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Agro-morphological and grain-quality responses of barley accessions to zinc foliar application

Abstract – The objective of this work was to evaluate the influence of zinc foliar application on the agro-morphological attributes and grain quality of 50 barley accessions. The experiment was carried out in a randomized complete block design with three treatments: 0.25 and 0.50% zinc as $ZnSO_4$; and the control, without zinc. Compared with the control, zinc foliar application at 0.25 and 0.50%, respectively, caused the following increases in the agro-morphological and grain quality traits of barley: 10.5 and 13.42% in plant height, 3.40 and 5.23% in the maturity index, 11.89 and 13.54% in spike length, 31.63 and 32.80% in spike weight, 28.96 and 33.06% in biomass, 6.74 and 19.03% in chlorophyll a, 0.73 and 0.57% in chlorophyll b, 12.20 and 12.90% in total chlorophyll, 31.83 and 43.68% in grain manganese content, and 23.06 and 38.08% in zinc. Therefore, zinc foliar application improved barley agro-morphological and grain quality performances, with variability among treatments and accessions. The foliar application of $ZnSO_4$ at 0.25 and 0.50%, especially at 0.50%, enhances the chlorophyll content, agro-morphological characteristics, and nutrient concentrations of barley grains.

Index terms: *Hordeum vulgare*, biofortificiation, foliar application, grain nutrients, zinc.

Respostas agromorfológicas e de qualidade de grãos de acessos de cevada à aplicação foliar de zinco

Resumo – O objetivo deste trabalho foi avaliar a influência da aplicação foliar de zinco nos atributos agromorfológicos e na qualidade de grãos de 50 acessos de cevada. O experimento foi conduzido em delineamento de blocos ao acaso, com três tratamentos: 0,25 e 0,50% de zinco como $ZnSO_4$; e controle, sem zinco. Em comparação ao controle, a aplicação foliar de zinco a 0,25 e 0,50%, respectivamente, ocasionou os seguintes aumentos nas características agromorfológicas e de qualidade de grãos de cevada: 10,5 e 13,42% em altura da planta, 3,40 e 5,23% no índice de maturidade, 11,89 e 13,54% em comprimento da espiga, 31,63 e 32,80% em peso da espiga, 28,96 e 33,06% em biomassa, 6,74 e 19,03% em clorofila a, 0,73 e 0,57% em clorofila b, 12,20 e 12,90% em clorofila total, 31,83 e 43,68% em teor de manganês nos grãos, e 23,06 e 38,08% em zinco. Portanto, a aplicação foliar de zinco melhora o desempenho agromorfológico e a qualidade de grãos de cevada, com variabilidade entre tratamentos e acessos. A aplicação foliar de $ZnSO_4$ a 0,25 e 0,50%, especialmente a 0,50%, aumenta o teor de clorofila, as características agromorfológicas e a concentração de nutrientes nos grãos de cevada.

Termos para indexação: *Hordeum vulgare*, biofortificação, aplicação foliar, nutrientes nos grãos, zinco.

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Introduction

Barley (*Hordeum vulgare* L.) is among the earliest domesticated crops commonly grown for livestock feed and is an important source of fermentable material for beer, as well as an essential component of different human health foods (Chutimanitsakun et al., 2013). Moreover, this crop's grain is a rich source of proteins, carbohydrates, phosphorus, calcium, vitamin B, and dietary mineral nutrients, including manganese and iron (Geng et al., 2022). Barley can also be used as suitable model to study the genomics of Triticeae due to its diploid nature (Griffey et al., 2010; Martin et al., 2020).

Although wild barley shows a high adaptation to a wide variety of climatic conditions, cultivated barley is comparatively more vulnerable regarding resistance to both biotic and abiotic stresses, which may be attributed to the presence of distinct alleles and gene regulators (Hao et al., 2021). Added to this challenge, barley production has declined qualitatively and quantitatively over the past years due to less productive landraces, the lack of diverse germplasm and improved varieties, and the availability of market-valued crops, as well as to land fragmentation and climatic variation (Tadesse & Derso, 2019).

Another factor that can reduce crop yield and may affect grain nutritional quality for humans is the deficiency of micronutrients, specifically of zinc (Khalid et al., 2013; Cakmak & Kutman, 2018; Ali et al., 2020). Zinc deficiency in the soil, for example, leads to a low level of the micronutrient in crop grains and to interactions with other soil nutrients (Imran et al., 2016).

In the context of crop nutrition, foliar application is considered an advanced way to supply micronutrients directly to the aerial parts of the plants. Ayenew et al. (2025) suggested that the foliar application of zinc as a nanofertilizer may enhance the absorption and bioavailability of this micronutrient, also reducing leaching. In this line, other authors reported a significant improvement in barley crop yield and grain quality, which they attributed to the direct absorption of zinc through leaf surface, without environmental and soil effects (Moshfeghi et al., 2019; Khalifa et al., 2022). In other crops, zinc foliar application has been shown to increase the concentration of this micronutrient by 73% (Zhang et al., 2012). However, research focusing on the effect of this application on barley agro-morphological and grain quality attributes is still limited for most accessions of this crop. To evaluate a large number of accessions, aiming to identify those with a high potential for zinc use, Cakmak (2008) highly recommend the adoption of a reliable germplasm screening procedure in a relatively short time.

The objective of this work was to evaluate the influence of zinc foliar application on the agro-morphological attributes and grain quality of 50 barley accessions.

Materials and Methods

The study was carried out in Swat, Pakistan (34°40'N, 72°12'E, at approximately 1,010 m above sea level). According to Köppen's classification, the climate of the area is subtropical humid (Cfa subtype) with an annual rainfall of 864 mm and mean annual temperature of 15°. The driest and wettest months are November (average precipitation of 22 mm) and August (average precipitation of 134 mm), respectively. The experimental site has a deep and well-drained basic soil, with characteristic smooth and fine-textured particles, pH 8.1, and electrical conductance of 0.23 dS m^{-1}. The physicochemical properties of the soil are 29.8% sand, 17% clay, 65% silt, 2.40% lime, and 1.08% organic matter. The analysis for micronutrients revealed 336 ppm potassium, 1.16 mg kg^{-1} zinc, 447 mg kg^{-1} manganese, 16.4 mg kg^{-1} copper, 0.36% nitrogen, and 7.0 ppm phosphorus.

The experimental design was in randomized complete blocks, with the following three treatments, with three replicates: foliar application of zinc as $ZnSO_4$ at 0.25 and 0.50%; and the control, without $ZnSO_4$.

The evaluated plants consisted of 50 barley accessions provided by Bio-Resources Conservation Institute of National Agriculture Research Centre, located in Islamabad, Pakistan (Table 1). Seeds from each experimental barley accession were first germinated on moist filter papers on petri dishes; after full germination, seedlings were transferred to the field. The seedlings were transplanted into 3.0 m long rows, each forming an experimental unit, with a 30 cm inter-row spacing. For a proper seedling growth, normal weeding and agricultural practices were performed according to Elshafei et al. (2024). Foliar $ZnSO_4$ was applied when the plants entered the tillering stage.

Table 1. Origin in Pakistan of the 50 experimental barley (*Hordeum vulgare*) accessions used in the study.

Accession number/code	Altitude (m)	Origin of the collected barely accessions
RU-1 / 4172	2,010	Kawas, Ziarat, 19 km from Ziarat to Kachi, Baloschistan
RU-2 / 4176	1,450	Seedstore, Loralai, Baloschistan
RU-3 / 4177	1,410	Seedstore, Loralai, Baloschistan
RU-4 / 4179	1,200	Seedstore, Loralai, Baloschistan
RU-5 / 4180	1,200	23 km from Shahrig to Kach, Sibi, Baloschistan
RU-6 / 4182	210	13 km northeast of Sibi, Khajjak, Sibi, Baloschistan
RU-7 / 4183	190	11 km northeast of Sibi, Khajjak, Sibi, Baloschistan
RU-8 / 4187	1,650	13 km from Quetta to Dagbari, Quetta, Baloschistan
RU-9 / 4188	1,550	10 km from Chaman to Damandi, Surkit Tahiri, Qila Abdullah, Baloschistan
RU-10 / 4191	2,320	24 km west of Sabura, Pishin, Kili Haji Khan, Baloschistan
RU-11 / 4193	1,810	4.5 km from Chinjan, Qila village, Pishin, Baloschistan
RU-12 / 4194	1,550	16 km north of Pishin, Dab Khanozai, Pishin, Baloschistan
RU-13 / 4195	1,550	15 km north of Pishin, Man Khanozai, Pishin, Baloschistan
RU-14 / 4196	1,550	Pishin market, Baloschistan
RU-15 / 4197	1,550	Pishin market, Baloschistan
RU-16 / 4198	1,500	64.5 km from Nushki, Kardagaab, Pishin, Baloschistan
RU-17 / 4199	910	12 km from Nushki, Chagai, Batto, Baloschistan
RU-18 / 4200	850	25 km from Dalbandin, Peeshok, Chagai, Baloschistan
RU-19 / 4201	930	Near the Afghan border, east of Isa Chah, Chagai, Baloschistan
RU-20 / 4204	1,780	112 km from Quetta, Qila Saifullah, Muslim Bagh, Baloschistan
RU-21 / 4205	1,660	Natar Tangi, Qila Saifullah, Baloschistan
RU-22 / 4206	1,640	30 km from Muslim Bagh, Qila Saifullah, Nasai, Baloschistan
RU-23 / 4207	2,050	11 km from Margha Faqir Zai, Qila Saifullah, Baka Cheena, Baloschistan
RU-24 / 4208	2,130	Margha Faqir Zai market, Qila Saifullah, Baloschistan
RU-25 / 4209	1,900	Sharan road market, Qila Saifullah, Baloschistan
RU-26 / 4210	1,860	08 km east of Sharan Jogizai, Qila Saifullah, Karm Zai Kacbh, Baloschistan
RU-27 / 4235	100	05 km from Qazi Ahmad Wala, Bashir Ki Goth, Nawabshah, Sindh
RU-28 / 4236	120	Haji Esa Bougai, 10 km from Moro west of Kacha, Naushahro Firoz, Sindh
RU-29 / 4237	125	Sukkur market, Sindh
RU-30 / 4238	125	Goth Mithal Khan, 30 km from Sukkur, Sindh
RU-31 / 4239	125	Ali Nawaz Bhatti, 12 km from Khairpur, Jacobabad, Sindh
RU-32 / 4240	100	Thul road, Rahimabad, Jacobabad, Sindh
RU-33 / 4241	100	Nandero, 18 km before Larkana, Sindh
RU-34 / 4242	125	Wegan market, Larkana, Sindh
RU-35 / 4243	115	Yaro Dero market, Larkana, Sindh
RU-36 / 4244	115	Dadu market, Sindh
RU-37 / 4291	20	03 km from Karachi-Thatta road, Bharoo, Karachi, Sindh
RU-38 / 4292	20	07 km from Karachi-Thatta Road, Bharoo, Karachi, Sindh
RU-39 / 4293	10	Ghulam Ullah, 15 km from Sangroo to Thatta, Sindh
RU-40 / 4294	0	Panjwi Chaka, 34 km before Badin, Badin, Sindh
RU-41 / 4295	90	04 km from the Bachal Pur village, near a river bank, Nawabshah, Sindh
RU-42 / 4296	90	Kacharo, 05 km from Kacharo road, Naushahro Firoz, Sindh
RU-43 / 4297	60	Somar Goth, 08 km before Sukkur, Sindh,
RU-44 / 4299	1,050	10 km before Panjgur, Zandaydaz, Baloschistan
RU-45 / 4300	100	Pasni market, Gwadar, Baloschistan
RU-46 / 4301	540	Kech, Dandar, Baloschistan
RU-47 / 4302	510	Awaran, Gashikor, 60 km before Awaran, Baloschistan
RU-48 / 4303	1,040	Awaran to Jibri road, Awaran, Jibri, Baloschistan
RU-49 / 4304	1,100	21 km south of Nal, Awaran, Aladam, Baloschistan
RU-50 / 4305	1,050	49 km from Khuzdar to Besima, Awaran-Nal, Baloschistan

Seven days after zinc foliar application, fresh leaves were harvested from each replicate to measure chlorophyll a, chlorophyll b, and total chlorophyll contents using the method of Hiscox & Israelstam (1979), with minor modifications. Approximately 0.5 g fresh leaf tissues were collected and then placed in test tubes containing 6.0 mL dimethyl sulfoxide and kept in a preheated water bath for about 35 min at 65°C. Afterwards, 4.0 mL dimethyl sulfoxide were added to all samples in order to reach a total final volume of 10 mL. The absorbance of the extract was read with the UV-1602UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 645 and 663 nm.

The evaluated agro-morphological attributes were determined according to Elshafei et al. (2024). Aerial dry biomass and the maturity index were obtained as described in Kahraman et al. (2024). Using the method of Hagenblad et al. (2019), the following characteristics were determined for spikes collected from each row: shape, attitude, glaucosity, weight, glume color, awn color, lemma awn barbs, glume and awn length, and grain coverings.

Seed micronutrient concentrations were determined using the methods of Shiri et al. (2019) and Hao et al. (2021). Briefly, samples of 1.0 g grain flour obtained from each accession were mixed with 5.0 mL HNO_3 and heated for 1 hour on an electric plate. This procedure was repeated after the addition of another 5.0 mL HNO_3 and heating for about 50 min. Then, 2.5 mL hydrogen peroxide (35% H_2O_2) were added to the reaction mixture and slightly heated until reaching a 25 mL volume. Afterwards, the reaction mixture was cooled and filtered through Whatman filter paper. The AAS vario 6 atomic absorption spectrometer (Analytik Jena GmbH+Co, Jena, Germany), equipped with cathode lamps, was used to measure zinc, manganese, and copper contents at 213.9, 279.5, and 324.8 nm, respectively. The micronutrients were analyzed following the standard method of Association of Official Analytical Chemists (AOAC) (Cunniff, 1995).

The collected data were subjected to the analysis of variance using the Statistix 8.1. software (Analytical Software, Tallahassee, FL, USA). A heatmap of Pearson's correlation was used to determine variability relationships. Box-violin plots were employed to depict a graphical summary of all studied traits using the ggplot2 package and the Jamovi software (Wickham et al., 2018).

Results and Discussion

Zinc foliar application greatly affected all evaluated barley traits (Table 2). Significant differences among treatments, accessions, and their interactions were depicted through the analysis of variance, specifically regarding spike weight, spike length, plant height, and the maturity index. Chlorophyll and micronutrient contents also differed between treatments (Table 3).

The studied barley accessions showed variable responses to zinc foliar application (Table 3 and Figure 1). Plant height increased by 10.5 and 10.4% when treated with 0.25 and 0.50% $ZnSO_4$, respectively. These results are in general agreement with those of Genc et al. (2004), Moshfeghi et al. (2019), Noreen et al. (2021), and Roshani et al. (2021), who found that zinc foliar application significantly enhanced the morpho-physiological traits of barley cultivars, including photosynthetic pigments, antioxidant enzyme activities, the harvest index, and yield.

An increased maturity index of 5.23 was recorded under 0.50% $ZnSO_4$ (Table 3), as similarly observed by Janmohammadi et al. (2016). Concerning spike weight, there was an increase of 32.8% with 0.25% $ZnSO_4$ in comparison with the control, which is in alignment with Sadeghzadeh et al. (2009).

The results obtained for spike length and biomass are shown in Tables 2 and 3. Spike length was the longest for plants treated with 0.50% $ZnSO_4$, but showed an increase of 11.9 and 13.5% under 0.25 and 0.50% $ZnSO_4$, respectively, when compared with control. Plant dry biomass increased from 97.5 g in the control to 145.7 g with the foliar application of 0.50% $ZnSO_4$.

Improvements in barley traits due to zinc foliar application were also reported by other authors, such as Moshfeghi et al. (2019) in their study on cultivars of this species. Similarly, Noreen et al. (2021) and Roshani et al. (2021) observed improved growth, antioxidant enzyme activities, and harvest index in barley grown under salt and drought stress conditions. This confirms the significant role that micronutrients, specifically zinc, play in plant biochemical and physiological processes. Ali et al. (2022), for instance, concluded that the proper supply of zinc significantly enhanced plant physiological activities, including photosynthetic pigments.

A high chlorophyll content was observed with the foliar application of $ZnSO_4$ at both concentrations, but especially at the lowest one (Tables 2 and 3). In the control treatment, chlorophyll a was 0.47 mg g^{-1}, increasing to 0.50 mg g^{-1} when 0.25% $ZnSO_4$ was applied, but decreasing to 0.40 mg g^{-1} with 0.50% $ZnSO_4$ (Figure 1). In the case of chlorophyll b, its content was enhanced by 16.2% due to the foliar application of 0.25% $ZnSO_4$, when compared with the control, representing a much more promising result. Total chlorophyll content also increased significantly with the foliar application of 0.25% $ZnSO_4$, showing a net reduction with 0.50% $ZnSO_4$ (Table 2 and Figure 2).

Regarding the observed correlations, the one between total chlorophyll content and chlorophyll b was highly positive for grown control plants treated with 0.50% $ZnSO_4$ (r=0.94 and 0.98, respectively), as shown in Figure 3. Likewise, the correlation between total chlorophyll content and chlorophyll a was also highly positive (r=0.95) in plants under 0.50% $ZnSO_4$. In addition, the correlation of spike weight and biomass was highly positive (r=0.80) in plants treated with 0.50% $ZnSO_4$, but was lower in the control (r=0.72). More associations between variables were also recorded for the other studied attributes, including grain micronutrients.

The photosynthesis process is regulated by various factors that affect it either directly, as light, carbon dioxide, and water, or indirectly, as soil pH and nutrient availability through altered enzymatic actions

Table 2. Summary of the statistics of the evaluated traits of the studied barley (*Hordeum vulgare*) accessions(1).

Source of variation	PH (d)	MI (d)	SL (cm)	SW (g)	Biomass (g)	Chla (mg g^{-1})	Chlb (mg g^{-1})	TChl (mg g^{-1})	Mn (µg g^{-1})	Zn (µg g^{-1})	Cu (µg g^{-1})
Mean	88.08	136.6	18.70	63.41	126.8	0.463	0.638	1.098	161.4	1757.9	248.6
Standard deviation	10.79	4.682	1.698	29.17	54.72	0.374	0.508	0.864	100.4	564.3	64.64
Variance	116.3	21.93	2.882	850.6	2994.4	0.140	0.331	0.747	10077	318461	4177.7
Standard error of the mean	0.501	0.221	0.080	1.375	2.580	0.018	0.027	0.041	4.732	26.60	3.047
CV (%)	12.24	3.428	9.077	45.99	43.14	80.67	90.12	78.70	62.20	32.10	25.99
Minimum	8.00	124.0	12.00	10.00	20.00	0.029	0.300	0.033	16.80	110.0	142.3
Maximum	114.0	143.0	23.30	175.0	440.0	2.242	6.228	5.971	579.6	4425.8	412.4
Treatment	Least significant difference of the traits										
0.50% $ZnSO_4$	93.26a	139.92a	19.72a	71.65a	145.70a	0.5008a	0.733a	1.241a	202.63a	2175.6a	258.82a
0.25% $ZnSO_4$	90.25a	137.26b	19.35b	70.43c	137.29b	0.473b	0.615b	1.088b	167.41b	1751.0b	245.10b
Control (without $ZnSO_4$)	80.75c	132.60c	17.05c	48.15c	97.53c	0.398c	0.5066c	0.964c	114.13c	1347.1c	241.92c

(1)PH, plant height; MI, maturity index; SL, spike length; SW, spike weight; Chla, chlorophyll a; Chlb, chlorophyll b; TChl, total chlorophyll; Mn, manganese; Zn, zinc; and Cu, copper.

Table 3. Variance analysis and percentage variations of the mean values obtained for the agro-morphological traits of 50 experimental barley (*Hordeum vulgare*) accessions regarding the influence of the foliar application of $ZnSO_4$(1).

Source of variation	DF	Mean square										
		PH	MI	SL	SW	Biomass	Chlorophyll content			Micronutrient content		
							Chla	Chlb	TChl	Mn	Zn	Cu
Replicate	2	2.8	5.8	1.4	72.6	820.2	0.09	0.22	0.51	1165	311045	927.4
Treatment (T)	2	6397***	2059***	313.7***	26253***	99278***	0.47***	1.12***	2.92***	29779***	215925***	12003***
Accessions (G)	49	408.4***	76.7***	5.44***	5593***	15673***	0.50***	0.94	2.45***	72606***	125545***	18522***
T × G	98	68.7**	13.1***	1.37**	363.6***	2086***	0.34***	0.83	1.88***	3721***	29720***	9593***
Error	298	42.6	2.27	0.88	65.6	576.5	0.01	0.06	0.078	15.0	766.34	1.0
		Mean value										
Control (without $ZnSO_4$)		80.8	132.6	17.1	48.2	97.5	0.5	0.6	1.1	114.1	1347.1	258.8
0.25% $ZnSO_4$		90.3	137.3	19.3	71.7	137.3	0.50	0.73	1.24	167.41	1750.9	241.9
0.50% $ZnSO_4$		93.3	139.9	19.7	70.4	145.7	0.40	0.51	0.96	202.6	2175.6	245.1
Change under 0.25% $ZnSO_4$ (%)		10.5	3.4	11.9	32.8	29.0	6.74	16.2	12.3	31.8	23.1	7.0
Change under 0.50% $ZnSO_4$ (%)		13.4	5.2	13.5	31.6	33.1	19.0	8.6	12.9	43.7	38.1	5.6

(1)DF, degree of freedom; PH, plant height; MI, maturity index; SL, spike length; SW, spike weight; Chla, chlorophyll a; Chlb, chlorophyll b; TChl, total chlorophyll; Mn, manganese; Zn, zinc; and Cu, copper.

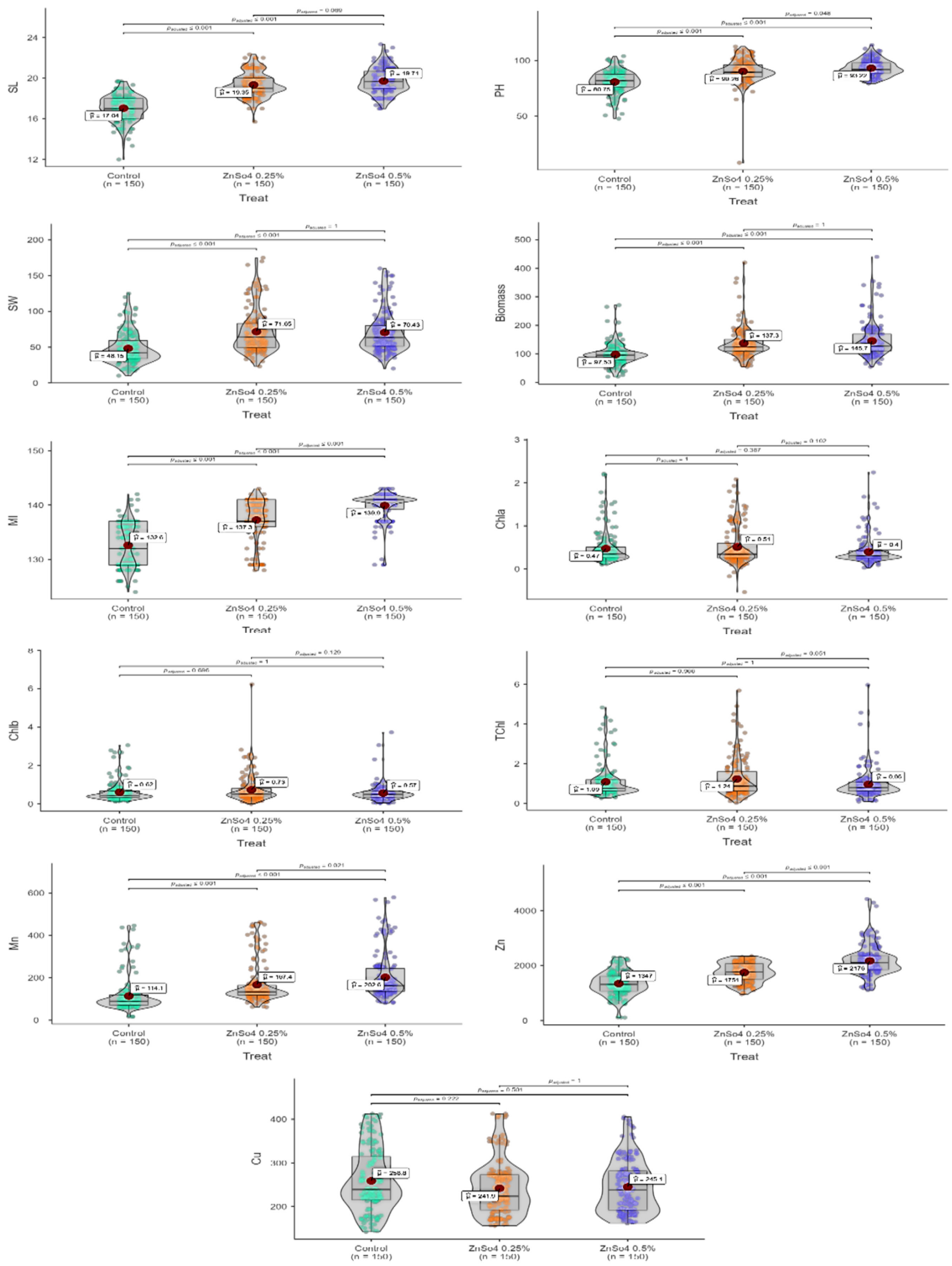

Figure 1. Box-violin plots presenting the studied morpho-physiological and agronomic attributes of 50 barley (*Hordeum vulgare*) accessions under the treatments: 0.25% $ZnSO_4$, 0.50% $ZnSO_4$, and control (without $ZnSO_4$). SL, spike length; PH, plant height; SW, spike weight; MI, maturity index; Chla, chlorophyll a; Chlb, chlorophyll b; TChl, total chlorophyll; Mn, manganese; Zn, zinc; and Cu, copper.

(Kinaci & Kinaci, 2005). In the present study, the photosynthetic pigments were significantly enhanced by the foliar application of zinc, which can act as a structural component of the enzymes and proteins involved in the synthesis of these pigments (Wani et al., 2022; Ali et al., 2022). The results obtained here are in agreement with those of Moshfeghi et al. (2019), Noreen et al. (2021), and Roshani et al. (2021), who found that zinc foliar application significantly increased barley photosynthetic pigments compared with the control. Similar findings have also been reported for other crop cultivars, including those of wheat (*Triticum aestivum* L.) by Sattar et al. (2022) and mung bean [*Vigna radiata* (L.) R.Wilczek] by Samreen et al. (2017).

Spike physiognomic characteristics showed variable responses to the applied treatments. Glaucosity was classified as completely absent, somewhat weak, strong, somewhat strong, and mostly strong (Table 4). Spike attitude went from erect to recurved, whereas spike shape ranged from tapering to parallel-parallel to fusiform-tapering to fusiform. The other spike attributes also varied, including grain covering, glume color, glume length, awn color, and awn length.

The foliar application of $ZnSO_4$ showed a significant effect on grain micronutrient concentrations (Table 3 and Figures 1 and 2). The recorded zinc contents in the grown control plants was 1,347 μg g^{-1}, which was further enhanced to 1,751 and 2,176 μg g^{-1} under 0.25 and 0.50 $ZnSO_4$, respectively. Moreover, grain zinc content was improved by 23.1 and 38.1% with the foliar application of 0.25 and 0.50% $ZnSO_4$, respectively, in comparison with the control. However, copper contents were negatively affected by both concentrations of $ZnSO_4$, being 258.7 μg g^{-1} in the control and reducing to 241.9 and 245.1 μg g^{-1} under 0.25 and 0.50% $ZnSO_4$, respectively. Similarly, manganese contents decreased from 1,14.1 μg g^{-1} in the control to 202.6 μg g^{-1} when treated with 0.50% $ZnSO_4$.

Foliar-applied zinc has been reported to enhance the concentration of micronutrients in cereal grains (Cakmak & Kutman, 2018; Drissi et al., 2018). Under field conditions, for example, a substantial increase of up to 85% was observed in zinc content (Ram et al., 2016; Aziz et al., 2019; Melash et al., 2019). In addition, Melash et al. (2019) reported an increase of 82.5% in micronutrient concentrations in response to zinc foliar application. These results are consistent with the findings of Shariatipour et al. (2020), who also observed a significant increase in zinc concentration due to zinc foliar application when evaluating wheat. Likewise, Moshfeghi et al. (2019) and Rehman

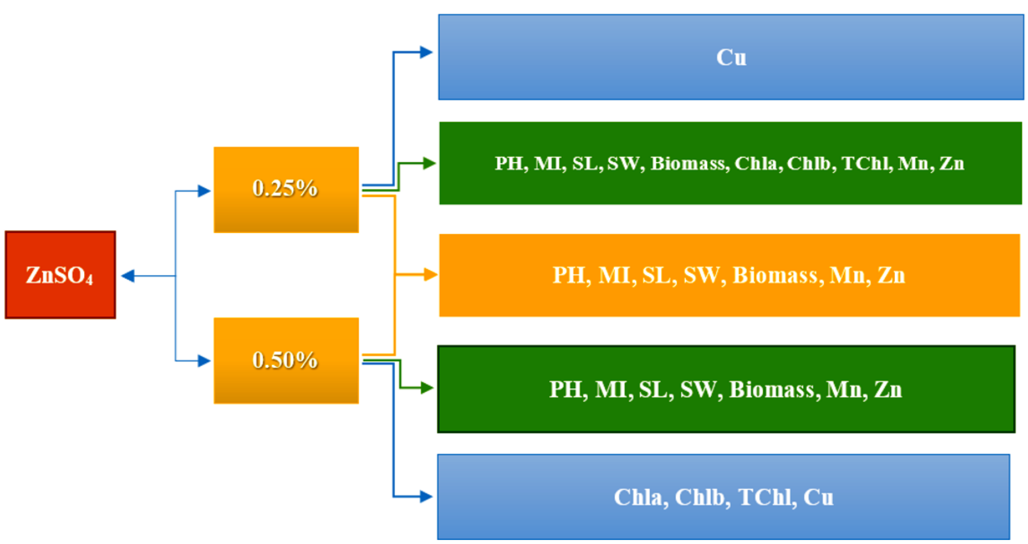

Figure 2. Overview of the effects of the foliar application of 0.25 and 0.50% zinc on the studied agro-morphological and physiological attributes of barley (*Hordeum vulgare*) accessions. Green, positive effect; blue, negative/decreased effect; and yellow, positive effect under both treatments. Cu, copper; PH, plant height; MI, maturity index; SL, spike length; SW, spike weight; Chla, chlorophyll a; Chlb, chlorophyll b; TChl, total chlorophyll; Mn, manganese; and Zn, zinc.

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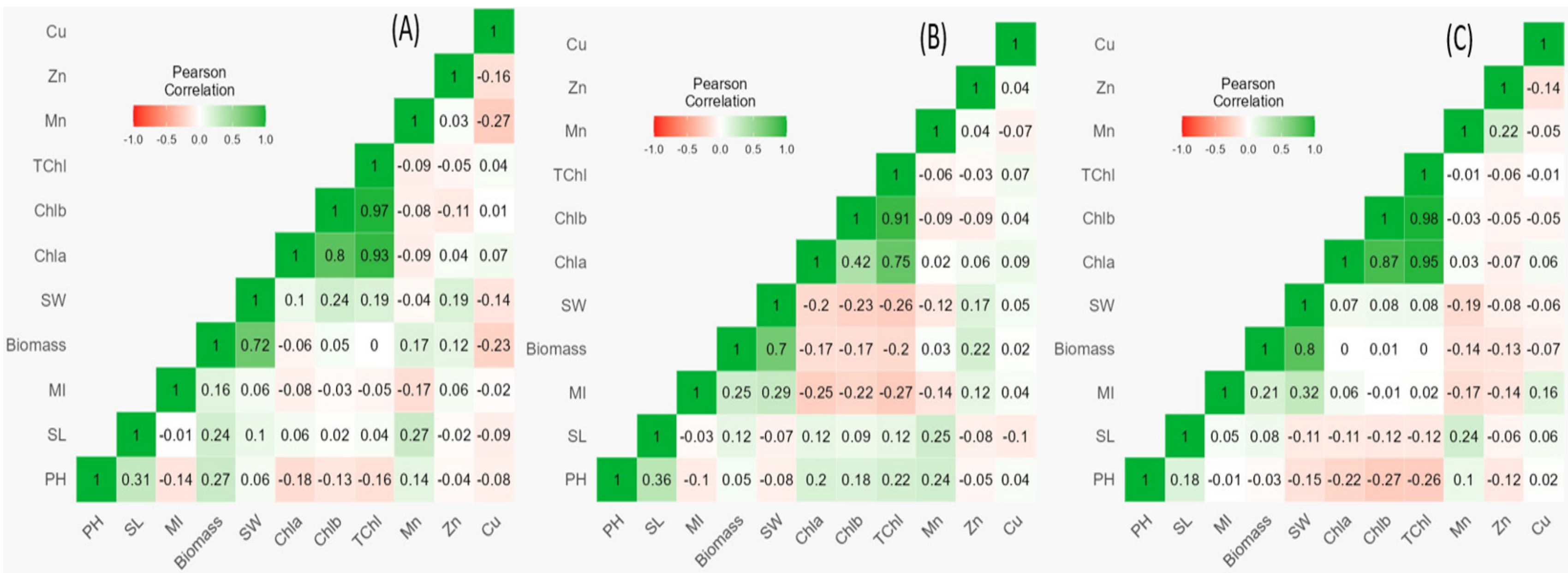

Figure 3. Heatmap of Pearson's correlation coefficient and associated probabilities among barley (*Hordeum vulgare*) accessions, showing the relationship of the studied traits (n = 10) under the following treatments: A, control, without $ZnSO_4$; B, foliar application of 0.25% $ZnSO_4$; and C, foliar application of 0.50% $ZnSO_4$. Significant correlations are shown in green (positive) and red (negative) at $p \leq 0.05$, ≤ 0.01, ≤ 0.001, respectively. PH, plant height; SL, spike length; MI, maturity index; SW, spike weight; Chla, chlorophyll a; Chlb, chlorophyll b; TChl, total chlorophyll; Mn, manganese; Zn, zinc; and Cu, copper.

Table 4. Spike qualitative characteristics of the 50 studied barley (*Hordeum vulgare*) accessions regarding the influence of the foliar application of $ZnSO_4$.

Accession	Glaucosity[(1)]	Attitude[(2)]	Shape[(3)]	Glume and awn length[(4)]	Glume color[(5)]	Awn color[(6)]	Awn length[(7)]	Lemma awn barbs[(8)]	Grain coverings[(9)]
1	Abst, SWk	Erct to Recrd	Tp to Prl	Srtr than Krnl	Wht to Ylw	Ylw to Grn	Lngr to Mdm	I to R	Sc
2	Strg	Erct to Recrd	Prl	Srtr than Krnl	Ylw	Ylw to Grn	Lngr to Mdm	I to Smt	Sc
3	Strg	Erct	Prl	Lngr than Krnl	Wht	Wht	Lngr to Mdm	R	N to Sc
4	MSt	Erct to Recrd	Prl	Lngr than Krnl	Wht to Ylw	Wht to Grn	Lngr to Mdm	I	N
5	Strg	Erct to Recrd	Prl	Lngr than Krnl	Wht	Wht	Lngr	R	C
6	Abst	Erct to Recrd	Prl to Fusi	Lngr than Krnl	Ylw to Brwn	Ylw	Lngr	R	Sc
7	Strg	Erct	Tp to Prl	Lngr than Krnl	Ylw	Ylw	Lngr	R	Sc
8	Strg to Wk	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Grn	Lngr	R	Sc
9	Abst	Erct	Prl to Fusi	Srtr than Krnl	Ylw	Ylw	Lngr	R	N to Sc
10	Abst to Wk	Erct	Prl to Fusi	Srtr than Krnl	Ylw to Brwn	Ylw to Grn	Lngr	R	C
11	Wk, SStrg	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Wht	Mdm	I	Sc
12	Abst to Strg	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Wht	Mdm	I	Sc
13	Abst	Erct	Prl	Srtr than Krnl	Ylw to Brwn	Wht to Grn	Lngr	R	Sc
14	Strg	Erct to Recrd	Tp to Prl	Srtr than Krnl	Wht	Grn	Lngr	R	C
15	Wk to Strg	Recrd	Prl	Lngr than Krnl	Wht to Ylw	Wht	Lngr	R	N
16	Wk to Strg	Erct	Tp to Fusi	Lngr than Krnl	Wht	Wht	Lngr	R	Sc
17	Strg	Erct	Prl	Srtr than Krnl	Wht	Wht	Lngr	R	Sc
18	Abst	Recrd	Prl	Lngr than Krnl	Wht	Ylw	Lngr	R	C
19	Abst	Erct	Tp to Prl	Lngr than Krnl	Ylw	Ylw	Mdm	R	C
20	Strg	Recrd	Fusi	Lngr than Krnl	Wht	Wht	Lngr	R	C
21	Strg	Erct	Fusi	Srtr than Krnl	Wht	Wht to Grn	Lngr	R	Sc
22	Strg	Recrd	Prl	Lngr than Krnl	Wht to Ylw	Wht to Ylw	Mdm	R	C
23	Strg	Erct to Recrd	Tp to Fusi	Lngr than Krnl	Wht	Wht	Lngr	R	Sc
24	Wk	Erct to Recrd	Tp to Prl	Srtr than Krnl	Ylw	Grn	Lngr	I	C
25	Strg	Erct	Prl	Lngr than Krnl	Wht	Wht	Lngr	R	Sc
26	MSt	Erct to Recrd	Prl	Lngr than Krnl	Wht to Ylw	Wht to Grn	Lngr to Mdm	I	N
27	Strg	Erct to Recrd	Prl	Lngr than Krnl	Wht	Wht	Lngr	R	Sc
28	Abst	Erct to Recrd	Prl to Fusi	Lngr than Krnl	Ylw to Brwn	Ylw	Mdm	R	C
29	Wk to Strg	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Grn	Lngr	I	C
30	Strg	Erct	Prl to Fusi	Srtr than Krnl	Ylw to Brwn	Ylw to Grn	Mdm	I	Sc
31	Wk, SStrg	Recrd	Prl	Srtr than Krnl	Ylw	Ylw to Wht	Mdm	R	C
32	Abst to Strg	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Wht	Lngr	R	C
33	Abst	Recrd	Prl	Srtr than Krnl	Ylw to Brwn	Wht to Grn	Lngr	I	Sc
34	Abst	Recrd	Fusi	Srtr than Krnl	Ylw	Ylw	Mdm	R	Sc
35	Strg	Erct to Recrd	Tp to Fusi	Srtr than Krnl	Wht	Wht	Lngr	R	Sc
36	Abst	Erct to Recrd	Prl to Fusi	Lngr than Krnl	Ylw to Brwn	Ylw	Mdm	R	C
37	Strg	Erct	Prl	Lngr than Krnl	Ylw	Ylw to Grn	Mdm	R	Sc
38	Abst to Wk	Erct	Prl to Fusi	Lngr than Krnl	Wht	Wht	Mdm	I	Sc
39	Abst	Recrd	Prl	Lngr than Krnl	Ylw	Ylw to Wht	Lngr	R	C
40	Abst to Strg	Recrd	Prl	Srtr than Krnl	Ylw	Ylw to Wht	Lngr	R	C
41	Abst	Erct	Prl	Srtr than Krnl	Ylw to Brwn	Wht	Lngr	I	C
42	MStrg	Erct to Recrd	Prl	Lngr than Krnl	Wht	Wht to Grn	Lngr to Mdm	I	N
43	Strg	Erct to Recrd	Prl	Srtr than Krnl	Wht	Wht	Mdm	R	C
44	Wk	Erct to Recrd	Prl to Fusi	Lngr than Krnl	Ylw to Brwn	Ylw	Lngr	R	Sc
45	Abst	Erct	Tp to Prl	Lngr than Krnl	Wht	Ylw	Mdm	I	Sc
46	Wk to Strg	Erct	Prl	Srtr than Krnl	Ylw	Ylw to Grn	Lngr	I	C
47	Wk, SStrg	Erct	Prl	Srtr than Krnl	Wht	Ylw to Wht	Lngr	R	Sc
48	Strg	Erct to Recrd	Tap to Prl	Lngr than Krnl	Wht	Grn	Lngr	I	Sc
49	Wk to Strg	Erct	Tp to Fusi	Srtr than Krnl	Ylw	Wht	Mdm	I	Sc
50	Abst	Erct	Tp to Prl	Lngr than Krnl	Ylw	Ylw to Grn	Lngr	R	N to Sc

[(1)]Abst, absent; SWk, somewhat weak; Wk, weak; Strg, strong; MStrg, mostly strong; and SStrg, somewhat strong. [(2)]Erct, erect; and Recrd, recurved. [(3)]Prl, parallel; Fusi, fusiform; and Tp, tapering. [(4)]Srtr than Krnl, shorter than kernel; and Lngr than Krnl, longer than kernel. [(5)]Ylw, yellow; Wht, white; and Brwn, brown. [(6)]Wht, white; Grn, green; and Ylw, yellow. [(7)]Lngr, longer; and Mdm, medium. [(8)]R, rough; I, intermediate; and Smt, smooth. [(9)]N, naked; C, covered; and Sc, semi-covered.

et al. (2020) found that zinc concentration increased through foliar application. Yagmur et al. (2017), Al Mutairi et al. (2020), and Roshani et al. (2021) also verified an increase in nutrient concentration due to zinc foliar application.

In the present research, the levels of zinc, manganese, and copper in the grains of the studied barley accessions increased in varying degrees, showing different responses in terms of concentration and bioavailability. These differences may be due to variations in barley accessions, soil types, and timing of zinc foliar application as previously concluded by Cakmak (2008) and Hao et al. (2021). Despite these variations, the nutrient content in the barley seeds increased as with the increase in zinc concentration through foliar application.

Although several approaches show potential to improve both the yield and nutritional quality of an agricultural crop for human diet (Aziz et al., 2019), biofortification stands out as an alternative, particularly in areas where people suffer from malnutrition (Ali et al., 2022). In this scenario, the foliar application of nutrients has been used to improve the yield of cereal crops, maintaining balanced nutrients and reducing the risk of nutrient losses (Aziz et al., 2019).

The results obtained in the present study allow of inferring that zinc foliar application is a promising way of improving the concentration of zinc and other micronutrients in barley grains, with a consequently better bioavailability for human consumption. However, organic matter, soil composition, and soil pH may affect the availability of these nutrients (Salim & Raza, 2020), explaining the different responses of the evaluated barley accessions to zinc foliar application. This is an indicative that further research is needed to explore the optimum levels of foliar-applied zinc.

Conclusions

1. The foliar application of 0.25 and 0.50% $ZnSO_4$, especially of the highest concentration, enhances the chlorophyll content, agro-morphological characteristics, and nutrient concentration of barley (*Hordeum vulgare*) grains.

2. Zinc foliar application improves barley performance considering the applied concentrations and used accessions.

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