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Zinc-induced enhancement in growth, ionic contents and yield of bio-fortified and standard wheat (*Triticum aestivum* L.) varieties

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Abstract
Zinc as a micronutrient is highly essential for animals and plants to attain optimum growth and development. Zinc-deficient soils result in stunted and abnormal growth of plants. Three wheat varieties, i.e., Zincol-2016 (biofortified), Galaxy-2013, and Punjab-2011 (both non-biofortified), were sown in pots containing fertile soil in a completely randomized design with four replicas. The seedlings were sprayed with four zinc solutions (Control, 0.03%, 0.06%, and 0.09% of Zn as $ZnSO_4.7H_2O$) 40 days after sowing. It was observed that the impact of 0.06% zinc spray was more pronounced on wheat plants as compared to that of 0.03% and 0.09%. More pronounced improvement in growth, chlorophyll content, total soluble proteins, and sugars was observed in cv. Zincol-2016 as compared to that in the other two varieties, Galaxy-2013 and Punjab-2011. Similarly, Zn foliar spray significantly enhanced root, straw, and grain K^+, Cu^{2+}, Zn^{2+}, and Fe^{2+} contents in all three cultivars. In contrast, grain phytate contents were reduced with increased supplementation of Zn. Data revealed that grain yield was improved significantly by exogenous application of zinc, especially at 0.06% in all three wheat varieties, but being more promising in Zincol-2016. Furthermore, Zincol-2016 accumulated higher zinc contents in grains as compared to that in Galaxy-2013 and Punjab-2011. Foliar application of zinc resulted in higher uptake and accumulation of this element from soil to seeds, thereby resulting in improved vegetative growth.

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Introduction

Of micronutrients, zinc (Zn) has a prime importance in cellular processes like chlorophyll synthesis, carbohydrate metabolism, and cell division and differentiation (Hacisalihoglu, 2020). Although soil Zn contents range from 40-120 mg/kg (Noulas et al., 2018), the agricultural soils are becoming Zn deficient (Kabata-Pendias, 2001; Moreno-Lora and Delgado, 2020). Moreover, arid and semi-arid areas of the world are Zn deficient (Sahrawat et al., 2008). Some estimates have shown that almost 70% of the agricultural land of Pakistan is Zn deficient (Ullah et al., 2019).

Most mineral nutrients like Zn are absorbed by plant roots, but it has been intensively investigated that foliar applied nutrients can be abruptly absorbed by plant leaves (Gupta et al., 2016). The soil application of Zn fertilizers requires a large quantity which is costly. Moreover, the poor mobility of Zn in

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the soil makes it less effective (Swietlik, 2002). Therefore, the foliar fertigation of Zn is practically more effective than that of soil treatment (Xie et al., 2020). The foliar application assures the targeted and fast uptake and delivery of nutrients to plant tissues involved in active metabolism (Fernández and Brown, 2013b).

However, the absorption of Zn from leaves is complex as different plant species have different leaf morphology, so their interaction with foliar applied Zn significantly differs (Xie et al., 2020). Still, the possible movement route and metabolic pathway of Zn can be traced (Zhang and Brown, 1999). The leaf surface is the main factor that affects the absorption of Zn as leaf surface has a large number of stomata with low trichome density, which promotes Zn absorption (Xie et al., 2020).

Zinc has an important role in plant metabolism as it works as a co-factor of many enzymes including RNA polymerase, superoxide dismutase, and alcohol dehydrogenase (Castillo-González et al., 2018; Ei et al., 2020). It can also enhance the resistance of plants to many abiotic and biotic stresses (Cakmak et al., 2010; Cabot et al., 2019). Zinc-mediated enhancement in growth and yield in different plant species is attributed to the improvement of different processes like photosynthesis (Sofy et al., 2020), pollen formation (Suganya et al., 2020), and metabolism of proteins and carbohydrates (Tsonev and Cebola Lidon, 2012). It has been previously described that foliar application of Zn enhanced the yield of many crops like wheat (Wu et al., 2020), maize (Xia et al., 2019), rice (Goloran et al., 2019; Lv et al., 2019), and strawberry (Soppelsa et al., 2019).

Wheat (*Triticum aestivum* L.) is a staple food of the majority of the population of the world. However, under Zn deficiencies in soils and different environmental stresses, wheat plants are unable to absorb enough Zn from soil to quench the thirst of humans. The reason being that presently grown wheat varieties contain 19 to 30 mg Zn kg^{-1}, whereas the daily dietary human requirement is around 40-50 mg Zn kg^{-1}. Apart from other interventions, bio-fortification technology offers its potential to enhance grain Zn content of wheat, thereby mitigating Zn deficiency in humans' diet through the food chain (Cakmak et al., 2010). Thus, the use of Zn spray could be a potential intervention to raise Zn content in wheat grain. Recently, a biofortified high Zn accumulator wheat variety Zincol-2016 has been introduced for general cultivation, which accumulates relatively high quantity of Zn than that of conventional wheat varieties. Cultivation of bi-fortified staple food crops is cost-effective in consumption contrary to fortified food products (Lim et al., 2012).

High yield can be attained by exogenous Zn application, which helps enhance Zn content in grains from 27 mg Zn kg^{-1} to 49 mg Zn kg^{-1} in wheat grains (Zou et al., 2012). The exogenous application of Zn can enhance grain Zn contents up to 50% in dietary food (Joy et al., 2015). Therefore, there is a need to understand the response of bio-fortified and non-bio-fortified wheat varieties to exogenously applied Zn for enhancing grain Zn content.

Materials and Methods

The experiment was designed and conducted at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan for two crop seasons 2014-15 and 2015-16. The treatments consisted of four Zn spray solutions (0, 0.03%, 0.06%, and 0.09% Zn as $ZnSO_4.7H_2O$) which were applied to three wheat varieties, i.e*.,* Zincol-21016 (bio-fortified) and Galaxy-2013 and Punjab-2011 (both non-biofortified) grown in equal weight plastic pots containing 8 kg of fertile garden soil, arranged in a completely randomized design (CRD) with four replicas of each treatment. Prior to sowing, the seeds were sterilized with 5.0 g L^{-1} sodium hypochlorite for five minutes. Ten healthy and equal-sized seeds of each variety were sown in each pot, while germinating seedlings were thinned to two plants per pot. Foliar spray of four Zn solutions was applied at day 40 after sowing at the vegetative growth stage. The nozzle of the sprayer was adjusted to dispense 10 mL solution on the plants in each pot.

The data for different morphological parameters (plant height, and root and shoot fresh and dry weights) was recorded after twenty days of Zn spray by careful uprooting one plant from each pot and leaving the other for yield determination. The chlorophyll constituents (Arnon, 1949), total soluble proteins (Bradford, 1976), total free amino acids (Hamilton et al., 1943), and total soluble sugars (Dubois et al., 1951) were determined on fresh leaf samples, while oven-dried samples were used to determine straw, root and grain K^+ contents by flame photometry. Copper (Cu^{2+}), Fe^{2+}, and Zn^{2+} of straw, root, and grain were determined using an atomic absorption spectrophotometer (Allen and Rae, 1986). The grain phytate contents were analyzed as described by García-Villanova et al. (1982). Data were analyzed statistically using statistical software IBM, SPSS Statistics 20. The mean values presented as bars with SE values.

Results

Growth attributes

The foliar fertigation of Zn significantly ($P \leq 0.05$) enhanced plant growth measured as shoot and root lengths, and fresh and dry weights in all three wheat varieties (**Table 1**). When compared to the control, shoot and root lengths were significantly enhanced (19% and 24%, respectively) at 0.06% Zn spray in Zincol-2016. Similarly, shoot fresh and dry weights also increased (16% and 25%, respectively) at 0.06% Zn spray in Punjab-11, while root fresh and dry weights (25% and 20%, respectively) at 0.06% Zn spray in Punjab-2011. However, of all wheat varieties, Zincol-2016 showed the highest growth attributes (**Table 1**).

Photosynthetic pigments

The photosynthetic pigments (Chl-*a*, Chl-*b*, total Chl, and carotenoids) were significantly ($P \leq 0.05$) increased in all three wheat varieties by foliar application of Zn (**Table 1**). The highest enhancement in these pigments was observed in Zincol-2016 as compared to that in the other wheat varieties wherein the increase in Chl-*a*, Chl-*b*, total Chl and carotenoids were 41%, 27%, 35%, and 80%, respectively, with foliar spray of Zn as compared to those by non-spray (**Table 1**).

Table 1. Mean square (ANOVA) values for shoot and root length (cm), shoot fresh and dry weights (g/plant), root fresh and dry weights (g/plant), chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoids (mg g^{-1} FW) of three wheat varieties after foliar spray of different Zn levels

Parameters	Intercept	Variety (V)	Treatment (T)	Interaction (V × T)
	df	2	3	6
Shoot length	229391.3***	853.5***	280.7***	11.59^{ns}
Root length	19899.5***	39.77***	32.36***	0.936^{ns}
Shoot fresh weight	692535.1***	3398.9***	623.6**	22.4^{ns}
Shoot dry weight	5173.6***	26.92***	8.48***	0.83^{ns}
Root fresh weight	2370.4***	0.36^{ns}	4.65^{ns}	0.15^{ns}
Root dry weight	22.9***	0.0006^{ns}	0.04***	0.0004^{ns}
Chlorophyll-*a*	357.7***	0.116^{ns}	1.436***	0.073^{ns}
Chlorophyll-*b*	191.4***	0.852***	0.459***	0.025^{ns}
Total chlorophyll	1072.5***	1.58***	3.46***	0.93^{ns}
Carotenoids	104.2***	0.611***	0.812***	0.796*

, * = significance level at $P \leq 0.01$ and 0.001; ns= non-significant; df = degree of freedom

Total soluble proteins, sugars and total free amino acids

A significant ($P \leq 0.05$) enhancement in total soluble proteins, total free amino acids, and total soluble sugars was observed in all three wheat varieties by foliar application of Zn except for Punjab-2011 wherein protein contents were only slightly enhanced at 0.03% Zn level, whereas a decrease in these parameters in this cultivar was observed at 0.06% and 0.09% Zn levels (**Figure 1**). It was observed that 0.06% Zn foliar spray resulted in a 31% increase in soluble proteins in Zincol-2016, and 15% in soluble sugars in Punjab-2011. Amino acid contents were highest in Punjab-2011 at 0.09% Zn spray (**Figure 1**).

K^+, Zn^{2+}, Fe^{2+}, and Cu^{2+} contents

Analysis of data showed that straw, root, and grain K^+ contents were significantly ($P \leq 0.05$) enhanced in all three wheat varieties by Zn foliar spray (**Figure 2**). It was observed that 0.06% Zn foliar spray resulted in an increase of 33% in straw K^+ contents in Zincol-2016, 59% in root K^+ in Galaxy-2013), and 24% increase in grain K^+ in Zincol-2016 as compared to those in the control (non-spray) plants. However, at 0.09% Zn spray, the K^+ contents in grains were decreased 3-9% compared to that of the control plants (**Figure 2**).

Similarly, the Zn^{2+} contents in straw, root, and grain were also enhanced in all three wheat varieties by foliar spray of Zn. It was observed that straw, root, and grain Zn^{2+} contents were enhanced by 77%, 24%, and 60%, respectively, in Zincol-2016 when compared to those of the non-sprayed wheat plants (**Figure 2**).

The foliar spray of varying levels of Zn ($P \leq 0.05$) affected the iron (Fe^{2+}) content of grains in the wheat varieties (**Table 2**). The spray of 0.06% Zn caused an increase of 21.3% over the control. The tissue concentrations of Fe^{2+} ranged from 26.44 to 50.53 mg Fe^{2+} kg^{-1} in plants subjected to varying levels of Zn. The wheat variety Punjab-2011 maintained 35.31 mg Fe^{2+} kg^{-1} compared to 34.07 Fe^{2+} kg^{-1} by Zincol-2016 (**Table 2**).

The foliar spray of Zn significantly ($P \leq 0.05$) affected grain Cu^{2+} contents of all three wheat varieties (**Table 2**). The Cu^{2+} content decreased progressively with each increment in Zn level. The crop sprayed with 0.06% Zn maintained 3.65 mg Cu^{2+} kg^{-1} compared to 4.47 mg Cu^{2+} kg^{-1} in the control non-sprayed plants. The wheat variety Punjab-2011 maintained higher Cu^{2+} content by 4.73 mg Cu^{2+} kg^{-1} compared to 3.92 and 3.61 mg Cu^{2+} kg^{-1} by Zincol-2016 and Galaxy-2013, respectively. The values of copper content ranged from 3.15 to 5.27 mg Cu^{2+} kg^{-1} at varying Zn treatments.

Table 2. Mean grain phytate contents of three wheat varieties fed through foliage with varying Zn concentrations of Zn

Zinc spray (%)	Varieties	Grain phytate (mg kg^{-1})
Control	Zincol-2016	11.51
	Galaxy-2013	13.10
	Punjab-2011	10.79
0.03	Zincol-2016	8.10
	Galaxy-2013	10.50
	Punjab-2011	9.11
0.06	Zincol-2016	7.71
	Galaxy-2013	9.10
	Punjab-2011	8.70
0.09	Zincol-2016	7.50
	Galaxy-2013	8.79
	Punjab-2011	8.00
ANOVA	T	0.06*
	V	0.09*
	V × T	1.00*

* = significance at $P \leq 0.05$, V= Varieties, T= Treatments

Grain phytate content

The foliar application of Zn significantly ($P \leq 0.05$) affected the phytate content in all three wheat varieties. The foliar spray of Zn caused a reduction in grain phytate content in all wheat varieties (**Table 3**). There was a substantial reduction in phytate content due to foliar spray of 0.09% Zn on the crop and resulted in a reduction of 31.1% over that of the control plants. The wheat variety Zincol-2016 maintained a minimum level of phytate content (8.71 mg PA kg^{-1}) followed by that in Galaxy-2013 and Punjab-2011. The values of phytate content ranged from 7.71 to 13.10 mg PA kg^{-1} under different Zn treatments.

Yield attributes

Different yield attributes (number of tillers/plant, number of spikelets/spike, 100-grain weight, and total seeds per plant) were significantly ($P \leq 0.05$) enhanced in all three wheat varieties by the foliar application of Zn (**Table 3**). It was observed that 0.06% Zn foliar spray resulted in increase in 17% in Zincol-2016, 16% in Galaxy-2013,, 16% in Galaxy-2013, and 24% in Zincol-2016 in number of tillers/plant, number of spikelets/spike, 100 grain weight, and total seeds per plant, respectively, as compared to their respective controls (**Table 3**).

Table 3. Different yield attributes (Means ± SE, *n*= 4) of three wheat varieties fed through leaves with varying Zn concentrations. Different letters on mean values represent a significant difference at *P* ≤ 0.05 (DMRT). In.: Intercept, V: Variety, T: Treatment, and V×T: Variety × Treatment Interaction

Varieties	Zn Spray	Number of fertile tillers/plant	Number of spikelets/spike	100-grain weight	Total seeds/plant
V1	Control	8.8±0.41^{abc}	21.1±0.24^{bc}	5.7±0.20^{a}	420.3±17.6^{bc}
	0.03%	9.3±0.41^{ab}	20.6±0.54^{ab}	5.8±0.19^{a}	451.0±15.5^{bc}
	0.06%	10.3±0.54^{a}	20.1±0.39^{a}	6.1±0.11^{a}	521.3±19.3^{a}
	0.09%	8.0±0.35^{bc}	19.7±0.45^{bcd}	5.2±0.24^{b}	385.8±25.3^{cde}
V2	Control	7.3±0.22^{c}	20.7±0.25^{fg}	4.4±0.08^{d}	349.5±17.6^{de}
	0.03%	8.0±0.35^{bc}	20.6±0.56^{defg}	4.8±0.14^{bcd}	409.5±17.6^{bc}
	0.06%	8.0±0.35^{bc}	21.0±0.38^{cde}	4.8±0.22^{bc}	416.0±16.5^{bcd}
	0.09%	7.3±0.41^{c}	20.5±0.45^{defg}	4.4±0.15^{d}	340.8±12.0^{e}
V3	Control	7.5±0.56^{bc}	20.0±0.53^{g}	4.6±0.08^{cd}	385.5±20.2^{cde}
	0.03%	8.0±0.61^{bc}	23.2±0.75^{efg}	4.7±0.06^{bcd}	406.3±20.2^{bcde}
	0.06%	8.5±0.56^{bc}	20.5±0.86^{def}	5.1±0.09^{bc}	462.0±22.5^{ab}
	0.09%	8.0±0.50^{bc}	19.1±0.37^{fg}	4.6±0.13^{bcd}	388.3±8.40^{cde}
ANOVA	In.	3250.5***	18135.2***	1216.4***	8121365.3***
	V	8.896***	69.93***	5.17***	17234.9***
	T	3.576*	13.30***	1.213***	21689.9***
	V×T	0.701^{ns}	1.215^{ns}	0.095^{ns}	1418.7^{ns}

, * = significance level at $P \leq 0.01$ and 0.001; ns= non-significant; V= Varieties, T= Treatments, In= Intercept

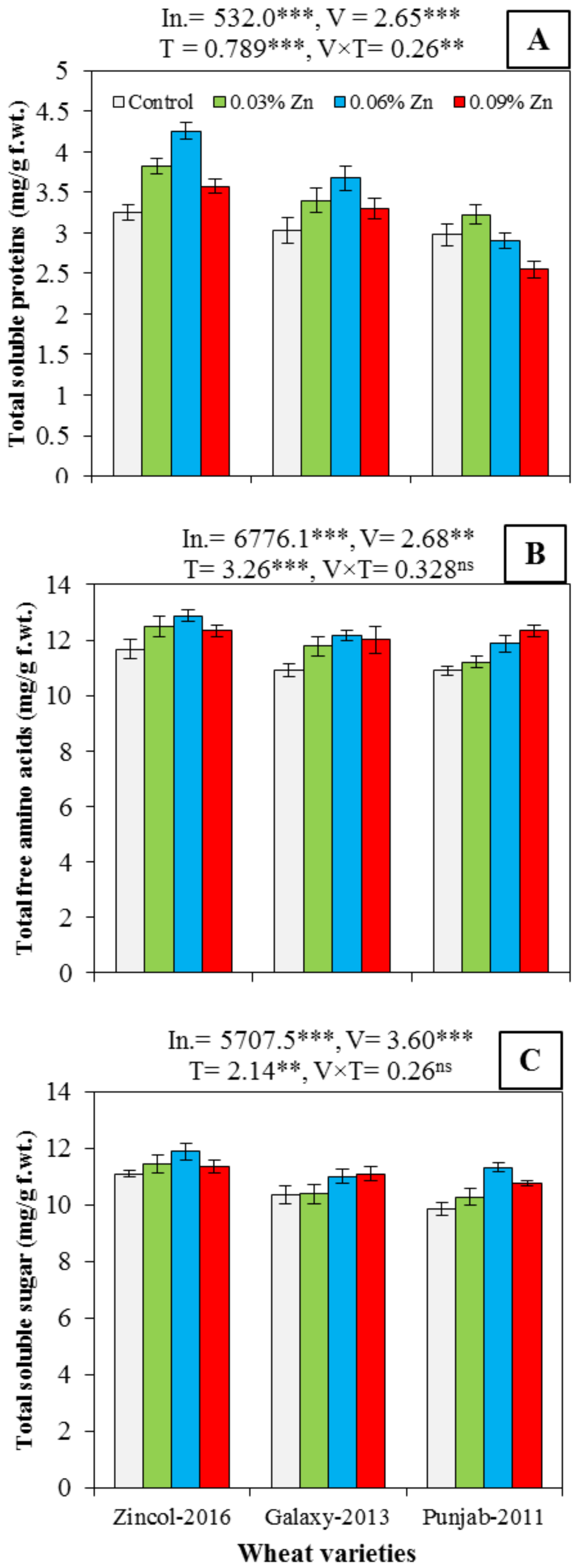

Figure 1. Total soluble proteins (A), total free amino acids (B) and total soluble sugars (C) (mg/g f.wt) in three wheat varieties in response to foliar spray of varying Zn levels. In.: Intercept, V: variety, T: treatment, V×T: variety × treatment interaction, *, **, * = significance level at $P \leq 0.05$, 0.01 and 0.001, respectively; ns= non-significant**

Discussion

Zinc, a micronutrient, is essential for algae, plants, and animals, having a pivotal role in growth and development (Cabot et al., 2019). Like other abiotic stresses, Zn deficiency can severely reduce the yield production of economically important crops (Helfenstein et al., 2015; Pawlowski et al., 2019; Yaseen and Hussain, 2021) as Zn deficient crops are easily prone to different types of diseases like pest and fungal attack (Helfenstein et al., 2015). So, there is a dire need of time to enhance Zn contents in food items of our inhabitants by bio-fortification of wheat grains with Zn (Zou et al., 2012; Lowe et al., 2018; Yaseen and Hussain, 2021). This deficiency can be met either by exogenous application of Zn to cereal crops or cereal grains can by Zn bio-fortified to reduce human Zn deficiencies (Imran et al., 2015).

The production of higher biomass due to foliar application of Zn particularly at 0.06% was observed in all wheat varieties; however, high growth in Zinclol-2016 after Zn spray is an outcome of inherently maintaining the higher amount of grain Zn content as compared to the other varieties tested in this investigation. Various researchers (Rawashdeh and Sala, 2015; Farouk and Al-Amri, 2019; Rizwan et al., 2019; Saleem et al., 2020) have reported that exogenous Zn supplementation can enhance biomass production in different crops. It has also been reported elsewhere that a greater quantity of biomass production was harvested by foliar spray of Zn (Fernández and Brown, 2013a). As growth is known to directly relate to the photosynthetic process in plants, so high amount of photosynthetic pigments may result in high photosynthetic efficiency, thereby causing improved crop growth and yield. This study revealed that chlorophyll constituents were enhanced with Zn spray in the wheat varieties, particularly Zincol-2016 synthesized maximal amount of photosynthetic pigments. It has been reported that exogenous application of Zn can enhance photosynthetic pigments in different crops like wheat (Wu et al., 2020), barley (Noreen et al., 2021), maize (Sofy et al., 2020), and rapeseed (Kamran et al., 2020).

Zinc has a vital role in protein and starch metabolism (Taheri et al., 2011). Furthermore, it also assists the protein synthesis and gene expression (Toor et al., 2020). In the present investigation, total free amino acids, total soluble proteins, and soluble sugars increased after foliar spray of 0.03% and 0.06% Zn, while higher level (0.09%) of Zn caused a reduction in these pigments in all wheat varieties. Likewise, in another study foliar application of Zn (1.0 g/L) to *Vicia faba* enhanced total soluble proteins and sugar contents (Mohamed et al., 2016). Similarly,

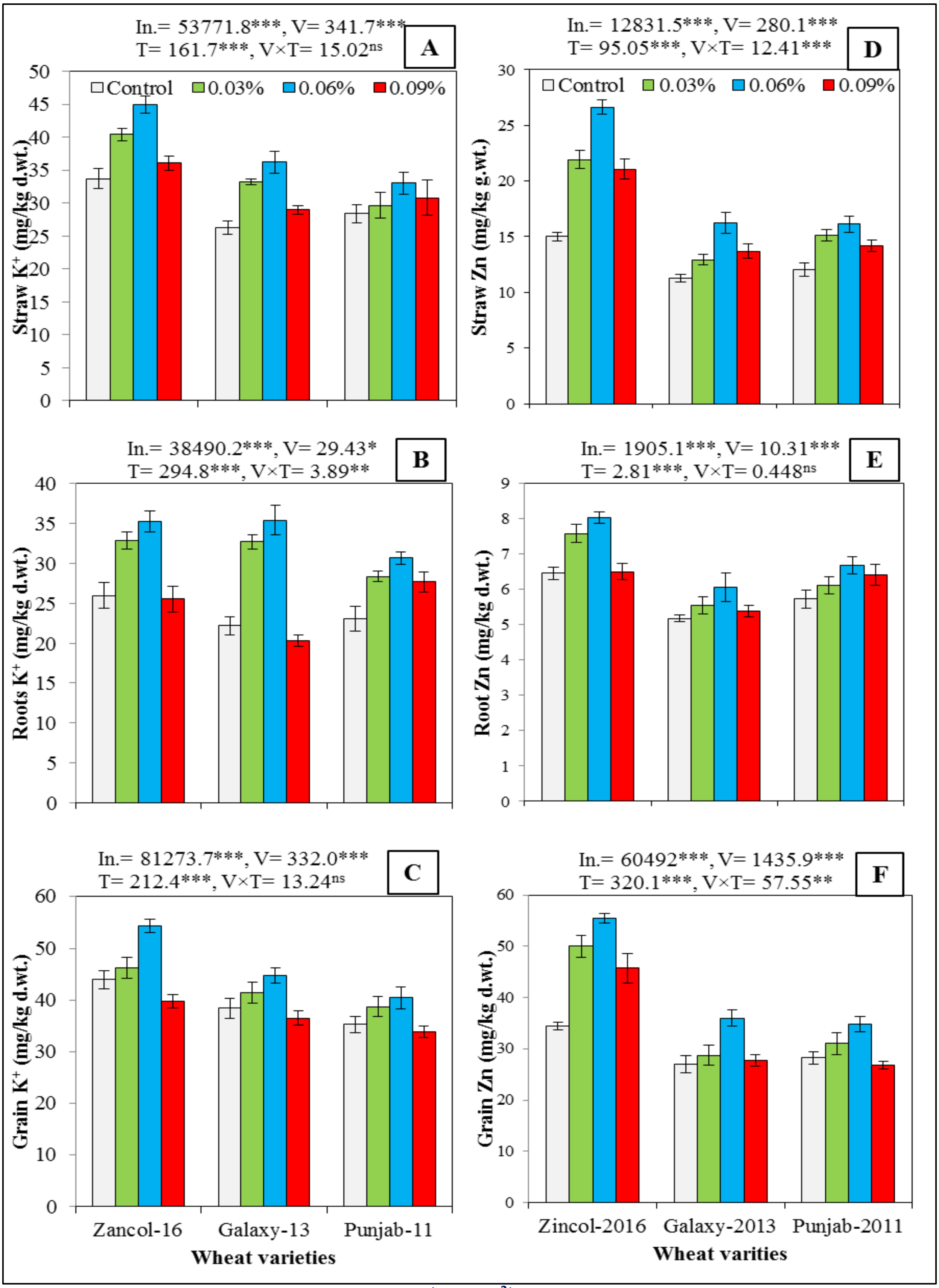

Figure 2. Straw (A, D), root (B, E) and grain (C, F) K^+ and Zn^{2+} contents, respectively, in three wheat varieties in response to foliar spray of varying Zn levels. In.: Intercept, V: variety, T: treatment, V×T: variety × treatment interaction, *, **, * = significance level at $P \leq 0.05$, 0.01 and 0.001; ns= non-significant**

increase in total soluble sugars is also reported in wheat (Yaseen and Hussain, 2021), and barley (Noreen et al., 2021).

The foliar spray of Zn caused a distinctive reduction in phytate contents in all wheat varieties. These results agree with those of Chattha et al. (2017) and Mabesa et al. (2013), who reported that phytate content could be lowered down in the wheat grain by maintaining higher zinc content in grains. The phytate contents in food have a complex interaction with Zn (Ghasemi et al., 2013).

The contents of Zn^{2+} in roots, straw, and grain were found to be improved due to maintenance of a greater quantity of Zn^{2+} by plants, derived either from already contained in cultivar Zincol-2016 or from externally applied Zn. Different wheat varieties varied in their capacity to accumulate Zn^{2+}, Cu^{2+}, Fe^{2+}, and K^{+} contents and responded differently to zinc application (Yang et al., 2011). In some earlier studies, it has been documented that copper contents decrease (Imtiaz et al., 2003), while those of Fe enhance (Mousavi, 2011) with increased concentration of Zn foliar spray. Rengel and Graham (1995) reported that K uptake in plants exposed to Zn deficient soil was reduced, whereas absorption could be restricted through increased supply of Zn to the plant either by cultivation of biofortified wheat varieties such as Zincol-2016 or through soil and/or foliar application.

The Zn nutrient is essential for formation of pollen tube, and grain formation and its filling process during reproductive stage. A significant improvement in grain yield and its components resulted because of Zn spray that may have caused a greater translocation of nutrients from leaves to grains, especially in the bio-fortified wheat variety 'Zincol-2016'. The results of our study are in accordance with those of other reports (Zhang et al., 2010; Chattha et al., 2017), which document that crop yield could be improved by application of Zn. The Zn content in straw and grain could be improved due to maintenance of greater quantity of Zn by the plant, derived either from already contained in bio-fortified varieties such as 'Zincol-2016' or from externally applied Zn. In an earlier study, a differential response in wheat varieties in order to accumulate Zn has been reported (Yang et al., 2011). The eco-edaphic environment and fertility of soil directly influence the ability of plant roots to uptake Zn and its transportation to areal parts in response to foliar spray of Zn (Cakmak et al., 2010; Yang et al., 2011; Fernández and Brown, 2013a).

Conclusion

Zinc deficiencies may lead to low growth and productivity of economically important cereal crops like wheat. The results of our study exhibited that the bio-fortified wheat variety (Zincol-2016) showed better growth either when Zn was applied or not, because the grains of Zincol-2016 already contained high amount of Zn which helps in better growth, however, Zn foliar spray further enhanced the growth of all wheat varieties including bio-fortified Zincol-2016. Similarly, the grain Zn contents were improved by the foliar application of Zn, especially at 0.06% of Zn, while higher level of Zn (0.09%) caused Zn toxicity in the wheat plants thereby resulting in a reduction in growth and yield. The standard wheat varieties (Galaxy-2013 and Punjab 2011) showed better growth after foliar application of Zn at 0.06%. However, the bio-fortified wheat variety, Zincol-2016 can accumulate high amount of zinc in its grains.

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This study does not involve Human/animal subjects and no ethical approval is needed.

Handling of bio-hazardous materials
The author(s) certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discorded to minimize any types of bio-contamination(s).

Availability of primary data and martials
As per editorial policy, experimental materials, primary data or software codes are not submitted to the publisher. These are available with corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Author's consent
All authors contributed in designing and execution of the experiment. All contributors have critically read this manuscript and agree for publishing in IJAEB.

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