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Effects of exogenously applied Zn on the growth, yield, chlorophyll contents and nutrient accumulation in wheat line L-5066

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Abstract

In order to explore the role of foliar Zn application as a supplement for obtaining higher yield of wheat (*Triticum aestivum* L.), pot experiments were conducted in the Old Botanical Garden, at University of Agriculture Faisalabad, Pakistan. Seeds of wheat line, L-5066 were sown in plastic pots arranged in completely randomized design (CRD). Three concentrations of Zinc i.e. 0, 4 and 6 m*M* were applied exogenously after the establishment of the seedlings. The data for different growth and yield parameters recorded during the course of study was subjected to statistical analysis for comparison purposes. Exposure of wheat line L-5066 to Zn foliar application increased the morphological and yield parameters. Plant height, fresh and dry weights, number of leaves, number of spikes and spikelets, number of tillars and 100 grain weight were significantly increased as compared to plants grown under zinc-free environment. Similarly, foliar Zn application also increased chlorophyll contents, ion uptake and accumulation. A marked increase ($P \leq 0.01$) in stem Na^+ and leaf K^+ contents was observed under Zn application. Maximum increase in most of the tested parameters was observed at 4m*M* Zn concentration. This study proves that foliar application of Zn at optimum level, through action as growth promoter, can increase the yield and ion uptake in wheat.

Key words: Zinc foliar spray; chlorophyll contents; ion uptake; crop yield; wheat (*Triticum aestivum* L.); line L-5066

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1. Introduction

Zinc is an essential micronutrient for biological systems and plays a crucial physiological role in protein synthesis and metabolism [1]. Besides, it is a structural constituent and regulatory co-factor in enzymes and proteins involved in many biochemical pathways [2]. Almost 40% of the Zn-binding proteins are transcription factors needed for gene regulation and 60% enzymes and proteins involved in ion transport [1]. Zn deficiency in cereal grains is aggravated by growing cereal crops on potentially Zn deficient soils. In such conditions, plants show a high susceptibility to environmental stress factors such as drought stress and pathogenic infections, and develop severe symptoms such as leaf necrosis and stunting growth. Consequently, Zn deficiency results in significant decrease in plant growth, productivity and yield, as observed in several crops [3-5].

The visual symptoms of zinc deficiency in plants are leaf mottling, interveinal chlorosis, and reduced plant growth. Zinc is involved in membrane integrity, enzyme activation, and gene expression [6]. Zinc plays important role towards increasing leaf iron concentration and also is an integral part of many enzymes and proteins. Meanwhile, a positive correlation between zinc fertilizer application and wheat grains and leaf iron concentration is reported [7]. Hence, foliar application of Zn fertilizer is a promising short-term approach to improve Zn concentrations in seeds and can also contribute to alleviation of Zn deficiency related health problems in the developing world [4,8].

Wheat (*Triticum aestivum* L.) is an important staple food crop across the world. However, the average yield of wheat is affected by nutrient deficiency of nitrogen and phosphorus followed by zinc, being a major cause of yield reduction [9]. In an earlier research, foliar application of zinc fertilizer increased leaf zinc accumulation and grain protein contents in wheat and pepper [10]. Researchers have presented mathematical model of Zn absorption as dependent on various dietary components, including Zn, and phytate as well as zinc molar ratio [11].

The present study was conducted to explore the potential role of optimized foliar Zn application as a supplement in a developing line (L-5066) of wheat (*Triticum aestivum* L.) by evaluating the growth, yield, chlorophyll contents and nutrient accumulation in the subject plant.

2. Material and Methods

2.1. Sample collection

The seeds of wheat (*Triticum aestivum* L.) line L-5066 were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan.

2.2. Chemicals and reagents

All chemicals and reagents/standards used in this study were analytical reagent grade purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Analytical procedures

2.3.1. Soil and irrigation water analysis

Soil texture was determined by a procedure as described by Hassan et al. [13]. Electrical conductivity (EC) of soaked soil was determined with WTW conductivity meter (Model LF-530) and pH by pH meter (Corning-130). Soil composition is presented in Table 1. Mean monthly climatic data for Faisalabad, Pakistan (2010-11) is also given in Table 2.

Table 1. Soil and irrigation water analysis (values are means of three replicates)

Parameter	Soil	Irrigation water
Sand contents	62%	-
Clay contents	24%	-
Silt contents	10%	-
Soil Texture	Loam (sand+clay)	-
Calcium carbonate ($CaCO_3$)	2.51%	-
Phosphorous (P) contents	9.4 ppm	-
Nitrogen contents (N)	0.73%	-
Organic matter	1.00%	-
CEC(Cation exchange capacity)	18.3 meq $100g^{-1}$	-
pH	7.60	7.50
EC (Electrical conductivity)	2.84 dS.m $^{-1}$	1.2 dS.m $^{-1}$
SP (Saturation percentage)	35%	
Soluble SO_4^{2-}	1.798 meq L^{-1}	-
Soluble NA^+	2.36 meq L^{-1}	4.4 meq L^{-1}
Soluble CA^{2+} +Mg $^{2+}$	15.12 meq L^{-1}	3.4 meq L^{-1}

Table 2. Mean monthly climatic data for Faisalabad, Pakistan 2010-11.

Month	Min Temp °C	Max Temp °C	Humidity %	Wind km/day	Sun light hours
October	17.1	32.7	57	86	8.9
November	10.8	25.7	64	78	6.3
December	7.0	22.1	64	61	8.0
January	6.0	16.2	82	104	4.1
February	9.5	22.0	62	112	6.6
March	16.5	30.4	57	86	8.7
Average	11.1	24.8	64.3	87.8	7.1

2.3.2. Chlorophyll determination

Plant material (0.1g leaf) was ground in 3 mL (80%) acetone using a pestle and mortar and placed overnight at room temperature. The absorbance of the supernatant was recorded at 480, 645 and 663 nm with a UV/Visible spectrophotometer (JENWAY 6300). Chlorophylls concentration was calculated by using following equation.

Chl. *a* (mg ml^{-1}) = [12.7 (OD 663) - 2.69 (OD645)] × V/300× W

Chl. *b* (mg ml^{-1}) = [22.9 (OD 645) - 4.68 (OD663)] × V/300 ×W

Total Chl. = [20.2 × (A645) + 8.02 (A663)] × V/300 × W

where V = Volume of the extract (ml)

W= Weight of the fresh leaf tissue (g)

Carotenoids = [(OD 480) + 0.114 (OD 663)-0.638 (OD645)]

E100% Cm = 2500

Carotenoids (g mL^{-1}) = Acar / Em30% × 100

2.3.3. Sample preparation for ion analysis

Oven-dried ground plant samples (0.2 g) of shoot and root were weighed in separate digestion flasks. Five mL of conc. H_2SO_4 (95-98%) was added to each of the flask. Then 1 mL of H_2O_2 was added into each of the flasks and the flasks placed on a hot plate for sample digestion purposes. The temperature of hot plate was steadily increased from 50-200 ^{0}C, and the heating continued until the samples were completely digested.

The digested material was mixed with an appropriate volume of distilled water and the resulting sample solution filtered by using filter paper. The volume was made up to 50 mL in a volumetric flask using distilled water. The diluted sample solutions were then used for the ions measurements [14].

2.5.3. Determination of cations (Ca^+, $Na^+$and K^+)

For analysis of sodium and potassium, 2 mL aliquot from the sample solution was taken and diluted up to 20 mL by using distilled water. The readings for Ca^+, Na^+ and K^+ were taken on a flame photometer (Jenway, PFP-7). A series of standards solutions for each cation were prepared and the standard calibration curves drawn after measuring their absorbance with a flame photometer. By using the standard curve for Ca^+, Na^+ and K^+ the concentration of each cation was worked out.

2.3. Experimental studies

2.3.1. Pot experiment

In order to appraise the effect of different concentrations of Zinc foliar application on wheat line L-5066, pot experiments were conducted in the Old Botanical Garden, University of Agriculture Faisalabad, Pakistan. Each treatment was replicated thrice. The experiment was laid out in completely randomized design (CRD), with factorial. Eight seeds of wheat line were sown in these pots. The germination of seeds started on the 5th day of the sowing and was completed in five days. The germinating seedlings were irrigated daily with tap water (Table 1). After 30 days of the germination of the crop, foliar Zn treatment was applied in the form of $ZnSO_4$ solution in three levels (0, 4, 6 m*M*). Data for vegetative growth was taken at 55 days after the germination.

2.3.2. Determination of vegetative and yield attributes

Data for plant height (cm), plant fresh weight (g), plant dry weight (g), and number of leaves per plant were recorded at the time of harvest being made after 55 days of the foliar Zn application. Different yield attributes *i.e.*

number of tillers per plant; number of spikes per plant, number of spikelet per spike and 100 grain weight were recorded at the time of final harvest taken at 96 days after the application of treatment.

2.4. Statistical Analysis

A two way analysis of variance was computed using the COSTAT (Cohort Software Berkeley, California, USA) for all the parameters.

3. Results and Discussion

3.1. Effect of foliar feeding of Zn on Vegetative parameters

Plant height, plant fresh weight, plant dry weight and number of leaves of wheat line L-5066 were increased as result of treatment with different concentrations of Zinc (Zn) as foliar spray, however significant ($P \leq 0.001$) increase in all these vegetative parameters was observed when 4 m*M* Zn foliar spray was applied (Table 3).

Table 3. Effect of foliar feeding of *Zn* on various vegetative parameters

Treatment of Zn	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Number of leaves (per plant)
Control	65.0±1.0	15.1±1.5	2.1±0.5	15.1±0.9
4m*M* Zn	72.0±0.5	22.0±0.4	3.3±0.1	23.5±0.5
6m*M* Zn	66.0±0.6	18.0±2.0	2.5±0.2	20.0± 2.0

The results are means of three replicates

3.2. Effect of foliar feeding of Zn on yield attributes

Number of tillers per plant, number of spikes, number of spikelets and 100 grain weight of wheat line-5066 grown under foliar feeding of different concentrations of Zinc (Zn) was increased, however significant ($P \leq 0.001$) increase in all the yield attributes was observed when 4 m*M* Zn was applied (Table 4).

Table 4. Effect of foliar feeding of feeding of *Zn* on various yield parameters

Treatment of Zn	Number of tillars (per plant)	Number of spikes (per plant)	Number of spikelets (per plant)	100 grain weight (g)
Control	2.7±0.3	19.5±0.9	16.5±1.5	3.5±0.2
4m*M* Zn	3.3±0.3	16.5±1.0	22.5±0.5	4.75±1.5
6m*M* Zn	2.7±0.3	14.0±2.0	20.9±1.1	4.5±0.3

The results are means of three replicates

3.3. Effect foliar feeding of Zn on chlorophyll (mg/g)

The contents of chlorophyll-*a*, and **c**hlorophyll-*b* of wheat line L-5066 grown under different concentrations of Zinc (Zn) as foliar spray is increased. However, a significant ($P \leq 0.001$) increase in chlorophyll-*a* and chlorophyll-*b* of wheat line was observed when 4m*M* Zn was applied (Fig.1).

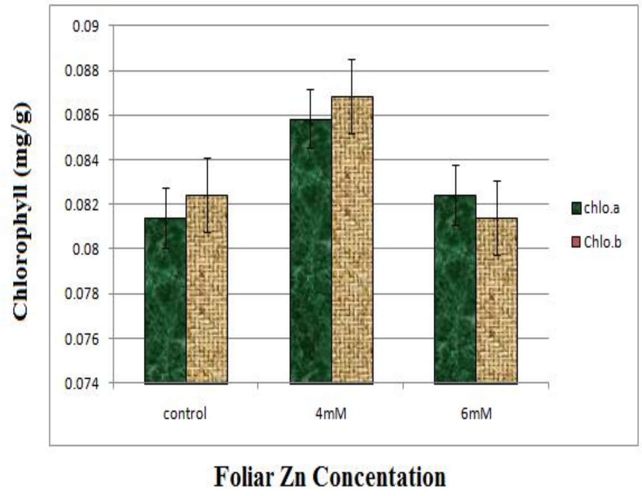

Fig. 1. Chlorophyll (mg/g) of wheat line L-5066 when 55 days old plants were grown under different concentrations of Zinc. Vertical bars indicate mean ± SE.

3.4. Effect foliar feeding of Zn on carotenoids (mg/g) fresh weight

Carotenoid contents of wheat line L-5066 were also increased as result of treatment with different concentrations of Zinc (Zn) foliar spray. A significant ($P \leq 0.001$) increase in the carotenoid was observed when 6m*M* Zn foliar spray was applied (Fig. 2).

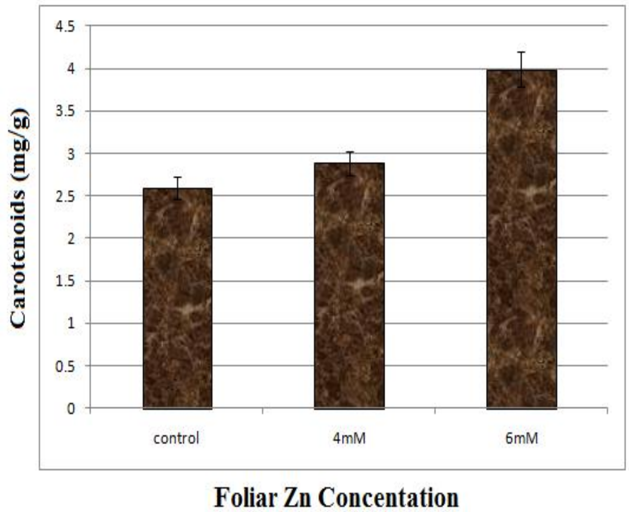

Fig. 2. Carotenoid (mg/g) of wheat line L-5066 when 55 days old plants were grown under different concentrations of Zinc foliar spray. Vertical bars indicate mean ± SE.

3.5. Effect on Stem and leaf Ca^+, Na^+ and K^+ contents (mg/g) of dry weight

The leaf Ca^+ and Na^+ contents of wheat line L-5066 were increased when 4m*M* and 6m*M* Zn was applied as foliar spray, however, a remarkable ($P \leq 0.01$) increase in stem Na^+ and leaf K^+ contents was observed when 4m*M* and 6m*M* Zn was applied (Fig. 3).

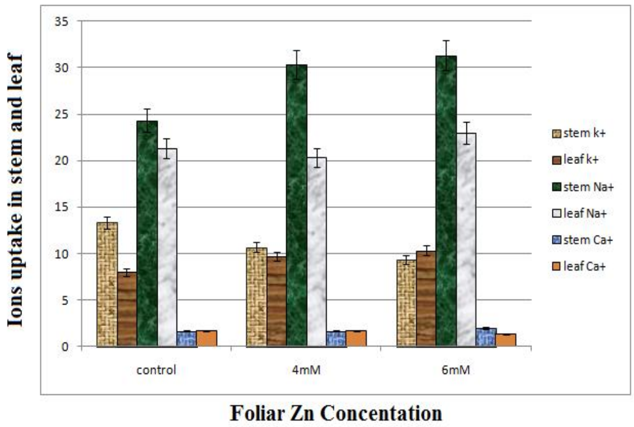

Fig. 3. Leaf and stem Ca^+, K^+, Na^+ contents (mg/g)) of wheat line L-5066 when 55 days old plants were grown under different concentrations of Zinc foliar spray. Vertical bars indicate mean ± SE.

General Discussion

Plants with Zinc application have increased photosynthetic rate due to synthesis of chloroplast, enhanced synthesis of chlorophyll, oxidation of water at PS-II, dominated activities of Calvin cycle enzymes, as well as enhanced absorption of CO_2 in consequence of stomatal opening and enhanced nutrient uptake [15].

Regarding photosynthetic pigments i.e. chlorophyll *a*, chlorophyll *b* and carotenoids, Zinc application showed an encouraging effect on chlorophyll contents and this increase in chlorophyll pigments is in accordance with the trends of previous studies by Quary *et al.* [7] and Broadly *et al*. [16]. These studies showed increasing effects in chlorophyll contents with increased concentration of Zn in wheat.

In our study, inorganic ion uptake and accumulation was facilitated by Zinc application and increase in stem Na^+ and leaf K^+ contents was observed under 4m*M* Zinc and 6m*M* Zn application. This increase in inorganic ions is in accordance with previous studies conducted by Martin-Ortiz *et al*. [17] and Chaab *et al*. [18], in which they also reported an increase in inorganic ions especially in K^+ uptake under Zinc application. This increase in K^+ takes place due to the opening of K^+ channels by high concentration of Zinc application. Similar results were reported by Cakmak *et al*. [19] in wheat where they reported an increase in leaf and shoot N^+ ion at low Zinc concentration.

Zinc application caused a significant increase in 100 grain weight of wheat and this increase in crop yield due to Zn application is in accordance with the previous report by Zeidan *et al.* [20] where they reported a significant increase in grain yield of wheat under different concentrations of Zn foliar treatment. Similar increase in yield was reported by Chaab *et al*. [18] in maize under Zinc application and this might be due to the maximum Zinc accumulation in aerial parts of plant. From the above discussions, this can be concluded that foliar application of Zinc enhanced stem fresh and dry weights, plant height and number of leaves in the subject wheat (*Triticum aestivum* L.) line. Besides, Zinc application also promoted minerals nutrients intake, especially of Na^+.

4. Conclusions

Foliar exposure of wheat line L-5066 to Zn increased the morphological and yield parameters of wheat. The plant height, fresh and dry weights, leaf area, number of spikes and spikelets were significantly increased as compared to control plant (grown under Zn-free environment). Photosynthetic pigments i.e. chlorophyll-*a*, chlorophyll-*b* and carotenoids were also increased. The marked increase in morphological and yield parameters at 4m*M* concentration was recorded. Similarly, Zn foliar application also caused an increase in ion uptake and accumulation. Marked increase in stem Na^+ and leaf K^+ contents was observed under Zn application. Maximum increase in all parameters was observed at 4m*M* Zn concentration. This study proved that Zn can be used as a growth regulator micronutrient to promoting and increasing growth, photosynthetic pigments production and regulating ion uptake and accumulation in the subject wheat line.

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