Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2018, 8(4):13-17

ISSN : 2249-7412
CODEN (USA): AJPSKY

Enhancement of Zn density in Barley (*Hordeum vulgare*) grain: A Physiological approach

Namita Yadav* and Yogesh Kumar Sharma

Plant Physiology Laboratory, Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India.

ABSTRACT

This research was designed to investigate the foliar zinc application effect for increasing the nutritional value of barley by the way of improving zinc grain density. At presowing soil application of NH_4NO_3 and $ZnSO_4.7H_2O$ (10 mg/kg soil each) and foliar application of 0.5% $ZnSO_4.7H_2O$ aqua solution at different plant growth stages were given. Barley (Hordeum vulgare L.) var. K551 were grown in earthen pots in glass house in October and subjected to six different treatments of foliar application of Zn at 35d (T1), 45d (T2), 55d (T3), 35+45d (T4), 35+45+55d (T5) and 45+55d (T6) with control (T). Observations were taken at 35,45 and 55 days after sowing for pigments analysis and enzyme activities while all other observation were taken on all treatment stages. The results demonstrate that all the growth parameters like plant height, flag leaf area, number of tillers plant^{-1} and chlorophyll content as well as enzyme activities of peroxidase and carbonic anhydrase in young leaves were increased significantly in comparison to control. Maximum plant height (72.80 cm), flag leaf area (79.17 cm^2), tiller plant^{-1} (12.66), number of grain (51.66), dry matter production (35.41 g) and more Zn translocation from flag leaf to grain and its accumulation were obtained at 35+45+55d (T5). Zn foliar application at T5 was observed more significant as compared to other stages of treatment.

Keywords: Zinc, Barley, Growth, Uptake, Foliar application

INTRODUCTION

The productivity of the crop may be related to physiological attributes such as flag leaf area, relative growth rate and photosynthesis rate. Higher photosynthetic rate indicates better growth and development which depends on flag leaf area. The interaction of these physiological traits with the nutrient should make it possible to work out the information required for obtaining yield. Zinc is an essential micronutrient hence its deficiency adversely affects crop productivity. It is generally presumed that soil deficient in zinc may adversely affect early vigour, chlorophyll content, photosynthesis and activity of carbonic anhydrase [1,2] which, in turn results in drastic reduction in crop yield. Grain yield reduction of up to 80% along with reduced grain Zn density has been observed under Zn deficiency [3]. Zinc deficiency leads to severe decrease in wheat production and nutritional quality of grain [4]. Barley grains are highly nutritious, carbohydrate and fiber rich food source for human. Nitrogen plays a key role in photosynthetic activity and contributes around 40% yield [5]. Nitrogen being a constituent of different proteins increases the food nutritive value as well [6]. Improving nutritional status of nitrogen in plants increases Zn accumulation in grain. It supports that nitrogen content of plants plays a significant role in Zn biofortification of cereal grain [7]. Present investigation was carried out to investigate the impact of soil Zn at presowing and foliar Zn applied at different interval of plant growth in barley for enhancement of grain Zn density. Because of widespread deficiency of Zn in soil in the India, its use as a fertilizer has become a regular feature of getting optimum yield of the crops.

MATERIAL AND METHODS

The experimental plant Barley (*Hordeum vulgare* L.) var. K551 was grown in pots each containing 10 kg soil, and subjected to combined treatment of zinc sulphate ($ZnSO_4.7H_2O$) and ammonium nitrate (NH_4NO_3) (10 mg kg^{-1}soil each pot) at pre-sowing and 0.5% $ZnSO_4.7H_2O$ aqua solution foliar spray at different stages of plant growth at an ambient temperature (25-30^{o}C) in a glass house. Experimental pot soil was loamy- sand having pH 7.30, electrical conductivity 0.354 dsm^{-1} and Diethylenetriaminepentaacetate (DTPA)–extractable Zinc (Zn) was 1.158 ppm before soil application. Analytical reagent grade ones chemical were used. The six foliar Zn treatments which applied to plants at 35d (T1), 45d (T2), 55d (T3), 35+45d (T4), 35+45+55d (T5) and 45+55d (T6) with control (no foliar application) and arranged in complete randomized block design.

There were three replicates in each treatment. After emergence, seedlings were thinned uniformly in each pot to maintain two plants per pot. The growth and yield parameter were taken such as flag leaf area (Systronic Leaf Area Meter *211*), number of tillers $plant^{-1}$, plant height, number of grain ear^{-1}, weight of grain ear^{-1}, dry matter and grain yield, and Zn concentration in different plant parts including the grains in all treatments with control. Carbonic anhydrase [8], peroxidase [9], and photosynthetic pigments [10] were observed in young leaves of all treated plants. At 120d plants were harvested and sampled for yield and separated into different plant parts such as root, stem, leaves and grains for determination of dry matter production and Zn tissue concentration by atomic absorption spectrophotometer (Thermo Jerrell Ash Video 12E: AA/AE Chicago, IL). On harvest the plant parts were separated, chopped and placed in paper bags after proper washing and rinsed with distilled water. The plant materials were oven dried at 70°C for 48 h and digested 1 g dried plant parts in nitric acid-perchloric acid (10:1) for Zn measurement by method of piper [11]. Soil pH, Electrical Conductivity (EC), organic carbon [12] and soil Zn concentration [13] were analyzed in soil at presowing and after harvesting the crop. The presented valves in all data are the mean of three replicates (± SE; n=3). The data were analyzed by one way ANOVA using software program Sigma Stats 3.5. It was followed by Holm Sidak method at $p \leq 0.05$.

RESULTS AND DISCUSSION

Significantly increased in plant height (5.59%, 9.65%, 18.74%, 20.74%, 23.05% & 21.24%) flag leaf area (16.63%, 35.08%, 38.55%, 37.37%, 61.76% & 59.76%) and tillers $plant^{-1}$ (30.54%, 39.16%, 43.69%, 52.21%, 65.27% & 47.91%) were achieved at 35d (T1), 45d (T2), 55d (T3), 35+45d (T4), 35+45+55d (T5) and 45+55d (T6) respectively by foliar Zn treatment as referred to Table 1. At T5 stage of zinc foliar application was obtained maximum plant height (72.80 cm), flag leaf area (79.17 cm^2) and tillers $plant^{-1}$ (12.66) (Table 1). Foliar application of Zn probably increase the biomass production, it might be due to indirect role of Zn in photosynthesis process, biochemical and physiological activities which reflect its importance in achieving higher yields [14]. The increase in flag leaf area and chlorophyll contents is also attributed to dry matter production [15] which increased the synthesis of photosynthetic products and their transport and accumulation to the site of grain production. Growth parameters such as plant height and flag leaf area improved probably, due to increase in amino acids which are main components in leaf area expansion. According to Firouzi, foliar spray of Zn increases flag leaf area due to an increase in length and width of leaves with increase in auxin [16].

Table 1: Effect of Zn foliar application at different days of plant growth on plant height, flag leaf area, grain yield and dry matter in barley.

Days of foliar spray with treatment	Plant height (cm)	Flag leaf area (cm^2)	No. of tiller $plants^{-1}$	No. of grain ear^{-1}	Weight of grains (g ear^{-1})	Dry weight (g $plant^{-1}$)
Control (T)	59.16 ± 0.28	48.94 ± 0.37	7.66 ± 0.66	37.00 ± 0.57	1.66 ± 0.024	22.60 ± 0.20
35d (T1)	62.47 ± 0.38*	57.08 ± 0.29*	10.00 ± 0.57*	46.66 ± 0.88*	2.32 ± 0.026	28.26 ± 0.13*
45d (T2)	64.87 ± 0.17*	66.11 ± 0.33*	10.66 ± 0.33*	47.66 ± 0.33*	2.43 ± 0.011	30.49 ± 0.04*
55d(T3)	70.25 ± 0.52*	67.23 ± 0.14*	11.00 ± 0.57*	48.33 ± 0.33*	2.46 ± 0.003	32.63 ± 0.12*
35+45d (T4)	71.43 ± 0.66*	67.81 ± 0.40*	11.66 ± 0.33*	50.00 ± 0.57*	2.53 ± 0.005	34.22 ± 0.07*
35+45+55d (T5)	72.80 ± 0.15*	79.17 ± 0.20*	12.66 ± 0.66*	51.66 ± 0.33*	2.66 ± 0.008	35.41 ± 0.07*
45+55d(T6)	71.73 ± 0.24*	78.19 ± 0.17*	11.33 ± 0.66*	48.00 ± 0.57*	2.51 ± 0.008	29.72 ± 0.31*

*Significant difference from the control at $p \leq 0.05$

Photosynthetic pigments were recorded at control (T), 35d (T1), 45d (T2) and 55d (T3) stage of treatment (Figures 1 A-D). The leaf chlorophyll content was recorded maximum at 35d (T1) of Zn treatment as compared to control and other treatments. Enzyme activities of Zn treated plant at three stage of treatment with control, the specific activity of carbonic anhydrase was found to be significantly increased at 45d (T2) stage as compared to control (Figure 2). The activity of carbonic anhydrase and leaf zinc showed a parallelism as both increased with zinc supply suggesting the enzyme activity as an indicator of Zn status in plants. Carbonic anhydrase is a hydrolase enzyme that catalyzes the reversible hydration of carbon dioxide, increasing absorption of carbon dioxide per unit leaf area,

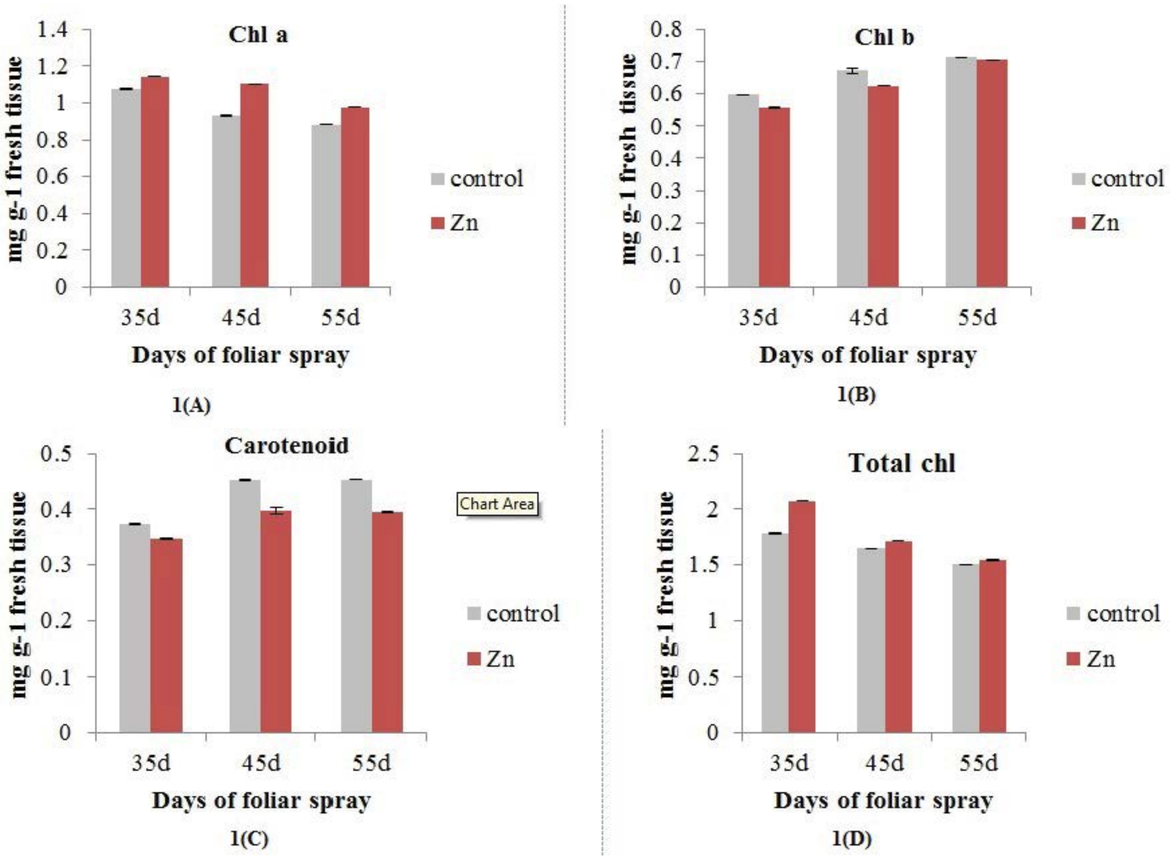

Figure 1(A-D): Effect of Zn foliar application at 35d, 45d and 55d on Chlorophyll a, Chlorophyll b, Carotenoid and total Chlorophyll (mg g^{-1} fresh tissue) in barley leaf. Values are mean of 3 replication ± SE.

thereby increasing photosynthesis and biomass production [17,18]. The increased enzyme activity of peroxidase at later stage might be attributed to the concentration of free radicals and phenol compound accumulate in leaves which is redundant for plant metabolism (Figure 3). It appears that zinc supply is more useful for plant growth at T5 stage because it includes tillering, booting and heading stage of plant growth and treatment at this stage significantly increased yield, quality and tissue Zn density in barley grain.

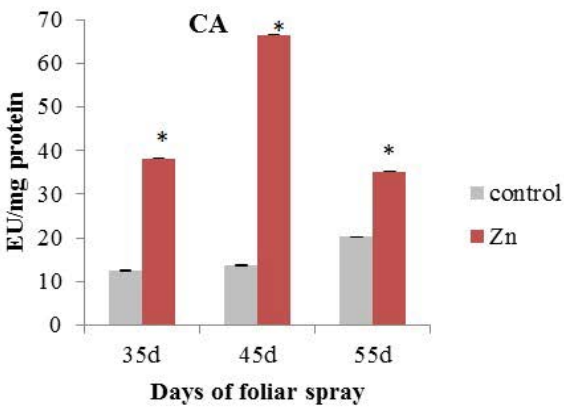

Figure 2

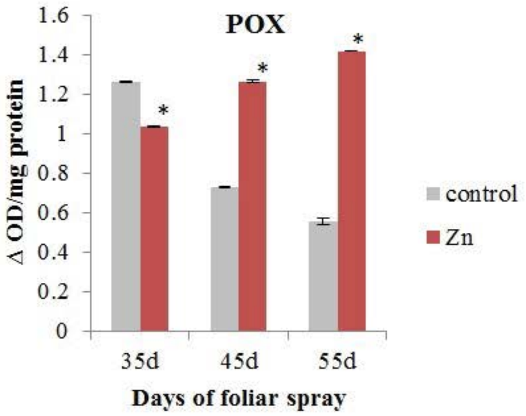

Figure 3

Figures 2 & 3: Effect of Zn foliar application at 35d, 45d and 55d on specific activities of carbonic anhydrase (measured as EU/ mg protein) and peroxidase (measured as ΔOD/mg protein) in barley leaf. Values are mean of 3 replication ± SE. * denotes significant difference from the control at $p \leq 0.05$.

Table 1 showed significant increase in the number of grain ear^{-1} (20.18%, 26.18%, 30.62% 35.13%, 39.62% & 29.72%), weight of grain ear^{-1} (39.75% 46.38%, 48.19%, 51.20%, 52.40% & 60.24%) and dry matter production (25.04%, 34.91%, 44.38%, 51.41%, 56.68%, 31.58%) at 35d (T1), 45d (T2), 55d (T3), 35+45d (T4), 35+45+55d (T5) and 45+55d (T6) respectively (Table 1). Maximum dry matter production and grain yield were obtained at T5 stage of treatment as compared to control. The data in Table 2 represent the Zn tissue concentration in root, stem, leaves and grain at different stages of Zn foliar treatment. Translocation and accumulation of Zn was from leaf to grain (25.41%, 28.24%, 30.17%, 34.85% & 49.88%, 29.38%) was recorded at 35d (T1), 45d (T2), 55d (T3), 35+45d (T4), 35+45+55d (T5) and 45+55d (T6) respectively. Reproductive growth was found most significant in barley & Zinc concentration was found higher in leaves, which in turn translocated more Zn to grains [19]. At T3 to T5 stage of Zn foliar application significantly increased Zn concentration up to 30.17% to 49.88% in grain and the highest Zn concentration were achieved with foliar Zn application that was applied three times at 35+45+55d (T5) stage of treatment. At this stage of treatments the zinc concentration decreased in the leaves, probably because zinc has been more translocated from younger leaves to grain. Single dose of foliar application of Zn was not more effective and less amount of Zn translocated into grain [20].

Table 2: Effect of Zn foliar application at different stages of treatment on tissue Zinc concentration in different plant parts of barley at harvest stage.

Days of foliar spray with treatment	Root	Stem	Leaves	Grain
	Zinc concentration(µg g^{-1} dry matter)			
Control (T)	41.39 ± 0.104	27.79 ± 0.090	30.29 ± 0.095	07.56 ± 0.155
35d (T1)	59.30 ± 0.110*	33.23 ± 0.165*	62.32 ± 0.085*	15.84 ± 0.103*
45d (T2)	62.82 ± 0.052*	52.41 ± 0.206*	61.43 ± 0.055*	17.35 ± 0.072*
55d(T3)	63.54 ± 0.035*	62.60 ± 0.106*	58.46 ± 0.081*	17.64 ± 0.118*
35+45d (T4)	68.43 ± 0.056*	85.45 ± 0.056*	52.37 ± 0.047*	18.25 ± 0.059*
35+45+55d (T5)	69.32 ± 0.115*	88.66 ± 0.064*	52.77 ± 0.055*	26.32 ± 0.061*
45+55d(T6)	68.36 ± 0.141*	93.69 ± 0.069*	59.36 ± 0.056*	17.44 ± 0.031*

*Significant difference from the control at $p \leq 0.05$

Some workers found that soil application of nitrogen and zinc improved plant height, flag leaf area, dry matter production and grain yield [21,22]. NH_4NO_3 maintains the pH of soil by acidifying effect which increases the availability of Zn to plants in treatment as compared to control [23,24]. Zinc availability to plant roots is highly dependent on pH and organic matter contents in soil as it is observed that available Zn increases with increase in organic matter in soil. Before mixing DTPA extractable zinc was 1.158 ppm in experimental soil and after mixing it varied from 7.216 ppm to 7.296 ppm it mean value 7.256 ppm in treatments. After harvesting of the crop it varied from 6.042 ppm to 5.234 ppm, the results indicate that 16.74% to 27.87% applied Zn was available to plant as compared to control. Soil pH values varied ranging from 7.30 to 6.77, EC from 0.354 to 0.718 dsm^{-1} and organic carbon from 1.380 to 2.022% and after harvest crop soil pH 7.75 to 7.11, EC 0.262 to 0.683 dsm^{-1} and OC 1.616 to 1.983% were obtained.

Combined application of soil and foliar Zn significantly increased yield as well as the Zn concentrations in grains of barley. Foliar Zn spray at T5 (35+45+55d) stage was most suitable stage and has positive impact on growth, yield and tissue concentration of barley in comparison to control.

ACKNOWLEDGEMENT

Author one is very grateful to Council of Scientific and Industrial Research (CSIR) for financial support and Department of Botany, University of Lucknow, Lucknow for necessary facilities to carry out the experimental work.

REFERENCES

[1] Kumssa DB, Joy EJM, Ander EL, Watts MJ, Young SD, et al. Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. *Sci Rep*, **2015**. 5.

[2] Rengel Z. Availability of Mn, Zn and Fe in the rhizosphere. *J Soil Sci Plant Nutr*, **2015**. 15: 397-409.

[3] Cakmak I, Torun B, Erenoglu B, Ozturk L, Marschner H, et al. Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica*, **1998**. 100: 349–357.

[4] Graham RD, Ascher JS, Hynes SC. Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil*, **1992**. 146: 241-250.

[5] Stewart WM, Dibb DW, Johnston AE, Smyth TJ. The contribution of commercial fertilizer nutrients to food production. *Agron J*, **2005**. 97: 1–6.

[6] Tome D, Bose C. Dietary Protein and Nitrogen Utilization. *J Plant Nutr,* **2000**. 130(7): 18685-18735.

[7] Cakmak I. Biofortification of cereals with Zinc and Iron through fertilization strategy. 19th World Congress of Soil Science, Soil Solutions for a Changing World 4. Brisbane, Australia, **2010**.

[8] Wilbur KM, Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. *J Biol Chem*, **1948**. 176: 147-154.

[9] Luck H. Peroxidase. In: methods of enzymatic analysed. H.U. Bergmeyer. New York: Academic Press, **1963**. 885-897.

[10] Lichtenthaler HK. Chlorophylls and carotenoids pigment of photosynthetic biomembranes. *Method in enzymology*, **1987**. 148: 350-382.

[11] Piper CS. Soil and Plant analysis monograph from waite Agric Res Ins The Univ Adelaide. Adelaide, **1942**.

[12] Walkley A, Black IA. An examination of the Degtijareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. *Soil Science*, **1934**. 37: 29-38.

[13] Lindsay WL, Norvell WA. Developments of a DTPA soil test for Zinc, Iron, Manganese and Copper. *Soil Sci Soc Amer Proc*, **1978**. 42(3): 421-428.

[14] Zeidan MS, Manal F, Hamouda HA. Effect of Foliar Fertilization of Fe, Mn and Zn on wheat Yield and quality in low sandy soils fertility. *World J Agric Sci*, **2010**. 6: 696-699.

[15] Sharafi S, Tajbakhsh M, Majidi M, Pourmirza A. Effect of iron and zinc fertilizer on yield and yield components of two forage corn cultivars in Urmia. *Soil and Water*, **2002**. 12: 85-94.

[16] Firouzi M. The effect of foliar absorption of microelements on growth and yield of foliagecorn. MS thesis in Agronomy, Agriculture College, Islamic Azad University, Khorasgan Branch, **2005**.

[17] Dimario RJ, Clayton H, Mukherjee A, Ludwig M, Moroney JV. Plant carbonic anhydrases: Structures, locations, evolution and physiological roles. *Mol Plant*, **2017**. 10: 30-46.

[18] Floryszak-Wieczorek J, Arasimowicz-Jelonek M. The multifunctional face of plant carbonic anhydrase. *Plant Physiol Biochem*, **2017**. 11: 362-368.

[19] Yuan L, Lianghuan W, Chunlei Y, Qian LV. Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *J Sci Food Agric*, **2013**. 93: 254-261.

[20] Cakmak I, Kalayci M, Kaya Y. et al. Biofortification and localization of zinc in Wheat grain. *J Agric Food Chem*, **2010**. 58: 9092-9102.

[21] Kutman UB, Yidiz B, Cakmak I. Effect of nitrogen on uptake, remobilization and partitioning of Zn and Fe throughout the development of durum wheat. *Plant and Soil*, **2011**. 342: 149-164.

[22] Xu YF, Yue SC, Zhang YQ, Cui ZL, Chen XP, et al. Grain and shoot zinc accumulation in winter wheat affected by nitrogen management. *Plant and Soil*, **2012**. 361: 153-163.

[23] Chaudhry FM, Loneragan JF. Effect of nitrogen, copper and zinc fertilizers on the copper and zinc nutrition of wheat plants. *Austr J Agri Res*, **1970**. 21: 865-879.

[24] Alloway BJ. In Zinc in Soil and Crop Nutrition. International Zinc Association. Brussels, Belgium, **2004**.