Effect of Foliar and Soil Application of Zinc at Booting and Anthesis Stages of Wheat Improves the Yield, Quality and Grain Zinc Biofortification

Impact of foliar and Soil Applied Zinc in Wheat

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Abstract

Hidden hunger is emerging as a major contest for agricultural researchers as world population is increasing at high pace and food production is augmenting. Efforts are being made to supplement wheat whole grain with the addition of zinc as a dietary complement. To address the issue field-based experiment was conducted during wheat cultivation season of 2022-23, in randomized complete block design (RCBD) with split plot arrangement having three replications at NARC, Islamabad to minimize the impact of hidden hunger (micronutrient deficiency) through agronomic biofortification. Four wheat genotypes including an advanced line (Pkistan-2013, Zincol-2016, Borloug-2016, Wafaq-2023 and NR-531) and six zinc management treatments viz. (T_1 = No zinc application in soil and no foliar application, T_2 = No zinc in soil + foliar application of 0.44 kg ha^{-1} at booting, T_3 = No zinc in soil + foliar application of 0.44 kg ha^{-1} at booting & after anthesis, T_4 = 11 kg ha^{-1} zinc in soil at planting, T_5 = 11 kg ha^{-1} in soil + foliar application of 0.44 kg ha^{-1} at booting and T_6 = 11 kg ha^{-1} zinc in soil + foliar application of 0.44 kg ha^{-1} zinc at booting & after anthesis were tested for this purpose. No application of zinc in soil and foliar application or their combinations served as the control. Yield and growth attributes were also significantly ($p < 0.05$) improved by combined application (11 kg ha^{-1} in soil + foliar application of 0.44 kg ha^{-1} at booting) as compared to the other zinc application treatments. Genotype wafaq-2023 was found more responsive regarding growth and yield attributes comparatively. The findings of the present study showed that the combined application methods soil and foliar application of Zn produced good quality grains (more Zn, protein and gluten contents) with a maximum productivity of bread wheat cultivars.

Keywords: Genotypes, biofortification, grain quality, micronutrient, wheat growth

Introduction

The global prevalence of dietary micronutrient deficiencies is extensive and represents a significant health issue for a population exceeding 2 billion individuals (Zulfiqar et al., 2020). Following the green revolution, scientists have mostly focused on enhancing production rather than prioritizing the quality of edible agricultural components (Cakmak et al., 2010). This is the rationale behind why addressing hunger is a primary objective for agricultural scientists (Ramzan et al., 2020). Globally, zinc (Zn) insufficiency is recognized as the most prevalent micronutrient disease. Zinc deficiency has been associated with various gastrointestinal disorders (Prasad et al., 2004), disruptions in reproductive biology, hindered physical growth (Levenson and Morris, 2011), DNA damage, and the development of cancer (Stein, 2010). Additionally, it has been linked to diabetes mellitus, hormonal imbalances, respiratory complications, hypertension and impacts on several facets of the immune system.

Zinc deficiency is among the top five micronutrient deficiencies and have rigorous impacts on one-third of the world's population, especially rural communities (Stein, 2010). Insufficient intake of food having low zinc amounts is a major contributor to the occurrence of zinc deficiency in humans. The daily Zn requirement of adult and pregnant/lactating women ranges from 8 to 11 mg and 11 to 13 mg, respectively (Bhowmik et al., 2010); while, 8-18 mg daily iron (Fe) intake is recommend depending on age, gender and body weight, and 27 mg/day. Zinc deficiency is associated with several health problems such as impaired learning, abnormal immune system, increased infection rate and impaired physical growth (Wessels and Rink, 2020).

The prevalence of malnutrition has been significantly accelerated by the monotonous and excessive use of wheat-based items. Wheat stands out as a prominent staple crop within the category of cereals, serving as a primary source of sustenance for around 1.2 billion individuals worldwide (Iqbal et al., 2018). The proportion of individuals residing in rural areas relied on wheat as a primary source of sustenance, obtaining over 70% of their daily caloric intake from this staple crop (Raza et al., 2019). Wheat constituted a fundamental component of the basic dietary patterns for almost 60% of the population surveyed. It is projected that the need for more wheat, in order to sustain the growing global population, would rise by about 40% by the year 2050, with the aim of ensuring food security (Akhtar et al., 2019). The soils of Pakistan approximately 25-30% exhibits characteristics of being calcareous and low in micro nutrients specially zinc (Zhao et al., 2014).

Globally, it has been estimated that around 50% of soils used for wheat growth exhibit zinc deficiency (Zhao et al., 2014). Zinc shortage is often seen in Pakistani soil characterized by elevated pH levels, the presence of free calcium carbonate and bicarbonate ions hinder the availability of zinc to plants (Akhtar et al., 2019). The prevalence of soils with insufficient levels of micronutrients is on the rise as a result of the frequent cultivation of high-yielding crops and the heavy use of fertilizers, namely nitrogen, potassium and phosphorus (Salim and Raza, 2020). Zinc serves as a structural component and activator of numerous enzymes in plants, which are responsible for various physiological processes such as protein synthesis, auxin synthesis regulation, carbohydrate metabolism, and maintenance of membrane integrity (Rehman et al., 2018). Additionally, Zn plays a crucial role in chlorophyll formation, pollen development and fertilization (Pandey et al., 2006). In order to address the issue of nutritional imbalance, plants use several strategies to minimize water loss and optimize water absorption. These strategies include a decrease in leaf surface area and osmotic adjustment, achieved by the utilization of liquid seaweed extract, organic compounds, and mineral components (Makawita et al., 2021).

Agronomic biofortification stands out as the most economically efficient, expeditious and enduring approach among the different fortification methods available. Zinc sulfate ($ZnSO_4$) is often used as an inorganic fertilizer for its abundant usage as a source of zinc. The use of $ZnSO_4$ has proven to be effective in improving the overall quality of wheat grains (Zulfiqar et al., 2020). The addition of micronutrients to soil has shown favorable outcomes in augmenting their levels in wheat grain (Ramzan et al., 2020). The use of exogenous and soil applied Zn treatments has the potential to enhance the quality of wheat grain while also increasing its yield. The primary purpose of this trial was to evaluate the effectiveness of the intervention in addressing the identified issue. The objectives of this study are: (i) to conduct a comparative analysis of the yield and physiological response of different wheat genotypes when subjected to foliar and soil-applied Zn (ii) to investigate the impact of individual and/or combined application of foliar and soil applied Zn on the grain quality and yield characteristics of different wheat genotypes.

Materials and Methods

Experimental particulates

The experimental work was conducted at field area allocated to national coordinated wheat program at National Agricultural Research Centre Islamabad, (33° 42' N, 73º 10' E) Pakistan, under rainfed conditions during winter season (rabi) 2022-2023. The weather data of different elements are presented (Figure 1).

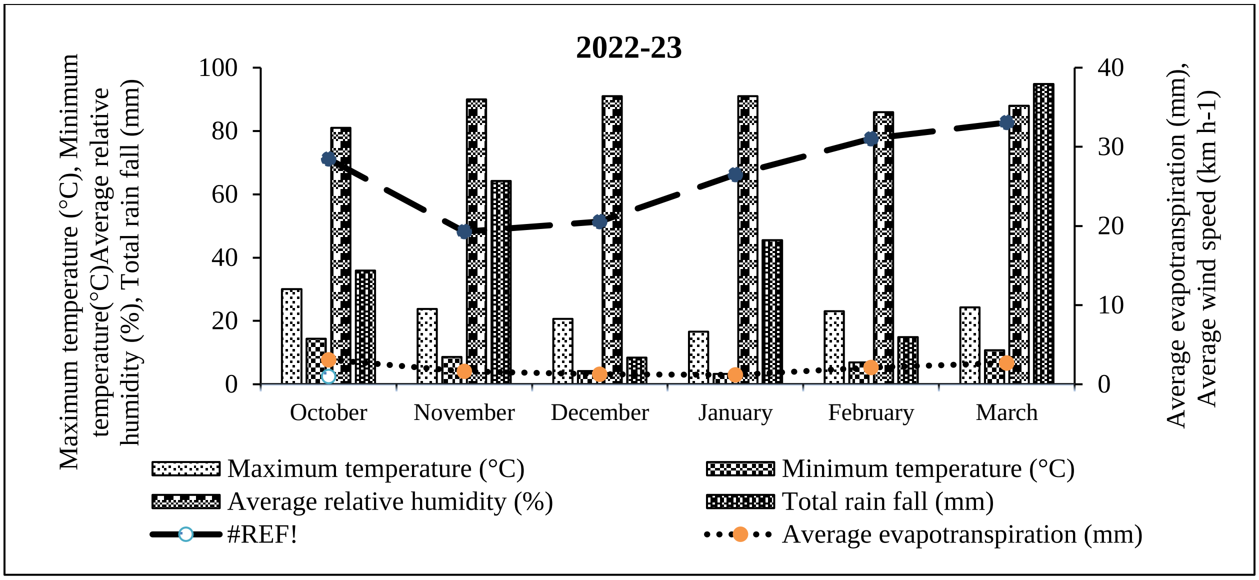

Figure 1: *Weather data of the experimental station during 2022-23 growing season*

The texture of experimental soil is clayey in nature. Soil samples were randomly collected from experimental site from depths of 0 to 15 cm and 15 to 30 cm with the help of augur, mixed samples of different depths distinctly, made composite and treated to record physiochemical characteristics of soil (ICARDA 2013) (Figure 2).

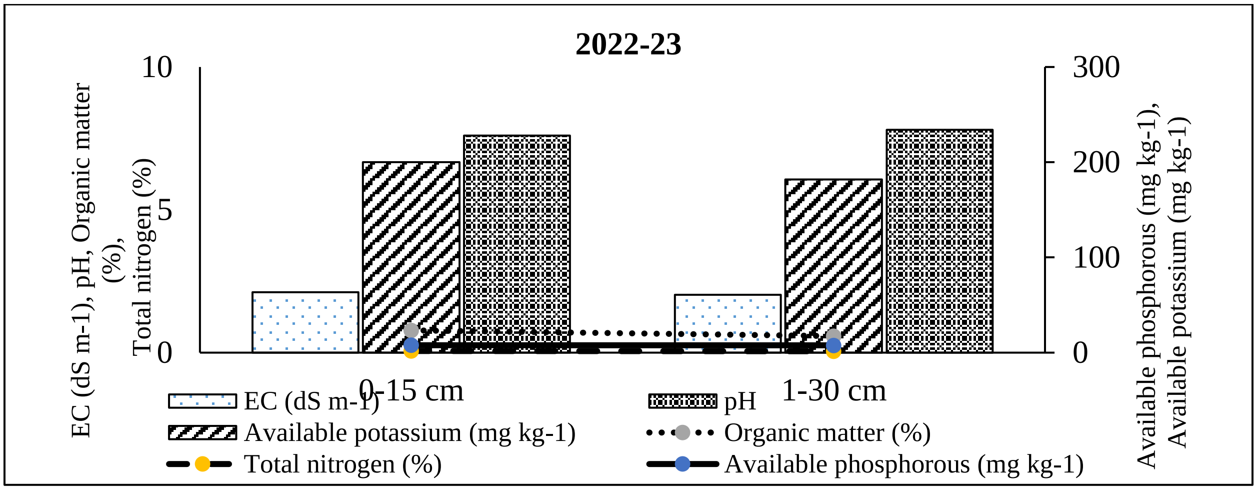

Figure 2: *Physical and chemical analysis of soil of the field trial site*

The experiment was comprised of following zinc foliar and soil applied treatments viz. control, foliar application of 0.44 kg ha^{-1} zinc at booting, foliar application of 0.44 kg ha^{-1} zinc at booting & after anthesis, 11 kg ha^{-1} zinc in soil at planting, 11 kg ha^{-1} in soil & 0.44 kg ha^{-1} zinc at booting and 11 kg ha^{-1} in soil & 0.44 kg ha^{-1} zinc at booting & after anthesis as main plot factor and 5 genotypes viz. Pakistan-2013, Zincol-2016, Borloug-2016, Wafaq-2023 and NR-531 as sub plot factor. The experiment was laid out in Randomized Complete Block Design (RCBD) with split plot arrangement and replicated thrice.

Crop husbandry

The crop was sown on 6 December 2022, with the help of Norwegian planter maintaining R × R distance 22.5 cm. The seed rate used during sowing was 100 kg ha^{-1}. Seed material of the cultivars was collected from seed bank of Wheat Program National Agricultural Research Centre (NARC) Islamabad, Pakistan. Each experimental unit was comprised of 5 m × 6 rows and net plot size was 5 m × 1.5 m. Fertilizer N:P:K at 120:96:60 kg ha^{-1} was uniformly applied in all experimental units. Urea, diammonium phosphate (DAP), K_2O, and $ZnSO_4$ were used as a sources of fertilizer. Half of nitrogen and full phosphatic and potassium fertilizer were applied at time of sowing. The remaining half nitrogen fertilizer was applied along with first irrigation at time of crown root initiation and zinc sulfate was manually spread in the experimental field with recommended fertilizer treatment doses. The crop was irrigated at four critical growth stages viz. crowning, tillering, spike initiation, and flowering. Weeds were managed using two manual hoeing 40 and 60 days after sowing. All other practices were kept uniform for all treatments during the growing season of crop.

Observations recorded

Morphological traits

Plant height from the base of plant to tip of spike and spike length were also measured with the help of meter rod at maturity. Spikes were manually harvested and spikelet per spike as well as grains per spike were calculated manually. Moreover, 1000 seeds were randomly taken from the seed lot of each experimental unit. Number of tillers (stem with spike) was counted before the harvest of crop.

Grain yield

The crop in each experimental unit was harvested, threshed and grain yield was weighed by using electronic balance and converted into kilogram per hectare.

Quality variables

Grains samples were collected after the harvesting of crop for grain Zn, protein and wet gluten analysis. After harvesting the crops spikes were separated and grains were carefully threshed and washed with deionized water for 30 s and were dried at 65°C in an electric oven having temperature 300°C higher than ambient. Grain Zn was determine by using atomic absorption spectrum (Skemadzu 7000) with wet digestion method (Rashid, 1986). Total soluble proteins were analyzed by using 0.1 g of grain was grounded using cooled phosphate buffer placed in ice bath. The homogenate was centrifuged at 15000 rpm at 4°C for 5 min. Enzyme extract of volume 40 µL + 160 µL Bradford Reagent was added in ELISA plate and recorded absorbance at 595 nm (Bradford, 1976).

Statistical analysis

The collected data were analyzed by using Statistix Software (STATISTIX 8.1) given by Gomez and Gomez (1984), and for comparison of various treatments means the Tukey's HSD test at ($p \leq 0.05$) was used. Correlation among the varying response variables was evaluated by using means of treatments and strength of relationship among recorded parameters using STATISTIX 8.1 software (Analytical Software, Tallahassee, Florida, USA) (Steel et al., 1997).

Results

Morphological traits

Plant height (cm), spike length (cm), 1000-grain weight (g), number of grains per spike and number of tillers (m^{-2}) were significantly ($p < 0.05$) affected by wheat genotypes (Table 1) but number of spikelets per spike remained non-significant. However, their interactive effect genotype × zinc levels method of application at different stages of wheat was significant for these parameters. Among the wheat genotypes, maximum plant height (75.03 cm) and spike length (12.11 cm) were recorded in wheat genotype Wafaq-23 which was statistically at par with Borloug-16, Zincol-16 and NR-531. Maximum 1000-grain weight (45.1 g), number of tillers (372) and grains per spike (47.9) were also recorded in Wafaq-23 which was statistically similar with Borloug-16 and also zincol-16 for only grains per spike (Table 1). Improvement in morphological traits was observed with increasing level of zinc in combination of soil and foliar application method. The zinc application method at booting (11 kg ha^{-1} zinc in soil + 0.44 kg ha^{-1} zinc as foliar) produced the maximum plant height (78.31 cm), spike length (12.40 cm), number of spikelet per spike (19.53), 1000-grain weight (43.8 g), number of tillers (354) and number of grains per spike (49.7) followed by 11 kg ha^{-1} zinc in soil + foliar application of 0.44 kg ha^{-1} zinc at booting & after anthesis. Minimum values of these parameters were recorded in control.

Grain yield

Grain yield was significantly ($p < 0.05$) influenced by the wheat genotypes and application of zinc fertilization techniques at different stages of wheat crop (Table 1). Interaction effect of wheat genotypes and zinc application tactics on grain yield was non-significant. Among the wheat genotypes, maximum grain yield was recorded in genotype Wafaq-23 (3444.7 kg ha^{-1}) which was statistically similar with Borloug-16 and NR-531 genotypes. Enhancement in grain yield was noted with combined soil and foliar application methods of zinc. Maximum grain yield (3327.8 kg ha^{-1}) was recorded with (11 kg ha^{-1} zinc in soil + 0.44 kg ha^{-1} foliar zinc) application at booting which was at par with (11 kg ha^{-1} zinc in soil + 0.44 kg ha^{-1} foliar zinc) at booting & after anthesis and no zinc in soil + foliar application of 0.44 kg ha^{-1} zinc at booting & after anthesis stages of wheat. Minimum grain yield of wheat was recorded in control.

Quality variables

There were significant differences ($p \leq 0.05$) in zinc, protein and wet gluten concentration among the wheat genotypes and ways of zinc application at different wheat stages while, their interactive effect was found significant. Significant interaction 'genotypes × soil and foliar zinc at different wheat stages' showed different response of zinc application methods on different stages to different genotypes for zinc, protein and wet gluten contents (Figure 1). Reasonably higher values of protein and wet gluten contents were recorded in wheat genotypes where zinc is applied at booting (11 kg ha^{-1} soil + 0.44 kg ha^{-1} foliar) closely followed by values obtained with application of (11 kg ha^{-1} zinc in soil + 0.44 kg ha^{-1} foilar zinc) at booting & after anthesis stage (Figure 1,2). In contrast grain zinc contents were higher where zinc applied at soil and foliar application at booting & after anthesis as compared to when applied at only booting stage (Figure 3).

Table 1: *Effect of foliar and soil application of zinc on plant height, spike length, number of spikelet per spike, 1000-grain weight, number of tillers, grains per spike and grain yield of wheat*

Treatments	**PH (cm)**	**SL (cm)**	**NS Spike^{-1}**	**1000-GW (g)**	**NT (m^{-2})**	**Grains Spike^{-1}**	**GY (kg ha^{-1})**
Genotypes							
Pakistan-2013	69.53 B	10.17 B	16.83	34.9 C	317 B	41.5 B	2772.1 B
Zincol-2016	71.53 AB	10.72 AB	17.11	39.8 B	340 B	43.9 AB	2751.1 B
Borloug-2016	73.47 AB	11.50 AB	17.44	42.5 AB	348 AB	45.8 AB	3050.5 AB
Wafaq-2023	75.03 A	12.11 A	18.06	45.1 A	372 A	47.9 A	3444.7 A
NR-531	71.22 AB	11.83 AB	17.61	39.3 BC	336 B	40.2 B	3014.9 AB
Tukey's HSD at p ≤ 0.05	**4.444**	**1.939**	**NS**	**4.48**	**31.1**	**6.27**	**566.46**
Foliar and Soil Applied Zinc							
Control	66.57 D	10.33 B	15.87 B	37.1 C	332 C	39.7 D	2708.6 D
Foliar 0.44 kg ha^{-1} zinc at booting	70.87 BCD	10.87 AB	16.67 AB	38.9 BC	335 BC	41.8 CD	2853.2 BCD
Foliar 0.44 kg ha^{-1} zinc at booting + After anthesis	73.37 ABC	11.40 AB	17.87 AB	41.2 ABC	347 ABC	44.3 BC	3090.9 ABC
11 kg ha^{-1} zinc in soil at planting	68.80 CD	10.80 AB	16.07 B	38.9 BC	336 ABC	41.8 CD	2836.7 CD
11 kg ha^{-1} zinc in soil + Foliar 0.44 kg ha^{-1} zinc at booting	78.31 A	12.40 A	19.53 A	43.8 A	354 A	49.7 A	3327.8 A
11 kg ha^{-1} zinc in soil + Foliar 0.44 kg ha^{-1} zinc at booting + After anthesis	75.03 AB	11.80 AB	18.47 AB	42.1 AB	351 AB	45.9 AB	3222.7 AB
Tukey's HSD at p ≤ 0.05	**5.306**	**1.750**	**2.930**	**4.20**	**18.2**	**3.73**	**381.16**

Any two means not sharing a letter in common and differ significantly at significance level (p ≤ 0.05)

PH: Plant Height; **SL:** Spike Length; **NS:** No of Spikelet per Spike; **1000-GW:** 1000-Grain Weight; **NT:** No of Tillers; **GY:** Grain Yield

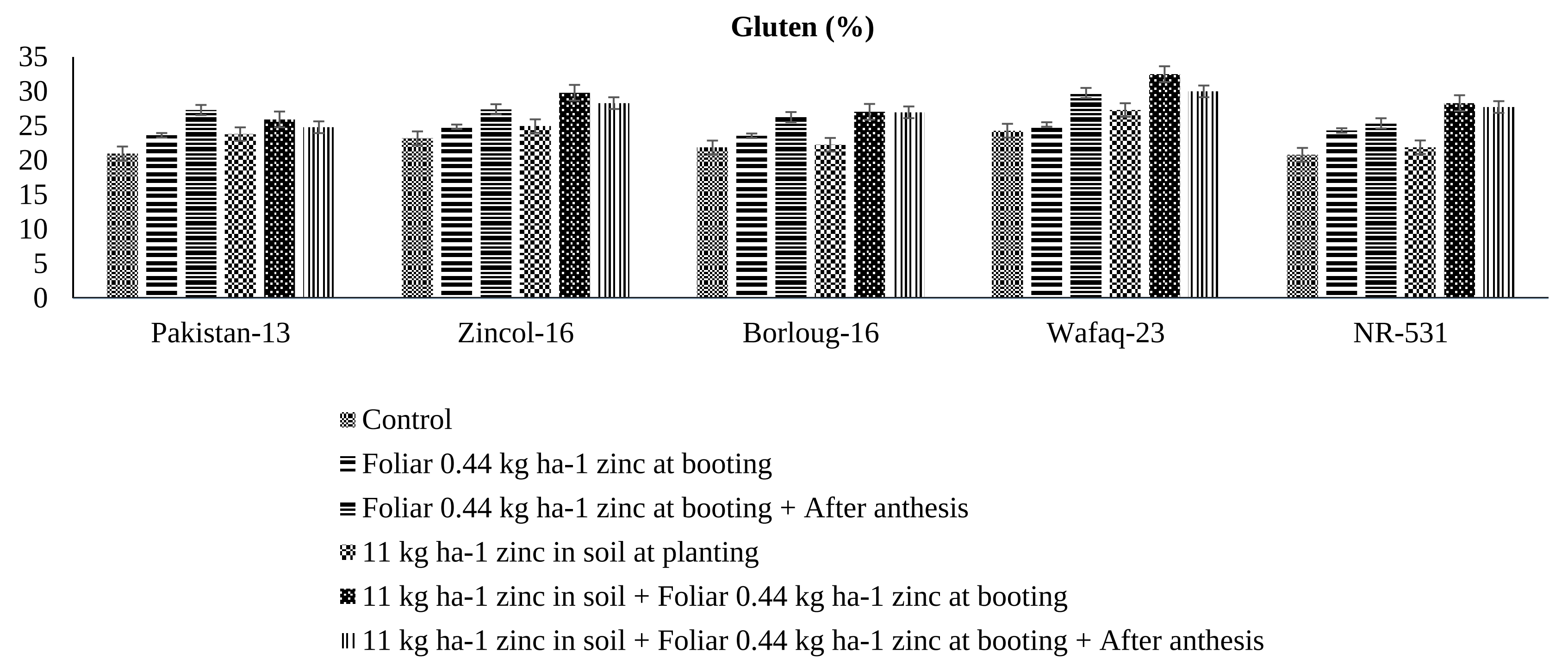

Figure 3: *Impact of soil and foliar applied different doses of Zn on grain gluten (%) of wheat genotypes grown during 2022-23 growing season. Means sharing the same letter did not differ significantly at p > 0.05. Error bars depict the standard error of means*

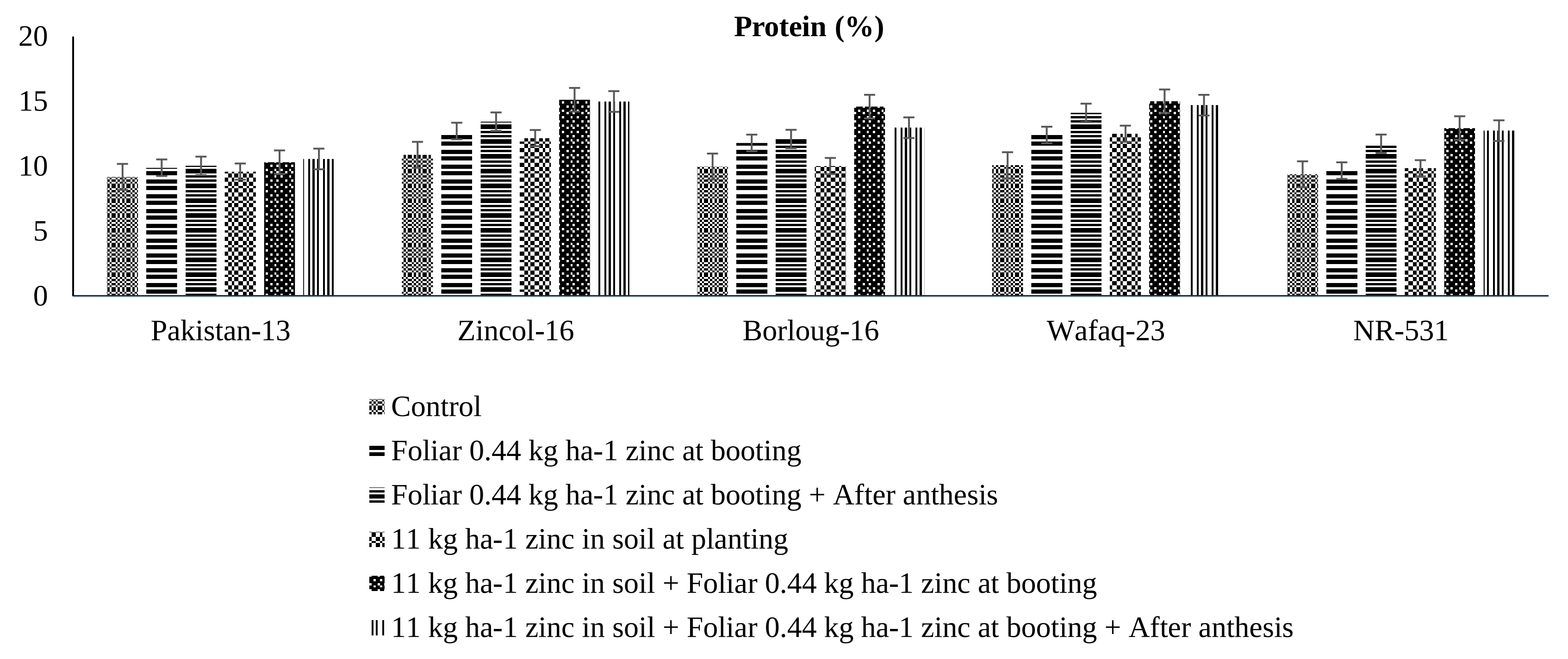

Figure 4: *Impact of soil and foliar applied different doses of Zn on grain protein (%) of wheat genotypes grown during 2022-23 growing season. Means sharing the same letter did not differ significantly at p > 0.05. Error bars depict the standard error of means*

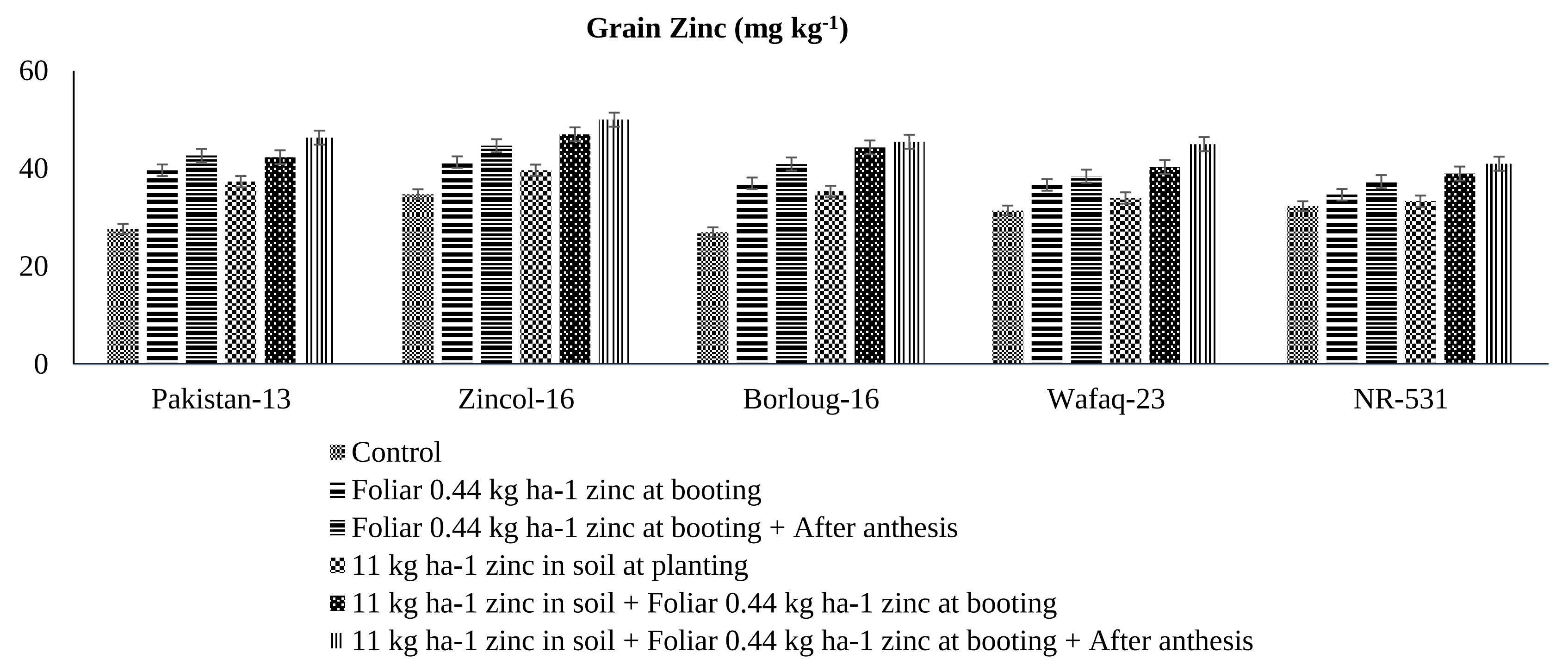

Figure 5: *Impact of soil and foliar applied different doses of Zn on grain zinc contents (mg kg*$^{-1}$*) of wheat genotypes grown during 2022-23 growing season. Means sharing the same letter did not differ significantly at* $p > 0.05$*. Error bars depict the standard error of means*

Discussion

This field study explored the effects of foliar and soil-applied zinc fertilization on crop yield and grain Zn contents in wheat genotypes, Zincol-16 (Zn enriched) and Pakistan-2013, Borloug-2016, Wafaq-2023 and NR-531 (Zn deficient). Micronutrient-deficient soils are becoming more common as a result of the increased use of fertilizer, namely nitrogen, potassium, and phosphorus (Salim and Raza, 2020). Soils with 0.5 mg kg^{-1} DTPA extractable Zn are considered potentially Zn deficient and may respond to soil Zn fertilization (Rashid et al., 2019). Zinc insufficiency is more common in Pakistani soil due to high pH, free $CaCO_3$, and HCO^{-3} levels, all of which impede Zn accessibility to plants (Akhtar et al., 2019). Plant height is one of the most important factors in determining crop yield.

Plant height is determined by the interaction of genetic and environmental variables. Significant changes in plant height were detected as a result of combined Zn treatment strategies during the booting stage. The combined method of application resulted in the highest plant and spike length (Table 1) in wheat. Plants height might be due to zinc role in cell division, cell expansion, meristematic tissue activities, and photosynthetic activities. The combined way of foliar and soil Zn application during booting is responsible for maximum number of grains per spike, 1000-grain weight, and spikelets per spike in the current experimentation (Hafeez et al., 2021) (Table 1). Zayed et al. (2011) discovered that a combination of soil and foliar Zn application greatly increased 1000-kernel weight in rice. According to the results of their experiments, Sher et al. (2022) concluded that the application of Zn considerably increased grain counts per spike, productive tillers, 1000-grain weight, and spike length from their experimentation. Hassan et al. (2019) discovered that Zn had a significant effect on the morphological parameters of the wheat crop. They claimed that the use of Zn enhanced Zn dietary standards, more 1000-grain weight, the maximum number of grains per spike, and spikelet per spike. This, perchance, is because of the fact that zinc is an important element that plays a critical role in controlling the auxin content throughout the plant body, as well as in the manufacture of indole acetic acid. Zinc also regulates the physiological and biochemical processes and stimuli that initiate primordia in terms of reproductive growth. It influences the movement of essential metabolites from the source to the sink of plants. A number of tillers play critical roles in achieving the final yield in a wheat crop. The combined foliar and soil application methods of 11 kg ha^{-1} in soil and 0.44 kg ha^{-1} foliar zinc at booting produced the maximum number of tillers per unit area. When compared to wheat genotypes, Wafaq-23 produced the more tillers as compared to other genotypes (Table 1). Jalal et al. (2020), supported our findings who reported that the combined application methods of Zn greatly increased the number of tillers per unit area. In current study, the combined soil and foliar treatment of Zn resulted in the highest grain yield (Table 1).

This increase in economic yield is due to zinc has catalytic and constructive involvement in physiological and biochemical activities, as well as respiration and photosynthesis processes, resulting in increased economic yield. Zain et al. (2015) observed that nutrient supplementation, particularly microelement supplementation, is responsible for increased grain yields associated with more tillers, number of grains per spike, and 1000-grain weight. The use of inorganic fertilizers and mineral elements is thought to be a beneficial practice in maintaining agricultural productivity with increased soil fertility in order to attain optimum plant development and economical yield under stressful situations (Zahid et al., 2021). The Zn also causes the conversion of nitrates to ammonia, which enhances the economics of a wheat crop. In the current study, the application of Zn at 11 kg ha^{-1} in soil and 0.44 kg ha^{-1} foliar zinc at booting boosted grain yield by facilitating the transfer of assimilates and photosynthates from the source to the sink, notably grains in the case of wheat. As a result, increased Zn content in grain and yield were anticipated with zinc soil fertilization.

Mineral buildup in grains is an important measure for assessing plants' ability to absorb beneficial nutrients that indicate plant biofortification potential. Zinc accumulation in grains of genotypes was greatly improved by foliar and soil applied treatments, and cultivar Zincol-16 was shown to be more effective in Zn accumulation than other genotypes (Figure 3). At booting, 11 kg ha^{-1} in soil and 0.44 kg ha^{-1} foliar zinc produced the greatest improvement. In terms of genotypes, Zincol-16 accumulated more Zn in grains than the other genotypes. Our findings were in line with Zou et al. (2012), who found that foliar and soil Zn applications increased Zn content in wheat grains. Our findings contradicted the findings of Keram et al. (2012) who found that soil-applied Zn significantly increased wet gluten. Protein content was also dramatically significantly increased by both foliar and soil-applied zinc treatments at various stages of wheat development. In contrast to our findings, Ramzan et al. (2020) found that soil-applied Zn and Fe considerably reduced the protein content of spring wheat.

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Conflict of Interest

The authors declare no conflict of interest

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