

Zinc-Induced Biochemical Constituents and Reproductive Yield of Wheat with Zinc Supply in Sand Culture Conditions

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ABSTRACT

In a sand culture experiment, the present study deals with the quantitative (growth and some biochemical constituents) and qualitative yield of grains (zinc and protein contents) of wheat (*Triticum aestivum* L., var. HD-2967) under the influence of various concentrations of zinc supply (0.01, 0.1, 1.0 and 5 mg l⁻¹). Zinc levels were prepared in a standard nutrient solution (omitting zinc). The zinc accumulation was dose dependent in vegetative parts of wheat, which was increased with increase in zinc supply levels. The low and high zinc content in wheat shoot inhibited pigments content and activity of antioxidative enzymes like catalase and peroxidase. The mobilization of zinc in grains was lower at elevated and low zinc-supply levels in contrast to their accumulation in vegetative parts. The zinc mobilization efficiency index (*ZnMEI*) was higher at normal doses of zinc as compared to low (0.01 mg l⁻¹) and elevated zinc levels (5 mg l⁻¹). In the present study, maximum increase of 15, 25 and 18% was observed in the dry matter yield, shoot length and total chlorophyll content, respectively following exposure of 1.0 mg l⁻¹ of zinc. At this zinc treatment, protein content in the seeds was elevated by 10% with zinc enrichment by 73%. The observations of this study may be helpful in the studies of zinc-enrichment in wheat production in Zn-deficient soil.

Keywords: Zinc-enrichment, Wheat grains, Zn-stresses, Pigments, Protein, *Triticum aestivum*.

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INTRODUCTION

Among the essential plant micronutrients, zinc (Zn) management acquired a greater importance in quantitative and qualitative crop production, world croplands (about 50% of the cereal grown areas) are facing zinc deficiency problems, limiting the crop production (Cakmak, 2008). In estimation, Indian agricultural croplands are 49% zinc deficient, and will increase to 49-63% by the year 2025 (Shukla *et al.*, 2018). One-third of the world's population is facing inadequate intake of zinc due to its deficiency in soil (Alloway, 2009; Shukla *et al.*, 2018). Cereal grains are satisfying zinc and other nutritional requirements of human beings, particularly in developing countries (Cakmak, 2008). Zinc deficiency causes impairments in physical development, poor immune system and brain development in human beings (Hotz and Brown, 2004). Above facts shows the importance of zinc in human diet. Zinc is a constituent of more than three hundred enzymes in plants affect growth and cellular metabolism of plants (Sharma, 2006; Pandey, 2018, 2020). Zinc deficiency in plants is influenced by various soil-plant factors (Pandey *et al.*, 2009). Multitudes of enzymes contain zinc as an activator or cofactor plays their roles in transcriptional regulation and stability of several regulatory proteins (Pandey, 2020). The requirement of zinc in the development of reproductive parts (Pandey *et al.*, 2006), regulatory proteins (Sharma, 2006; Pandey 2020) and defense mechanism of plants have been reported (Pandey *et al.*, 2002; Cakmak, 2008). Zinc is a constituent of several proteins involved in structural and catalytic functions of many enzymes (Pandey, 2018). Zinc also plays essential role in photosynthesis, carbohydrate metabolism and biosynthetic pathway of auxin influence growth and development of apical buds (Salami and Kenfic, 1970). Zinc deficiency in plants show non-protein nitrogen accumulation, decrease in ribonucleic acid content and poor development of anther and sporogenous tissue (Kobayashi *et al.*, 1998). The total protein and Zn contents

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give a big clue regarding nutritional quality of grains and influenced greatly with Zn supply status in growth medium.

Wheat is a Zn-responsive crop and a staple food grains of most of the countries in the world. This is a commonly growing as winter crop in India which covers about 68% of total crop area. To solve the Zn deficiency problems in plants, it becomes imperative to know the nutritional supply status and provide tissue-Zn influence on growth and yield along with wheat grains quality. The present experiment was conducted to study the effects of various levels of zinc supply in refined sand on growth, growth supporting some biochemical constituents such as pigments (chlorophyll a, b and total chlorophyll contents) and the activity of catalase and peroxidase, qualitative yield of seeds (protein and zinc contents) and reproductive yield (length, number and weight of spike and grain weight) of wheat (*Triticum aestivum* L., var. HD-2967).

MATERIALS AND METHODS

The wheat (*Triticum aestivum* L., var. HD-2967) plants were raised in refined sand culture medium as described by Agarwala and Sharma (1976). The clay pots (10 kg size), internally coated with

bitumen black paint were filled with refined sand. Experiment was conducted in triplicates. Initially, only distilled water was used for irrigation up to 7 days of the sowing. Thereafter, various concentrations of Zn (as ZnSO₄) from deficient (0.01 mg l⁻¹) to elevated levels (1.0 and 5.0 mg l⁻¹) were supplied regularly (six days in a week) till the maturity of grains at 112 days of the treatment, except one day/week (used only distilled water). The variable zinc levels supplied in standard nutrients solution (omitting zinc) which was prepared as: 4 mM KNO₃, 4 mM Ca (NO₃)₂, 2 mM MgSO₄, 1.5 mM NaH₂PO₄ for macronutrients; and Fe-EDTA (Fe, 5.6 ppm), MnSO₄ (Mn, 0.55 ppm), CuSO₄ (Cu, 0.65 ppm), H₃BO₃ (B, 0.33 ppm), NaCl (Cl, 3.5 ppm), Na₂MoO₄ (Mo, 0.05 ppm), CoSO₄ (Co, 0.012 ppm) and NiSO₄ (Ni 0.012 ppm) for micronutrients supply.

Growth observations and visible symptoms appeared on the test plants were monitored, regularly. At day 25 and 50 days after treatment, plants were harvested for their length and dry matter yield (dried at 70°C for 48 hours). Wheat leaves (3rd and 4th) were sampled at 50 days after treatment when moderate visible symptoms appeared (such as intervenial spots and yellowing of leaf tips) for the determination of pigments content (Lichtenthaler and Welburn, 1983), activities of catalase (Euller and Josephson, 1927) and peroxidase (Luck, 1963). At the maturity plants were harvested for reproductive yield (length, number and weight of spike); number of grains per spike and weight of 100 seeds. Wheat grains were also analyzed for zinc concentration and protein content for nutritional value of wheat grains. Protein content was determined by the method of Lowry *et al.* (1951). Dry matter of plant tissue and grains were digested in nitric and perchloric acids in the ratio 3:1 (v/v) (Piper, 1942) for determination of tissue-Zn using atomic absorption spectrophotometer (AAA, Parkin Elmer-250). Zinc mobilization efficiency index (ZnMEI) was determined by the method as described by Srivastava *et al.* (2009) as follows:

$$ZnMEI = \frac{ZnG (\mu g \text{ g}^{-1} \text{ dryweight})}{ZnS (\mu g \text{ g}^{-1} \text{ dryweight})}$$

Where:

ZnG=zinc concentration in grains; ZnS=zinc concentration in shoot.

All the data presented in the table are mean values (n=3) and statistically tested for its significance for least significant difference L.S.D. (at P=0.05) with use by the following statistical formulae:

$$LSD = t_n - 2 \sqrt{2} * \frac{MS_{within}}{n} = t_n - 2 * q$$

Where: MS=Mean square; q=Studentized range; n=Treatment

RESULTS AND DISCUSSION

Growth and Visible Symptoms

Low level of zinc supply (0.01 mg l⁻¹) in sand developed some visible symptoms on wheat (*Triticum aestivum* L., var. HD-2967) plants at 45 days of treatment (DAT). These symptoms were stunted plant growth; sub terminal leaves showed mild chlorosis which spreads from base to the apex. Eventually, the apical portion turned yellowish. These symptoms resembled with earlier reports on zinc deficiency in chickpea and wheat (Sharma *et al.*, 1987; Agarwala and Sharma, 1979). These Zn-deficiency induced symptoms were not appeared on wheat supplied with critical normal range of Zn supply (0.1 to 1 mg l⁻¹) in sand (Agarwala and Sharma, 1976). Whereas, at excess Zn supply (5 mg l⁻¹) retarded plant growth, induced chlorosis of young leaves resembled with heavy metals toxicity (Vassilev *et al.*, 2007). Maximum dry matter yield (+77%) and shoot length (+11.8%) was determined at 1 mg Zn l⁻¹ supply which accumulated 56.6 µg Zn g⁻¹ dry weight in shoot (+96.5%) compared with plants supplied with normal Zn (Table 1). Suppression in dry matter yield by 35% was observed at deficient level (0.01 mg l⁻¹). The increased growth parameters were observed could be attributed due to adequate Zn in tissues as reported 25-60 µg Zn g⁻¹ dry weight by Nautiyal and Agarwala (1986), which could promote carbonic anhydrase activity and photosynthesis in wheat (Rengel, 1995; Pandey and Sharma, 2000).

Pigments

Deficient zinc supply (0.01 mg l⁻¹) inhibited total chlorophyll, chlorophyll 'a' and chlorophyll 'b' by -26.4, -24.7 and -27%, respectively in wheat. Zinc deficiency inhibited pigments content may be due to the synergistic effects of Zn on the uptake of iron and magnesium in wheat, which play significant role in biosynthetic pathways of pigments synthesis (Pandey, 2020). In addition, degradation of thylakoids under zinc stresses could also decline pigments content (Vassilev *et al.*, 2007). Both reduction in growth and pigments content correlated with reduction in the activity of carbonic anhydrase (Rengel,

Table 1: Effects of various concentrations of zinc supply in sand on growth and pigments in wheat (*T. aestivum* L., var. HD 2967) at 50 DAT.

| Parameters | Zinc supply mg l ⁻¹ | | | | LSD (P = 0.05) |
|---|--------------------------------|----------------------|------------------------|------------------------|-------------------|
| | 0.01 | 0.1 (Control) | 1.0 | 5.0 | |
| Shoot length (cm) | 28.6 ± 1.0 (-29.4) | 40.5 ± 1.5 (0.0) | 50.8 ± 2.0 (+25.4) | 48.5 ± 2.0 (-19.8) | 13.8 |
| Dry weight (g) | 4.43 ± 0.1 (-35) | 6.81 ± 0.1 (0.0) | 7.85 ± 0.5 (+15.3) | 7.12 ± 0.5 (+4.6) | 0.5 |
| Total chlorophyll (mg g ⁻¹ f. wt.) | 2.40 ± 0.20 (-26.4) | 3.26 ± 0.50 (0.0) | 3.84 ± 0.50 (+17.8) | 2.12 ± 0.20 (-35) | 1.21 |
| Chlorophyll 'a' | 1.62 ± 0.10 (-24.7) | 2.15 ± 0.25 (0.0) | 2.7 ± 0.50 (+25.6) | 1.50 ± 0.30 (-30.2) | 0.81 |
| Chlorophyll 'b' | 0.91 ± 0.1 (-27.2) | 1.25 ± 0.20 (0.0) | 1.51 ± 0.20 (+20.8) | 0.74 ± 0.10 (-40.8) | 0.46 |

Table 2: Effect of various concentrations of zinc supply on reproductive yield of wheat.

| Parameters | Zinc supply (mg l ⁻¹) | | | |
|--|-----------------------------------|---------------------|-------------------------|------------------------|
| | 0.01 | 0.1 (Control) | 1.0 | 5.0 |
| Length of spike | 5.6 ± 0.2* (-17.7) | 6.8 ± 0.2 (0.0) | 7.6 ± 0.5* (+11.8) | 4.5 ± 0.5 (-33.8) |
| Spike pt ⁻¹ | 2.5 ± 0.5 (-44.4) | 4.5 ± 1.0* (0.0) | 4.5 ± 1.0* (0.0) | 2.5 ± 0.5 (-44.4) |
| Spike weight (g) | 2.6 ± 0.1* (-25.7) | 3.5 ± 0.2 (0.0) | 4.3 ± 0.2 (+22.9) | 2.1 ± 0.1** (-40) |
| Grains spike ⁻¹ | 28 ± 2 (-31.7) | 41 ± 5 (0.0) | 48 ± 5* (+17.1) | 20 ± 2 (-51) |
| 100 grains weight | 3.15 ± 0.1 (-25.70) | 4.24 ± 0.1 (0.0) | 4.8 ± 0.1 (+13.2) | 2.8 ± 0.1 (-34) |
| Tissue Zn (shoot) (µg g ⁻¹ dry weight) | 14.6 ± 0.1* (-49.3) | 28.8 ± 0.5 (0.0) | 56.6 ± 1.0** (+96.5) | 116.7 ± 1.5* (+305) |
| Zn in grains (µg g ⁻¹ dry weight) | 6.2 ± 0.1 (-72.3) | 22.4 ± 0.1 (0.0) | 38.8 ± 1.0** (+73.2) | 58.6 ± 1.5 (+161.6) |
| Protein% (grains) | 8.5 ± 0.1* (-49.4) | 16.8 ± 0.1 (0.0) | 18.5 ± 0.2** (+10.1) | 6.5 ± 0.2* (-61.3) |

Note: Parenthesis indicate percentage decrease (-) or increase (+) over control. ± - S.E (n=3); ** - value significant at 0.01 and * - value significant at 0.05 level.

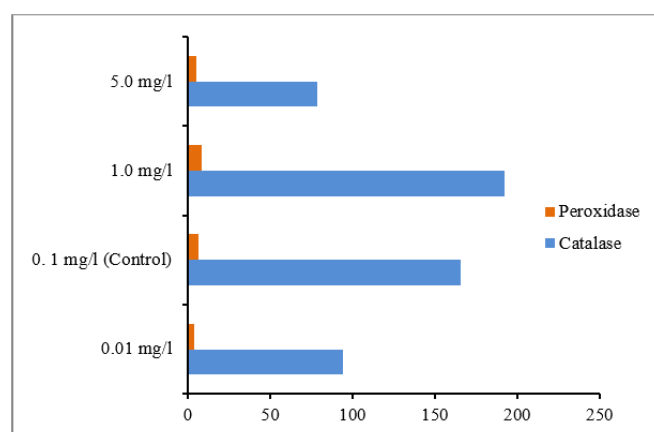


Fig. 1: Effect of various concentrations of zinc supply in sand on the activities of catalase and peroxidase (mMol H₂O₂ decomposed g⁻¹ fr.wt.) in wheat at 50 DAT.

1995), auxin via low tryptophan (Salami and Kenfic, 1970) and photosynthesis in test plants (Hu and Sparks, 1991). Excess zinc (5 mg l⁻¹) inhibited pigments contents are might be due to its toxic effects on biosynthetic pathways (Sharma, 2006) and cellular metabolism of plants (Hu and Sparks, 1991). Both deficient and elevated Zn levels were observed inhibitory on chlorophyll 'b' than the chlorophyll 'a' content in accordance with earlier report of Pandey and Sharma (2000).

Activity of Enzymes

Normal zinc doses supplied (0.1 to 1.0 mg l⁻¹) in sand, the maximum enhanced activity of catalase (+16%) and peroxidase (+37%) in wheat observed at 0.1 mg l⁻¹, while maximum inhibition by 52.5 and 22.6% determined at elevated zinc level (5 mg Zn l⁻¹), respectively. Deficient zinc level (0.01 mg l⁻¹) retarded activity of catalase and peroxidase by 43 and 38.7%, respectively were observed (Fig. 1). The inhibition in these enzymes activity could be attributed due to the decrease in unsaturated fatty acids and

phospholipid contents, particularly reactive sulphydryl (-SH) group due to zinc deficiency-induced oxidative damage through enhanced peroxidation of the cellular membrane (Sharma, 2006; Cakmak, 2008). At 5 mg Zn l⁻¹ supply inhibited these antioxidants enzymes activity due to toxic effects on cellular defense system and in turn disturbed cellular metabolism and biosynthetic pathways of biomolecules (Vassilev *et al.*, 2007; Cakmak, 2008; Pandey, 2020).

Tissue Zinc

Zinc content in wheat (shoot and grains) increased with increase in zinc concentrations supply, results were in accord with Nautiyal and Agarwala (1986) who reported dose dependent tissue-Zn accumulation. But, Zn translocation up to the shoot and its mobilization to the grains was different to its uptake responses, which indicated the influence of some other plant factors in mobilization of zinc (Srivastava *et al.*, 2009) from shoot to the grains (Table 2). The low zinc content at deficient (14.6 µg Zn g⁻¹ dry weight) and elevated content at 5 mg Zn l⁻¹ supply (116.7 µg Zn g⁻¹ dry weight, +305%) was determined. However, Zn transportation up to the grains was very low at deficient (-72.34%), normal (+73.2) and excess Zn supply (+161.6%) as compared to Zn content in wheat shoot, respectively (Table 2). The zinc mobilization efficiency index was higher at normal Zn supply (control, 0.1 mg l⁻¹) with a straight decrease at 1.0 mg l⁻¹ Zn supply. Zinc content in wheat shoot was in a critical normal range at the Zn supply of 0.1 to 1.0 mg l⁻¹ (28.8 to 56.6 µg g⁻¹ dry weight) as reported earlier (Nautiyal and Agarwala, 1986). Zinc status in grains was low at deficient Zn and normal at 0.1 to 1.0 mg l⁻¹ supply level as reported 20-30 µg Zn g⁻¹ dry weight is the critical normal range in wheat grains (Agarwala and Sharma, 1979; Cakmak, 2008).

Reproductive Yield

Development of reproductive parts of wheat (Spike numbers and weight) was observed at 0.1 (control) to 1.0 mg Zn g⁻¹

supply in sand. Whereas decreased at deficient Zn on (spike length by -17.7 and its weight by -25.7% and excess Zn (5 mg Zn l⁻¹) by -33.8 and -40%, respectively. These results showed, excess Zn supply suppressed more reproductive development (length and number of spike and grains production) than deficient Zn (Table 2). The inhibitory effects on the reproductive development with zinc deficiency could be affected by losing the controlling capacity of zinc finger protein (TF III A-type) on the development of floral parts (Sakai *et al.*, 1995). Low and high zinc decreased spike development and grain yield. Deficient Zn decreased grains per spike by +31.7 % and weight/100 grains by -27.7% was recorded. These results may be attributed by the inhibition in microsporogenesis (Kobayashi *et al.*, 1998). It has also been reported that, Zn as zinc phytate and other zinc-proteins are needed for embryo development (Otegui *et al.*, 2002), thus low Zn might be decreasing reproductive yields. The maximum grain yield, protein content (+10%) and Zn content in grains (38.8 µg Zn g⁻¹ dry weight, +73.2%) was determined at 1.0 mg Zn l⁻¹ supply in sand indicated adequate Zn supply limit for qualitative grains yield of wheat (*Triticum aestivum* L., var. HD-2967).

CONCLUSION

Wheat (variety HD 2967) grown in sand culture and supplied with various Zn concentrations showed maximum growth and reproductive yield at 1.0 mg l⁻¹ supply of zinc. Where shoot Zn content was 56.6 µg g⁻¹ dry weight. The inhibition in growth and reproductive yield (spike length, weight and numbers) was at 14.6 µg Zn g⁻¹ dry weight on low Zn (0.01 mg l⁻¹) and 116.7 µg Zn g⁻¹ dry weight on excess Zn supply (5 mg Zn l⁻¹). The maximum grain production was similar to the results as above and show high quality of grains with respect to Zn (22.4 µg Zn g⁻¹ dry weight) and protein content (18.5 µg g⁻¹ dry grain weight) at 1.0 mg Zn l⁻¹ supply level. Thus, the mobilization of Zn was lower from shoot to grains, and Zn MEI (Zn mobilization efficiency index) was higher at normal doses supply levels of zinc (0.1 to 1 mg l⁻¹) and lower under zinc stresses.

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