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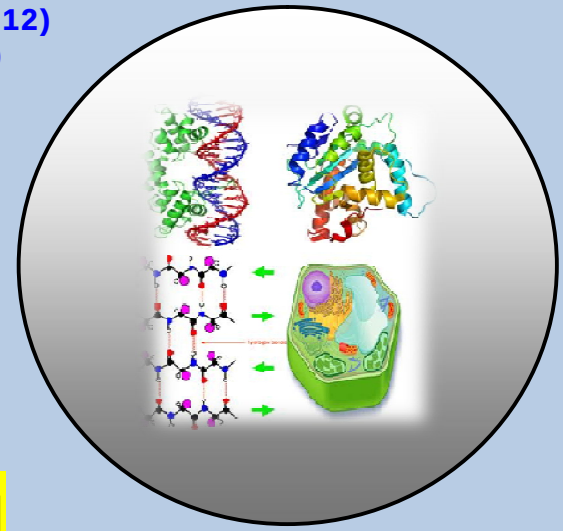
ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)



J. Biol. Chem. Research
Volume 31 (1) 2014 Pages No. 408-415

Journal of Biological and Chemical Research

(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 1: 408-415 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)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RESEARCH PAPER

Received: 31/01/2014 Revised: 25/02/2014 Accepted: 26/02/2014

Assessment of Four Crops for Hyper Accumulation of Zn, Growth Response and Enzymes Activity, Grown in Alluvial Soil

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ABSTRACT

This study investigate the growth, yield, accumulation of Zn and certain metabolic activities of wheat, mustard, fenugreek and broad bean plants in response to soil treatment with 0, 5, 10, 25, and 50 kg ha^{-1} of Zn. The treated plants showed significant stimulation in most of the growth characteristics i.e. length of shoots and roots, plant biomass and number of pods/plant. Treatment with Zn significantly increased the content of photosynthetic pigments and soluble proteins in all the undertaken experimental plants. Treatment with Zn greatly increased the activities of catalase (CAT) and peroxidase (POX) enzymes and accumulation of Zn in root and shoot. Results showed that broad bean and fenugreek is the hyper accumulator of Zn. Therefore it may be suggested that regular cultivation of these crops create Zn deficiency in soil. Therefore, to maintain soil, rich with Zn need crop rotation with less accumulator of Zn and proper application of Zn in soil.
Key word: Soil, Broad Bean, Catalase, Protein and Zinc.

INTRODUCTION

The growth and development of crop needs optimum ratio of micronutrient in soil. Deficiency or efficiency of these nutrients in soil affecting the plant growth leads to severe loss in the productivity and yield.

Nutrient deficiency of soil can be due to absence of nutrient rich parent rock to which soil is formed, leaching out with running water, erosion, excessive cultivation and over grassing etc. For proper growth and yield it is necessary to maintain soil fertility. Annually a large amount of micronutrient was added to soil to maintain their fertility. Many agricultural agencies and scientific organization suggested to farmer that how to maintain soil fertility and how to manage their loss. Here, study was focused on an essential micronutrient Zinc, which functions as co-factor in many enzymes that participated in many metabolic and defense process. Zn deficiency leads to reduction in growth and yield. Wheat grown under such deficient condition has low yield and micronutrients contents in grain. Each element of these micronutrients has its own function in plant growth for example. Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism– uptake of nitrogen and protein quality; (ii) photosynthesis– chlorophyll synthesis, carbon anhydrase activity; reported that Zn-deficient plants reduce the rate of protein synthesis and protein content drastically Mn is required for biological redo system, enzyme activation, oxygen carrier in nitrogen fixation (Romheld and Marachner, 1995). Zinc deficiency in plants is most frequently corrected by application of Zn to soils. Zinc sulfate (ZnSO4) is used extensively as a source of Zn fertilizer, because of its higher solubility in water and existence in both crystalline and granular forms (Mordvedt and Gilkes, 1993). In most instances, Zn deficiency in crop species is corrected by applying 4.5 to 34 kg Zn ha^{-1} as broadcast $ZnSO_4$ (Martens and Westermann, 1991). But limited source of this compound feels a problem of future when the present sources become ended. So, for the avoidance of excessive use of Zn it is necessary to prevent excessive loss from soil. Growth of various cereal crops including wheat showed a drastic decline when Zn is deficient in soil (Hemantaranjan and Trivedi 1997 and Pandey *et al*. 2002). Zinc deficiency in plants is most frequently corrected by application of Zn to soils (Mordvedt and Gilkes, 1993; Mortvedt, *et al*. 1999). Since the accumulation of nutrients in different crop is different. Some plants are the hyperaccumulator of particular element that helped in phyto-remidiation of heavy metals but when accumulated element are essential then regular cultivation of that crop created a situation of deficiency. So, that type of deficiency can overcome by applying regular crop rotation technique in agriculture. The present investigation was undertaken to assess the hyper accumulator of Zn along with growth and certain metabolic responses of wheat, mustard, fenugreek and broad bean plants in response to soil treatment with Zn.

MATERIAL AND METHODS

A pot culture experiment was conducted to study the effect of different levels of Zn application on growth and biochemical responses of wheat (*Triticum aestivum* L.), mustard (*Brassica juncea* L.), fenugreek (*Trigonella foneum-grecum* L.) and broad bean (*Vicia faba* L.) plants grown in the alluvial soil Badshah bagh of Lucknow district. The seeds were surface sterilized with 0.01% $HgCl_2$ for 5 min and rinsed extensively in running distilled water (H_2O). Healthy and equal-sized seeds were chosen and soaked in distilled H_2O for 24hr and germinated in dark at (25^0C).

Treatments consisted of various levels of Zn (0, 5, 10, 25 and 50 kg ha^{-1}) added to soil as a basal dose. The plants were irrigated with tap water as and when required. After 65 days, plants were sampled; separated into leaves and root washed; and dried in an oven at 70 ◦C for 48 h. The dry-matter yield was recorded. Apart from the determination of biomass and tissue Zn, pigment synthesis, protein contents and activity of CAT and POX were also analyzed.

For tissue analysis, oven-dried samples were digested in a nitric and perchloric acid mixture (10:1). Zinc concentrations were estimated in clear digests by atomic absorption spectrophotometry (Elemant AS AAS4141; Electronics Corporation of India Limited, Hyderabad, India). Chlorophyll was estimated by the method of Arnon (1949). Leaves were plucked and washed with distilled water and blotted. 100 mg leaves were taken and ground in 10 ml chilled acetone (85% v/v). Extract was centrifuged at 2000 rpm for 10 minutes. The absorbance of supernatant was read at 663, 645, 510 and 480 nm for chl a, chl b, chl T and carotenoids respectively using the double beam UV-VIS spectrophotometer UV5704SS. The content was expressed in mg g^{-1} fresh weight tissue.

Protein content was estimated by the method of Lowry *et al.* (1951). About 500 mg of test plants were crushed in 5ml of 10% trichloroacetic acid and centrifuged at 10,000 rpm for 10 minutes. After decanting the supernatant, pellets were washed with 5ml of 1N NaOH twice, again centrifuged in 5ml of 1N NaOH and final supernatant was collected. Reagent A (50 ml) and B (1ml) were added to reagent C to make 100ml. 5ml of above (A+B+C) solution was added to final supernatant (0.5ml) and kept for 10-15 minutes at 30 ºC. Reagent D (0.5ml) was finally added and thoroughly mixed. After 45 minutes, the absorbance was recorded at 750 nm. Bovine serum albumin (sigma) was used as standard.

CAT activity was assayed following to the method prescribed by Euller and Josephson (1927). The reaction mixture for CAT containing 0.01 mM phosphate buffer (pH 7.0) and 0.5mM H_2O_2 in 10 ml was incubated with suitable aliquot from the extract. The reaction was run for 5 minutes at room temperature (25^{o}C) and was stopped by the addition of 5ml 2N H_2SO_4, Corresponding zero hour blanks with added H_2SO_4 was also run. The mixture was titrated against 0.1 N $KMnO_4$ and the activity of CAT were expressed as µmol H_2O_2 decomposed / 100 mg fresh weight tissue.

The POX activity was determined by the method of Luck (1963). The assay system for POX contained 0.5 mM phosphate buffer (pH 6.0), 0.01% (v/v) H_2O_2, 5 mg p-phenylenediamine and extract in 8 ml. The reaction was run at 25^{0}C for 5 minute and stopped with 2ml 5N H_2SO_4. Blanks with added H_2SO_4 were also taken. After centrifugation, O.D. was measured at 485 nm on double beam UV-VIS spectrophotometer UV5704SS. The enzymes activity was measured as change in optical density per 100 mg FW. The experimental data were tested for significance by using least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test were performed with analysis tools from Microsoft office excel 2007.

RESULTS AND DISCUSSION

Application of Zn showed a considerable increase in growth and different yield attributes in comparison to control plants without Zn applications. The increase in Zn supply also increased the Zn concentration in different plant parts as has been reported earlier (Pandey *et al*. 2002).

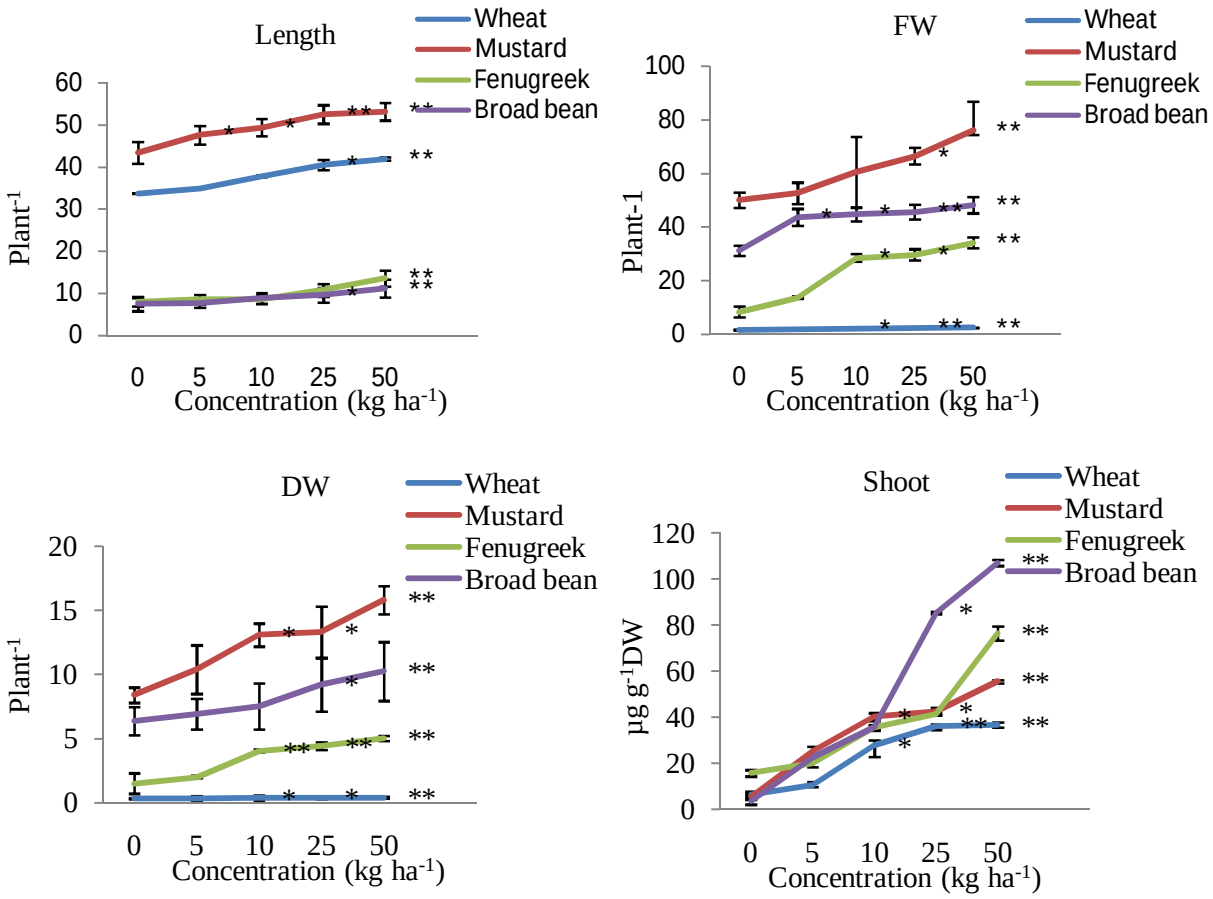
Accumulation of Zn in root and shoot tissue increased at all levels of Zn supply (figure 1) but in all the four experimental plants broad bean plant appear to be hyper accumulator of Zn. The value of Zn concentration in root tissue of control plants of wheat, mustard, fenugreek and broad bean was 9.8, 9.8, 5.8 and 9.8 $\mu g\ g^{-1}$ which were increased to 50, 49, 39 and 134 $\mu g\ g^{-1}$ DW at 50 kg ha^{-1} Zn application. In case of mustard and fenugreek, accumulation of Zn was more pronounced in the shoot than in root while in case of wheat and broad bean it was in root than shoot. These results suggested that Ariel part of mustard and fenugreek are the good source of Zn compared to wheat and broad bean. In this study application of Zn promote the growth of plants up to 50 kg ha^{-1} level of Zn supply. The response of Zn application in terms of plant height, fresh weight and dry weight were different in wheat, mustard, fenugreek and broad bean (figure 1). Plant height increased by 24, 49, 53 and 69% in wheat, mustard, fenugreek and broad bean grown in soil amended with 50 kg ha^{-1} compared to plant grown without Zn supplement. The values of FW and DW in response to Zn application (50 kg ha^{-1}) increased by 304 and 234% in fenugreek which was comparatively higher than 44 and 25% in wheat respectively. These finding was also supported by Zeidan *et al*. (2010). The order of increment in biomass production in response to Zn application was found as wheat < broad bean < mustard < fenugreek. The stimulative effects of Zn on plant growth were also obtained by many workers (Tobbal, 1999; Hemantaranjan *et al*. 2000; Rizk and Abdo, 2001; Wanas, 2002 and Gomaa, 2003). Mahmoud *et al*. (2006) found that, treating broad bean plants with B (25 - 50 ppm) significantly increased plant height and total dry weight.

The increase in Zn supply also increased the pigment concentration in the leaves of wheat, mustard, fenugreek and board bean. Chlorophyll concentration increased to 50 kg ha^{-1} Zn supply. Total chlorophyll increased by 462% in the leaves of fenugreek at 50 kg ha^{-1} of Zn supply compared to control followed by 66, 58 and 25% in board bean, wheat and mustard respectively (figure 1, 2). Similar results were obtained for chl a and b. These results are in agreement with the results obtained by Rashid and Ahmed (1997) on broad bean plants. They found that, chlorophyll contents were increased by using foliar application with Zn (50 ppm). Hassanein *et al*. (2000) found that, spraying cow pea (*Vigna sinensis*) plants with 10, 50 and 250 mg/L of B or Zn caused high significant increases in the contents of chlorophyll (a) and chlorophyll b. In the present study, protein content showed an increasing trend with increase in Zn supply (figure 2). Compared to control plant protein content significantly ($P<0.01$) increase by 82.5, 153, 110 and 72% in leaves of wheat, mustard, fenugreek and broadbean treated with 50 kg ha^{-1} of Zn. Tobbal (1999) reported that, the contents of soluble proteins in fenugreek and chickpea plants were increased in response to the treatment with Zn (100ppm) as foliar spraying. Role of Zn in providing defense strength to plant were also analyzed by analyzing activity of CAT and POX.

Activity of CAT increased up to 50kg ha^{-1} Zn supply which was compared to control significantly increased by 88, 41, 114 and 55% in the leaves of wheat, mustard, fenugreek and broad bean respectively (figure 2). The activity of POX increased up to 25 kg ha^{-1} of Zn supply in wheat and mustard then decreasing trend was observed to higher dose while in fenugreek and board bean it was increased up to 50 kg ha^{-1} of Zn supply (figure 2). Tobbal (2006) and Gamal El-Gebaly *et al*. (2003) reported increased activity of CAT and POX enzymes in plants treated with Zn.

CONCLUSION

The present investigation was undertaken to assess the hyper accumulator of Zn along with growth and certain metabolic responses of wheat, mustard, fenugreek and broad bean plants in response to soil treatment with Zn. Present study suggests that soil amended with Zn has good response toward biomass production in plants. Indeed some crop plants accumulate more Zn than other so to maintain Zn level in soil regular crop (less accumulator/hyper accumulator crops) rotation was needed.



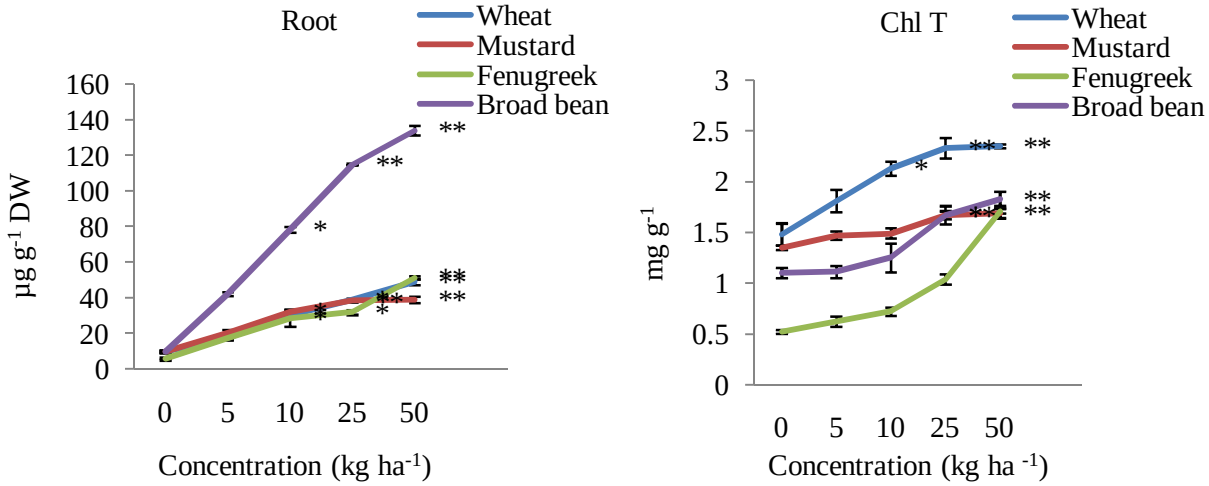
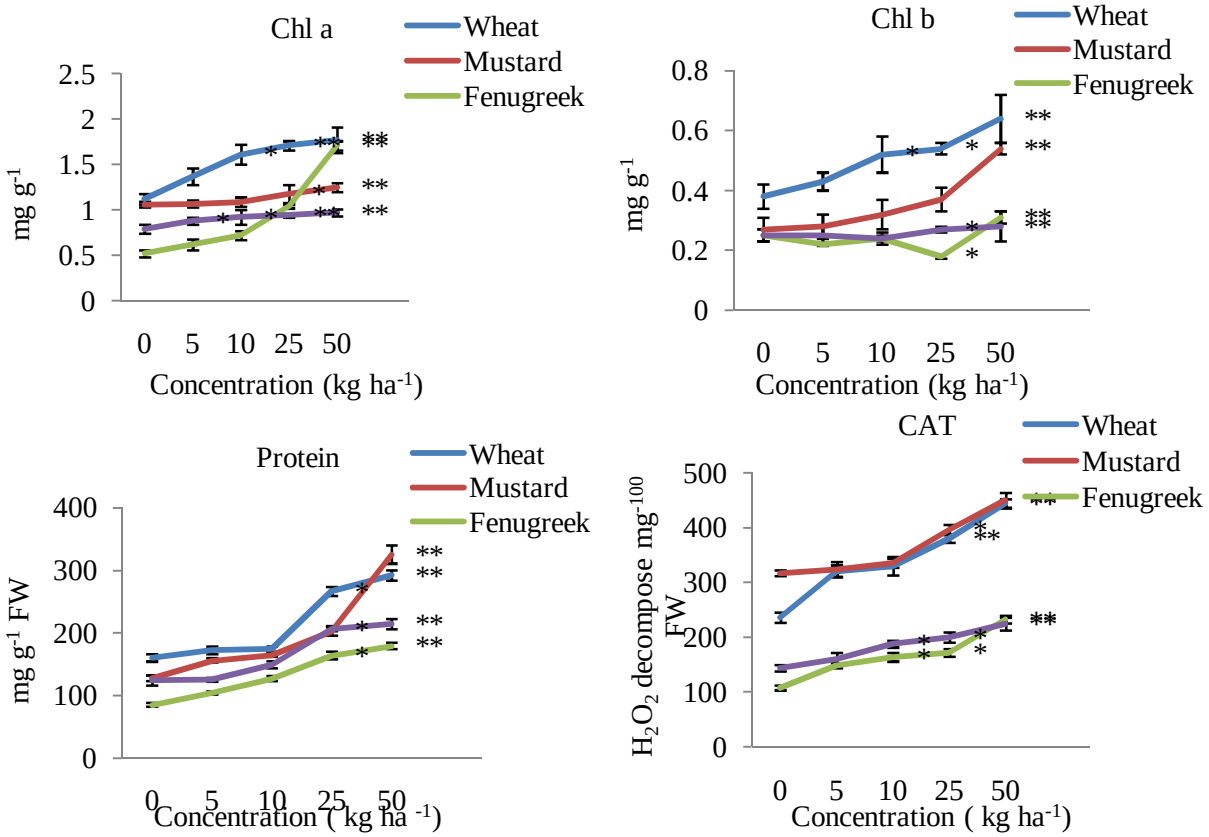


Figure 1. Effect of various levels of Zn on shoot length, FW, DW, Chl T and tissue concentration in wheat, mustard, fenugreek and broad bean plant observed at 65 days. Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.



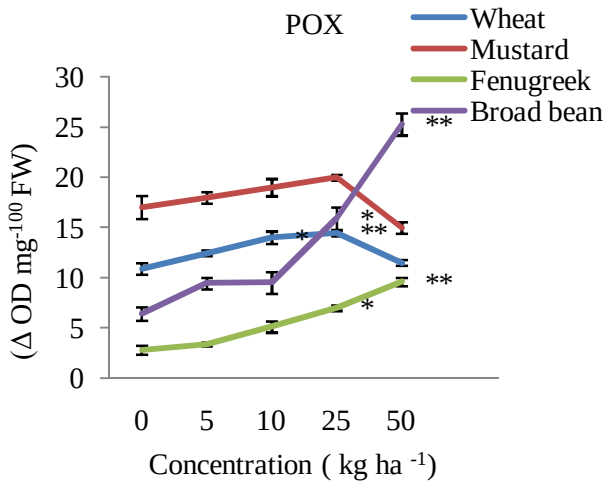


Figure 2. Effect of various levels of Zn on Chl a, Chl b, protein content and activity of CAT and POX in the leaves of wheat, mustard, fenugreek and broad bean plant observed at 65 days.

Bar represent SE value. *- value significant at P<0.05 and **- value significant at P<0.01 levels.

ACKNOWLEDGEMENTS

The author would like to acknowledge Council of Scientific and Industrial research, New Delhi, for financial support and Dr. Rajeev Gopal for the critical reading of the manuscript.

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