil zinc application. Control method of Akbar-19 was recorded with minimum number of grains spike⁻¹ (54.66) while seed coating of Akbar-19 was observed with minimum 1000 grain weight (35.26 g). The interactive effect between genotypes and zinc application method was also significantly impacting the number of grains per spike and 1000 grain weight (Figure 2).

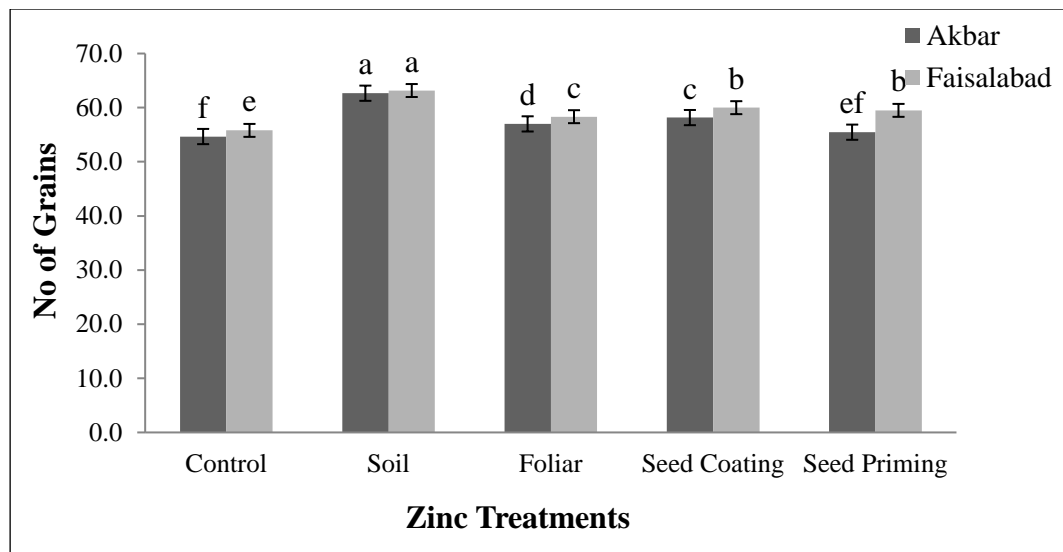


Figure 1: No. of grains per spike in different wheat genotypes under different Zinc treatments. Data represent mean \pm SD, followed by different letters on the top of bars indicate significant differences as per Tukey’s HSD test ($P \leq 0.05$).

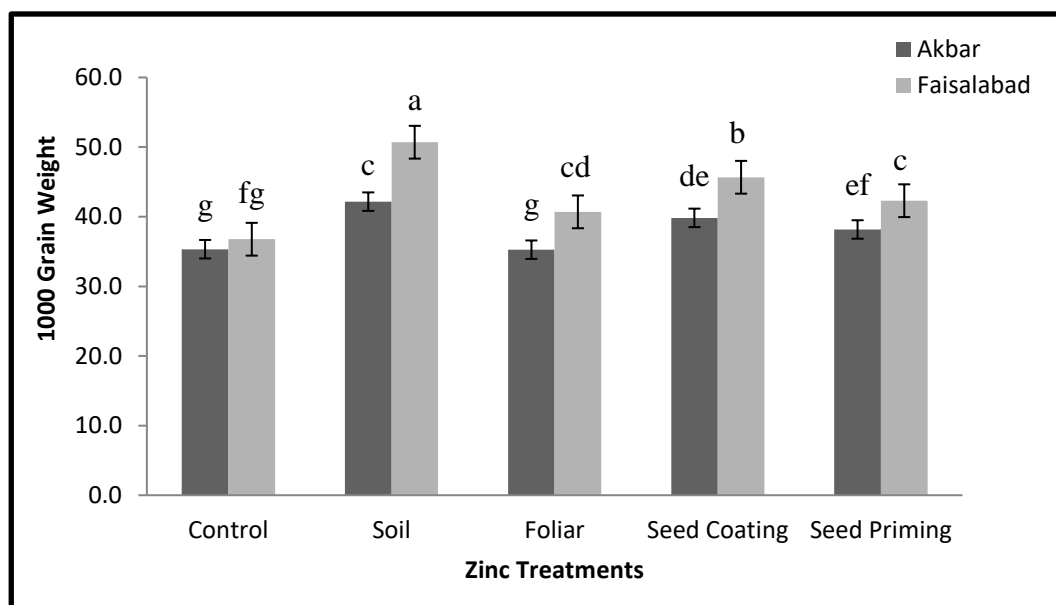


Figure 2: 1000 grain weight in different wheat genotypes under different Zinc treatments. Data represent mean \pm SD, followed by different letters on the top of bars indicate significant differences as per Tukey’s HSD test ($P \leq 0.05$).

No of spikelets Spike⁻¹ and Grain weight Spike⁻¹ (g)

Maximum number of spikelets and grain weight spike⁻¹ was noted from Faisalabad-2008 with soil zinc treatment (19.06, 2.28 g respectively). But this trend was statistically non-significant (Table 5).

Table 5: No of spikelets Spike⁻¹ and Grain weight Spike⁻¹ (g) in different wheat genotypes under different Zinc treatments

Wheat Genotypes				
Zn Treatments	No of spikelets		Grain weight Spike⁻¹ (g)	
	Akbar-19	Faisalabad-2018	Akbar-19	Faisalabad-2018
Control	16.9±1.01	17.53±0.42	1.717±0.07	1.72±0.05
Soil Application	18.66±0.42	19.06±0.42	2.11±0.29	2.28±0.20
Foliar Application	17.33±1.17	17.33±0.81	1.59±0.02	1.59±0.35
Seed Coating	18.20±0.60	18.33±0.42	1.80±0.38	1.83±0.35
Seed Priming	17.80±2.55	18.00±0.35	1.91±0.66	1.84±0.31

Biological Yield (t ha⁻¹) and Grain yield (t ha⁻¹)

Table 6 showed the impact of wheat genotypes and zinc application methods on biological and grain yield also called economic yield. Wheat genotypes and zinc application methods significantly affected the both biological and grain yield. Faisalabad-2008 treated with soil application of zinc provided the highest biological and grain yield (17.46, 5.22 t ha⁻¹). Lowest biological and grain yield was obtained from Akbar-19 with foliar zinc application (13.63, 3.86 t ha⁻¹).

Table 6: biological and grain yield (t ha⁻¹) in different wheat genotypes under different Zinc treatments

Wheat Genotypes				
Zn Treatments	Biological Yield (t ha⁻¹)		Grain yield (t ha⁻¹)	
	Akbar-19	Faisalabad-2018	Akbar-19	Faisalabad-2018
Control	14.56±1.12	15.19±0.47	4.33±0.06	4.34±0.11
Soil Application	15.34±0.91	17.46 ±2.88	4.90±0.11	5.22±0.64
Foliar Application	13.63±0.81	14.39±1.06	3.86±0.09	4.34±0.36
Seed Coating	14.64±1.07	15.51±0.27	4.38±0.13	4.62±0.11
Seed Priming	14.66 ±0.12	14.79±0.30	3.92±0.38	4.41±0.25

Harvest Index (%)

Results of this study for harvest index showed the improvement when crop was zinc treated. Harvest index had significantly impacted by genotypes and zinc treatments but their interactive affect provided no significant impact (Table 7). Faisalabad-2008 was noted with highest harvest index (34.17%) when treated with soil zinc application and lowest harvest index was recorded from Akbar-19 with control treatment (26.74%).

Table 7: Harvest index (%) in different wheat genotypes under different Zinc treatments

Zn Treatments	Wheat Genotypes	
	Akbar-19	Faisalabad-2018
Control	26.76±3.49	32.00±2.61
Soil Application	33.17±3.76	34.17±2.04
Foliar Application	29.87±0.84	28.36±1.71

Seed Coating	32.18±1.19	32.51±2.33
Seed Priming	29.68±0.60	27.04±1.31

Zinc Contents (mg kg⁻¹)

Table 8 showed that significance of treatments or combination of treatments on zinc contents. Different zinc application methods (no zinc, soil application, foliar application, seed coating, and seed priming) provided the highly significant effect on zinc contents. Varieties i.e. Akbar-19 and Faisalabad-2008 provided the significant effect while the interactive factor ($V \times Zn$) between varieties and zinc application method had given the non-significant impact on zinc contents.

Akbar-19 provided the maximum grain zinc contents (44.33 mg kg⁻¹) when treated with foliar zinc spray and Faisalabad-2008 provided the minimum grain zinc contents (29.37 mg kg⁻¹) under soil zinc treatment.

According to the results of this study, foliar application method provided the greatest zinc contents due to the fact that wheat crop had enough Zn availability during reproductive stage. Foliarly applied is phloem mobile and easily translocated into emerging grains. Minimum value was recorded from soil application method for zinc contents because of the fact that soil had high pH.

Table 8: grains zinc contents (mg kg⁻¹) in different wheat genotypes under different Zinc treatments

Zn Treatments	Wheat Genotypes	
	Akbar-19	Faisalabad-2018
Control	31.27±2.87	30.42±2.50
Soil Application	29.52±1.58	29.37±1.99
Foliar Application	44.33±1.62	42.33±2.10
Seed Coating	37.63±1.17	35.16±2.79
Seed Priming	33.96±3.26	33.96±3.53

Discussion

This study focused on the evaluation of different crop parameters under the treatments of wheat genotypes and zinc genotypes. Findings showed that wheat genotypes and zinc treatments had substantial effect on different agronomic traits of crop while their interaction did not give significant effect mostly. Akbar-19 was noted for optimal plant height potential and Faisalabad-2008 was observed with optimal overall yield parameters including number of productive tillers, no of grains, spikes, grain weight, biological yield and grain yield. This reflects the importance of selecting optimal genotype and zinc treatment combination for maximum crop growth, yield and quality improvement because different genotypes have different potentials for crop productivity and nutrient uptake and genotypes have different interaction with various zinc treatments. Rizwan et al. (2017) similarly stated that crop variety and zinc method selection is vital for optimum biofortification and crop productivity.

Results of this study revealed that wheat genotypes had clear substantial effect on plant height. Akbar-19 had shown the maximum potential for plant height. Even though zinc application methods had no significant impact but the trend showed that soil application could provide the maximum potential for plant height. These findings are similar to previous study where soil treatment found with maximum plant height potential by improve functions of plant, growth regulators, vegetative growth (Kaya and Higgs, 2002).

Although both genotypes and zinc treatments were not statistically significant impacting the spike length but Akbar-19 with soil zinc application provided the optimum spike length.

Similarly, Hassan et al. (2019) studied that spike length enhanced in wheat with soil zinc treatment due to improvement of cell growth and division. Habib (2009), also noted that the use of Zn treatments promoted the spike length in wheat.

Yield parameters including number of productive tillers, no of grains spike⁻¹, number of spikelets, grain weight spike⁻¹, 1000 grain weight, biological yield grain yield, and harvest index were significantly outperformed by soil zinc treatment. This showed that soil application provided the nutrient for whole crop period. While seed treatments (seed coating, seed priming) provided the minimum yield potential because they can provide the zinc supply during early crop stages like germination but unable to provide the nutrient supply during late growth stages such as reproductive stage. This study explained that zinc treated crop had given positive influence for biological and grain yield by showing the improvement in seedling, tillers, spikelets, grains and biomass production. These results concur with the findings of Chattha et al. (2017) where soil application was predicted as the best for crop yield improvement. Soil application increased the readily available zinc to crop, ultimately improving the yield (Cakmak, 2008).

Faisalabad-2008 showed significant optimum potential for all yield parameters except for number of spikelets and grain weight spike⁻¹, not statistically proven. Results of Hassan et al., (2019) matches to these findings, where Faisalabad-2008 increased significantly the number tillers, no of spikelets per spike, grain weight under soil application because of boosted nutrient uptake. Availability of sufficient amount of Zn enhanced the plant growth, biomass production grain weight and yield, because it reduced the uptake of toxic metal and provided the less stress to crop, resulting in increased zinc bioavailability in people (Mousavi, 2011).

According to the results of this study, foliar application method provided the greatest zinc contents due to the fact that wheat crop had enough Zn availability during reproductive stage. Foliarly applied is phloem mobile and easily translocated into emerging grains. Minimum value was recorded from soil application method for zinc contents due to the fact that soil had high pH. Maximum grain zinc accumulation was observed in Akbar-19, which suggests that this genotype has more potential for zinc uptake. This makes the Akbar-19 suitable crop to include in future biofortification activities. Cakmak and Kutman (2018) also similarly found the foliar application as the optimum treatment for enhancing the grain zinc contents.

This experimental study recommends that soil zinc application can be a cost effective solution to improve the crop quality and yield. In addition, soil application can become a sustainable solution to enhance the grain zinc contents and to address the prevalent zinc deficiency in Pakistan, ultimately lead to improve the human health. Akbar-19 shows the potential for optimum zinc biofortification.

This research has some limitations as it was conducted at one location, so its findings are not reliable for different climate and soil condition. Also this research was performed one time, so it has limited replicability. Further research is needed to check the impact of genotype and zinc methods over multiple years in different climatic conditions to ensure the findings more reliable.

Conclusion

It is concluded from the results of this experiment that Faisalabad-2008 improved the wheat production overall and out of all zinc application methods; soil zinc application method enhanced the wheat yield. Foliar application method provided the highest grain zinc contents.

Overall, the study focused on the importance of interaction and selection of suitable zinc methods in accordance with wheat genotypes for optimal zinc biofortification in crop and offering a sustainable way to cope up with human zinc deficiency.

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