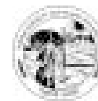
Indian J. Plant Physiol., Vol. 14, No. 1, (N.S.) pp. 60-66 (Jan.-March, 2009)



ZINC HOMEOSTASIS IS CRITICAL FOR OPTIMISED ANTIOXIDANT DEFENSE IN FABA BEAN

G.C. PATHAK, D.K. PANDEY, B. GUPTA AND N. PANDEY*

Department of Botany, University of Lucknow, Lucknow-226 007

Received on 24 April, 2008

SUMMARY

Faba bean (*Vicia faba* L.) plants were grown with five levels of Zn supply ranging from 0.01 to 10 µM. Best growth and maximum biomass were obtained with 1 µM Zn. Leaves of plants with sub- and supra- optimal levels of Zn showed accumulation of lipid peroxides and alterations in the concentration of antioxidants (ascorbate, carotenoids). Activities of carbonic anyhydrase (CA) and total and Cu/Zn superoxide dismutase (SOD) increased with increase in Zn concentration. Activities of ascorbate peroxidase (APX) and catalase (CAT) were lower at both sub- and supra- optimal levels of Zn. Leaf tissue concentration of Zn (~30 µg Zn g^{-1} dw) was found optimum for biomass yield of faba bean and also provided defense against oxidative damage. Sub-optimal tissue Zn increased sensitivity to oxidative damage because of decreased activity of SOD that implied enhanced accumulation of superoxide. Supra-optimal tissue Zn produced similar, though less pronounced effect because of stimulation of SOD activity associated with inhibition of APX activity, which could impair efficacious scavenging of H_2O_2. The data shows that Zn homeostasis is critical for optimized defense against ROS. Deviation from this induces alterations in antioxidant enzyme activities resulting in weakening of antioxidant defense against the ROS.

Key words: **Antioxidant defense, ascorbate peroxidase, faba bean, glutathione reductase, superoxide dismutase, zinc**

INTRODUCTION

The sedentary life-style of terrestrial plants exposes them to extremes of environment that produce metabolic perturbations, a major and common consequence of which is accelerated generation of ROS such as the $^1O_2$, O_2^- and H_2O_2 (Alscher *et al.* 1997, Asada 2006). As such, or by giving rise to even more reactive hydroxyl radicals ($OH^·$), the ROS cause oxidative damage to the cellular constituents, viz. membrane lipids, proteins and the genomic DNA (Halliwell and Gutteridge 1989). Survival under stressful environment is, however, made possible because of the inherent capability of plants to up-regulate the antioxidant system, providing a mechanism for efficient detoxification of the ROS (Foyer and Mullineaux 1994, Asada 2006). The antioxidant system consists of low molecular weight compounds such as ascorbate, glutathione, α-tocopherol and carotenoids (Deming-Adams 1990, Fryer 1992, Noctor and Foyer 1998) and enzymes such as the superoxide dismutase (SOD) (Scandalios 1993), ascorbate peroxidase (APX) (Nakano and Asada 1981, Shigeoka *et al.* 2002), glutathione reductase (GR) (Pastori and Trippi 1992) and catalase (CAT) (Willekens *et al.* 1997). Three of these enzymes, SOD, APX and CAT, are metalloenzymes with cationic micronutrients (Zn, Cu, Fe, Mn) as cofactors and their activities are influenced by the plant nutrient status

*Corresponding author, E-mail: nalini_pandey@rediffmail.com

of the constituent metal micronutrient (Sharma 2006). Micronutrient status of plants may therefore has determining influence on plant response to oxidative stress. Strong evidence for this has come from researches on oxidative metabolism of plants subjected to deficiency and overload of Zn. Cakmak (2000) reviewed evidences substantiating a role of Zn in offering protection against damage from ROS. Several subsequent studies have affirmed this (Obata *et al*. 2001, Pandey *et al*. 2002 b, Pathak *et al*. 2005). Increased damage from ROS has also been reported in plants subjected to Zn-toxicity (Weckx and Clijsters 1997, Prasad *et al*. 1999, Rao and Sresty 2000). We postulated that physiological or homeostatic concentration of Zn is critical to optimal functioning of the antioxidant defense and explored the changes in lipid peroxidation (MDA), a parameter of oxidative damage, chlorophyll (Chl), carotenoid (Car), ascorbate (Asc), enzyme carbonic anhydrase (CA) and the antioxidant enzymes- SOD, APX, GR, CAT and peroxidase (POX) in relation to changes in leaf tissue concentration of Zn in faba bean (*Vicia faba* L.) grown with Zn supply varying from deficiency to excess.

MATERIALS AND METHODS

Faba bean (*Vicia faba* L.) was grown under glasshouse conditions in acid purified silica sand (Sharma 1996) in polyethylene pots (5 L) with a central drainage hole, covered with glasswool underneath an inverted watch glass that allowed free drainage. The nutrient solution contained 4 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.33 mM H_3BO_3, 0.1 mM Fe-EDTA, 10 µM $MnSO_4$, 1.0 µM $CuSO_4$, 0.1 µM Na_2MoO_4, 0.1 mM NaCl, 0.1 µM $CoSO_4$, 0.1 µM $NiSO_4$ and Zn at five levels viz. 0.01, 0.1, 1, 2 and 10 µM. The nutrient solution was supplied six days a week. At the week-end, pots were flushed with glass-distilled water to avoid accumulation of nutrients in the rooting medium. For each treatment, there were three pots, each containing five plants. Thirty eight days after sowing (DAS), when leaves of plants grown with 0.01 µM Zn displayed initial symptoms of Zn deficiency, plants were quantified for biomass yield and leaf tissue concentration of Zn for working out its optimal concentration for maximum biomass yield and the critical concentrations for thresholds of deficiency and toxicity (corresponding to 90% optimal yield) (Sharma 2006). Simultaneously, the two fully expanded terminal trifoliates were assayed for thioarbituric acid reactive substances (TBARS), Chl a and b, Car, Asc, CA, total and Cu-Zn SOD, APX, GR, CAT and POX.

Biomass and Zn concentration: Plants were separated into roots and shoots and total biomass was determined by oven drying (70^{0}C) the samples. Leaf tissue concentration of Zn was determined by Atomic absorption spectrophotometery (Perkin Elmer A Analyst 300) in HNO_3: $HClO_4$ (10:1) digests.

Lipid peroxides: Lipid peroxides were measured in terms of thiobarbaturic acid reactive substances (TBARS). Fresh leaf material was extracted in 1% trichloracetic acid (TCA). The supernatant was centrifuged at 10,000 g for 10 min and treated with 0.5% thiobarbaturic acid in 20% TCA. The reaction mixture was incubated in boiling water bath for 30 min and TBARS were measured spectrophotometrically at 532 nm after adjusting for non-specific absorbance at 600 nm (Heath and Packer 1968).

Chlorophylls and carotenoids: Chlorophyll (a+b) and carotenoids were extracted in 80% acetone and measured spectrophotometrically (Perkin Elmer UV/VIS Lambda Bio 20). Chlorophyll was measured at 645 and 663 nm and carotenoids at 480 and 510 nm (Lichtenthaler 1987).

Ascorbate dehydroascorbate: Ascorbate was extracted in 10% TCA and assayed according to Law *et al*. (1983) by following the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid and measuring the color intensity of the Fe^{2+}-α,α-bipyrridyl complex at 525 nm. Dehydroascorbate (DHAsc) was determined as the difference between total Asc, after reduction with dithiothreitol.

Enzyme activities: For assay of SOD and GR, fresh leaf tissue was ground in potassium phosphate buffer (50 mM, pH 7.0), containing EDTA (1mM) and PVP (2%). The extracts were centrifuged at 15000 g for 10 min and the supernatant was assayed for the enzyme activities.

SOD was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich 1971). One unit of enzymes is defined as the amount of enzyme causing 50% inhibition of NBT reduction. The difference after inhibition of the cytochrome C oxidase activity with KCN (3 mM) was taken as the measure of Cu/Zn SOD activity. For determining APX activity, 1mM ascorbate was added to the above mentioned buffer and the enzyme activity was assayed by following the oxidation of Asc at 290 nm (Nakano and Asada 1981). Assay for GR was carried out by following the oxidation of NADPH and monitoring the decrease in absorbance per min at 340 nm in a reaction mixture containing 100 mM phosphate buffer pH 7.0, 1mM oxidized glutathione (GSSG), 1mM EDTA, 0.1mM NADPH and 25 to 50 µl of the enzyme extract (Jablonski and Anderson 1978).

Catalase and non-specific peroxidases were extracted by homogenization of the fresh leaf tissue in ice cold glass distilled water (1:10) in a chilled pestle and mortar. Catalase was assayed by a modification of the method of Euler and Josephson (1927) and PO according to Luck (1963). Carbonic anhydrase was extracted by grinding fresh leaf tissue in pre- chilled 0.02 mol L^{-1} veronal buffer (pH 8.15) containing 0.1 µmol L^{-1} EDTA and mercaptoethanol and assayed according to Rickli *et al.* (1964). Enzyme activities are expressed on the basis of total soluble proteins in the enzyme preparations, as determined by the dye binding method of Bradford (1976).

Statistical analysis: All measurement were made on samples drawn in triplicate and the data were statistically analysed (ANOVA). Significant differences (LSD; P= 0.05) from plants grown with 1 µM Zn (that showed optimum growth and biomass yield) are indicated by asterisk (*) in the figures.

RESULTS

Growth, visible symptoms and biomass yield: The effect of variation in Zn supply was reflected in differences in plant growth after 28 DAS. Height, branching and leaf size of plants grown with 0.01µM Zn appeared restricted and their terminal leaves appeared clustered. The young leaves turned chlorotic with their margins turning greyish-brown and scorched. Late in the growing period (90 DAS), growth of plants supplied 10 µM Zn also appeared restricted and the old leaves of these plants showed chlorosis and necrosis of the apical part. Application of 1 µM Zn produced best growth of plants. The effect of Zn on growth was also reflected in their biomass yield. Plants grown with 1µM Zn produced maximum biomass yield. Compared to this, biomass yield was significantly low at all the other levels of Zn supply. Plants grown with 0.01µM Zn showed the least (<50% of the maximum) biomass yield.

Zinc concentration: Leaf tissue concentration of Zn was related to the level of Zn supply. It increased from 7.1 µg g^{-1} dw in plants supplied 0.01µM Zn to 109.6 µg g^{-1} dw in plants supplied 10 µM Zn (Fig. 1). Plants grown with 1µM Zn, that produced maximum biomass yield, contained ~30 µg Zn g^{-1} dry wt. Plot of biomass yield relative to 1 µM Zn (relative yield) against leaf tissue concentration of Zn (Fig. 2) revealed 26 and 48 µg Zn g^{-1} dw as the critical levels (90% max. yield) for threshold of deficiency and toxicity, respectively. Zinc concentration ranging 26 to 48 µg g^{-1} dw represent sufficiency or homeostatic concentration of Zn.

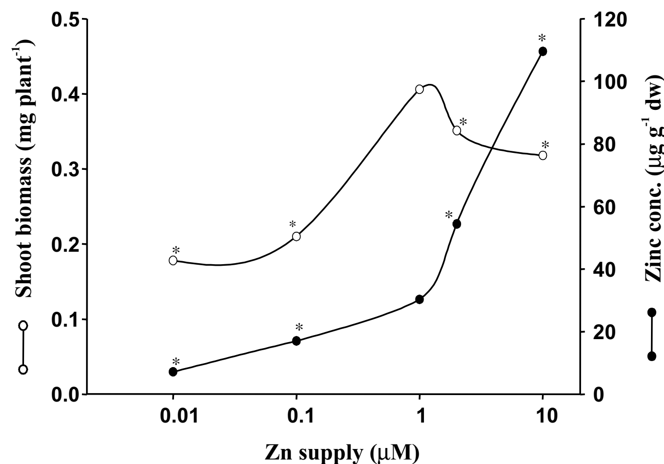


Fig. 1. Effect of Zn supply on shoot biomass (O-O) and leaf tissue concentration of Zn (●-●). Asterisk (*) indicates significant difference (P=0.05) from optimal Zn supply

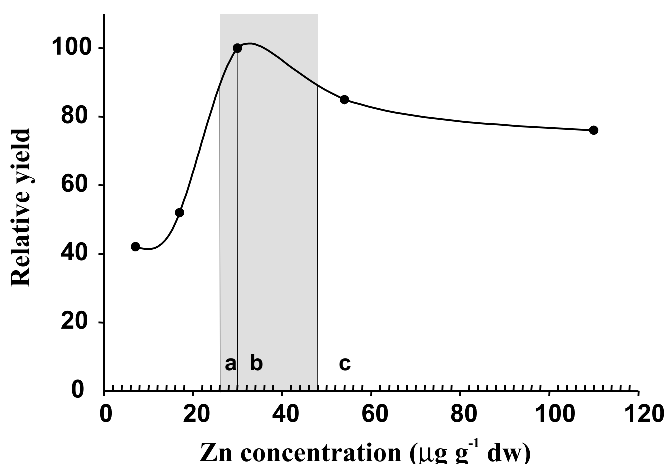


Fig. 2. Plot of relative shoot growth *versus* Zn supply, showing critical concentrations of Zn for threshold of deficiency (a) optimal growth (b) and threshold of toxicity (c). The shaded portion depicts sufficiency concentration of Zn.

Thiobarbituric acid reactive substances: Plants grown with 1 µM Zn, with leaf tissue concentration of ~30 µg Zn g^{-1} dw, showed the minimum concentration of TBARS. Leaves of plants grown with less or more than 1 µM Zn contained significantly higher concentrations of TBARS (Fig. 3 a).

Chlorophyll and carotenoids: Plants grown with 1µM Zn, that showed best growth and maximum biomass yield, contained the highest concentration of Chl and Car (Fig. 3 b,c). Both at sub- and supra- optimal levels of Zn, concentration of each Chl a and Chl b were significantly lower. Greater decrease in Chl a than Chl b led to decrease in Chl a:Chl b ratio. At sub-optimal levels of Zn, Chl a:Chl b was reduced by ~ 10% and at supra- optimal leaves of Zn by >20%. Carotenoids followed a trend similar to Chl, but Zn effect on Car was relatively less. This caused an increase in Car:Chl ratio in leaves of plants grown with sub- and supra- optimal levels of Zn.

Ascorbate and dehydroascorbate level: The level of Zn supply changed the leaf tissue concentration of ascorbate (Asc). Plants grown with 0.01µM Zn, with 7 µg g^{-1}dw Zn in leaves showed the highest concentration of Asc. Increasing Zn supply from 0.01 µM to 1.0 µM decreased the Asc level. Increase in Zn supply above 1 µM increased asc concentration (Fig. 3 d). A similar trend was observed in the DHAsc concentration (Fig. 3 d).

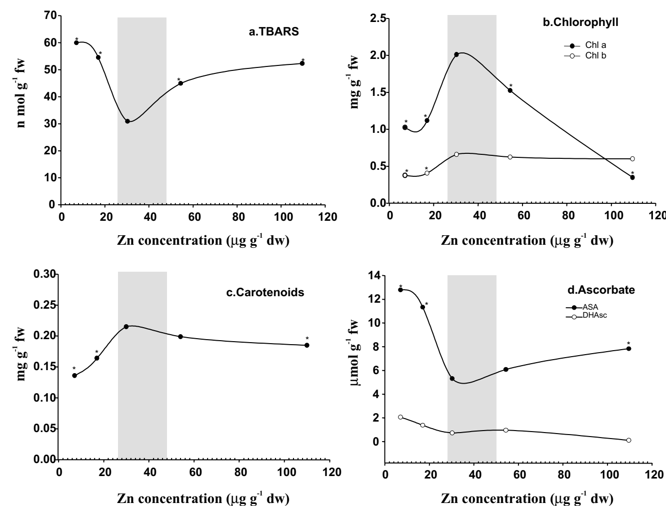


Fig. 3. Effect of leaf tissue Zn on the concentration of (a) thiobarbituric acid reactive substances (TBARS), (b) chlorophyll (Chl), (c) carotenoids (Car) and (d) ascorbate (Asc) dehydroascorbate (DHAsc). The shaded portion depicts sufficiency concentration of Zn. Asterisk (*) indicates significant difference (P=0.05) from the, respective, values at optimal Zn supply.

Activity of carbonic anhydrase and antioxidant enzymes: The activity of the Zn containing enzyme CA showed a steady increase with increase in the level of Zn supply from 0.01 to 10 µM (Fig. 4 a). Total as well as Cu/Zn- SOD activities followed similar trend (Fig. 4 b). The activities of the other antioxidant enzymes increased or decreased with increase in Zn supply from 0.1 to 1 µM, beyond which the trend was reversed. Thus, maximum activity of APX and CAT were detected in the leaves of plants grown with 1.0 µM Zn (Fig. 4 c, d). At both sub- and supra- optimal levels of Zn, both APX and CAT activities were significantly reduced. An opposite trend was shown by POX and GR (Fig. 4 e, f). Compared to that at 1 µM Zn supply, the activities of POX and GR were significantly enhanced at sub-optimal levels of Zn.

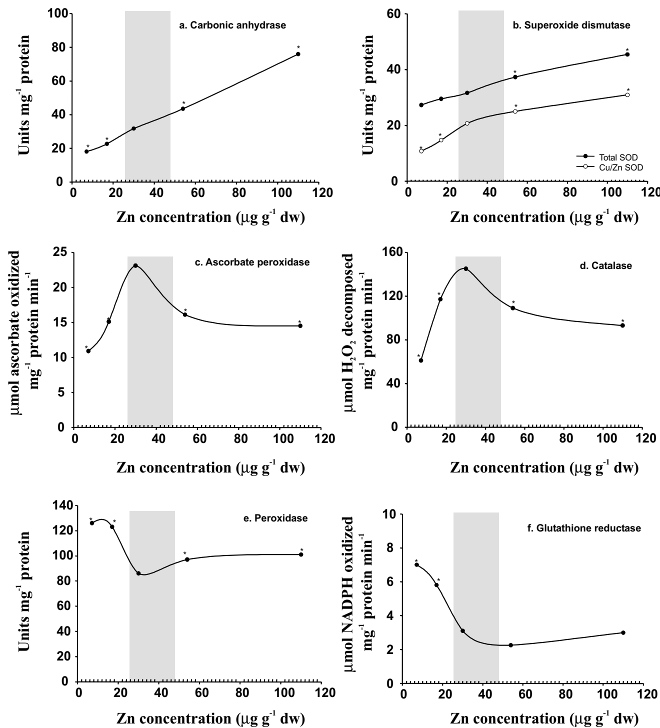


Fig. 4. Effect of leaf tissue Zn on the activities of (a) carbonic anhydrase, (b) superoxide dismutase, (c) ascorbate peroxidase, (d) catalase, (e) non-specific peroxidases and (f) glutathione reductase. The shaded portion depicts sufficiency concentration of Zn. Asterisk (*) indicates significant difference (P=0.05) from the, respective, enzyme activities at optimal Zn supply

DISCUSSION

The study of oxidative metabolism in leaves of faba bean, grown over a wide range of Zn supply (0.01 to 10 µM) affirmed increased damage from ROS as a sequel to Zn-deficiency (Cakmak and Marschner 1993, Yu and Rengel 1999, Obata *et al*. 2001, Pandey *et al*. 2002 a, b, Pathak *et al*. 2005) and Zn-excess (Weckx and Clijsters 1997, Prasad *et al*. 1999, Rao and Sresty 2000). Importance of homeostatic concentration of Zn for optimized functioning of the antioxidant defense system of plants was also revealed. We observed decrease in chloroplast pigments and increased accumulation of TBARS at both sub- and supra-optimal levels of Zn that indicated accelerated generation of ROS and increased lipid peroxidation. Similar to CA, SOD activity increased with increase in Zn concentration from the lowest to the highest. Low levels of Cu/Zn SOD under Zn deficiency, have also been reported earlier (Cakmak and Marschner 1993, Yu and Rengel 1999, Pandey *et al*. 2002 a, b, Pathak *et al*. 2005) and could limit dismutation of O_2^- to H_2O_2 which could be effectively detoxified *via* the ascorbate- glutathione cycle (Foyer *et al*. 1997). Increased buildup of O_2^- can also contribute to production of highly toxic OH· radicals (Foyer 1996) and inhibition of catalase (Kono and Fridovich 1982, Shimizu *et al*. 1984), a key enzyme involved in detoxification of peroxisomal H_2O_2. Increased sensitivity of plants raised at supra-optimal Zn supply to oxidative damage seems to be an outcome of increased dismutation of O_2^- to H_2O_2 due to elevated levels of SOD and inhibition of APX and CAT involved in the detoxification of H_2O_2 in different cellular compartments. These observations are in consonance with high levels of SOD and increased accumulation of H_2O_2 reported in leaves of wheat plants subjected to excess supply of Zn (Sharma *et al*. 1999). Thus, both sub- or supra- optimal levels of Zn results in weakening of defense against excessive production of ROS as a result of altered stoichiometry of the antioxidant enzymes. Lack of coordination in the antioxidant enzyme activities under Zn deficiency has also been reported by Chauhan *et al*. (2005). These observations led us to conclude that Zn homeostasis is critical for optimizing the antioxidant defense of plants, which contributes to their adaptive potential against abiotic stresses. Enhanced sensitivity of Zn- deficient plants to high light intensity (Marschner and Cakmak 1989) and ozone toxicity (Wenzel and Mehlhorn 1995) lends support to our premise.

ACKNOWLEDGEMENT

The present study formed a part of the research project funded by the Indian Council of Agricultural Research, New Delhi.

REFERENCES

Alscher, R.G., Donahue, J.L. and Cramer, C.L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. *Physiol. Plant.* **100:** 224-233.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* **141:** 391-396.

Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem.* **44:** 276-287.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* **72:** 248-254.

Cakmak, I. (2000). Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.* **146:** 185-205.

Cakmak, I. and Marschner, H. (1993). Effect of Zn nutritional status on activities of superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. *Plant Soil* **155/156:** 127-130.

Chauhan, S., Patil, R.V. and Khanna-Chopra R. (2005). Lack of coordination in antioxidant enzymes is a consequence of partial Zn-deficiency in wheat leaves. *J. Plant Biol*. **32:** 19-23.

Deming-Adams, B. (1990). Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthins. *Biochem. Biophys. Acta*. **1020:** 1-24.

Euler, H. Von and Josephson, K. (1927). Über Katalase I. *Leibigs Ann.* **452:** 158-181.

Foyer, C.H. (1996). Oxygen processing in photosynthesis. *Biochem. Soc. Trans.* **24:** 427-433.

Foyer, C.H. and Mullineaux, P.M. (1994). Causes of Photooxidative Stress and Amelioration of Defense System in Plants. Boca Raton: CRC Press.

Foyer, C.H., Lopez- Delgado, H., Dat, J.F. and Scott, I.M. (1997). Hydrogen peroxide- and glutathione- associated mechanism of acclimatory stress tolerance and signaling. *Physiol. Plant.* **100:** 241-254.

Fryer, M. (1992). The antioxidant effects of thylakoid vitamin E (á-tocopherol). *Plant Cell Environ.* **15:** 381-392.

Halliwell, B. and Gutteridge, J.M.C. (1989). Free Radicals in Biology and Medicine. Oxford: Clarendon Press.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125:** 189-198.

Jablonski, P.B. and Anderson, J.W. (1978). Light dependent reduction of oxidized glutathione by ruptured chloroplasts. *Plant Physiol.* **61:** 221-225.

Kono, Y. and Fridovich, I. (1982). Superoxide radical inhibits catalase. *J. Biol. Chem.* **257:** 5751-5754.

Law, M.Y., Charles, S.A. and Halliwell, B. (1983). Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of paraquat. *Biochem. J.* **210:** 899-903.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, In: L. Packer and R. Douce (eds.), Methods in Enzymology, 148, pp. 350-382. Academic Press Inc., New York.

Luck, M. (1963). Peroxidase. In: H.U. Bergmeyer (ed.), Methods in Enzymatic Analysis, pp. 895-897. Academic Press, New York.

Marschner, H. and Cakmak, I. (1989). High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium deficient bean (*Phaseolus vulgaris*) plants. *J. Plant Physiol.* **134:** 308-315.

Nakano, Y. and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22:** 867-880.

Noctor, G. and Foyer, C.H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49:** 249-279.

Obata, H., Kawamura, S., Shimoyama, A., Senoo, K. and Tanaka, A. (2001). Free radical injury in rice leaf under Zn deficiency. *Soil Sci. Plant Nutr.* **47:** 205-211.

Pandey, N., Pathak, G.C., Singh, A.K. and Sharma, C.P. (2002 a). Enzymic changes in response to zinc nutrition. *J. Plant Physiol.* **151:** 1151-1153.

Pandey, N., Singh, A.K., Pathak, G.C. and Sharma, C.P. (2002 b). Effect of zinc on antioxidant response in maize leaves, *Indian J. Exp. Biol.* **40:** 954-956.

Pastori, G.M. and Trippi, V.S. (1992). Oxidative stress induced high rate of glutathione reductase synthesis in a drought- resistant maize strain. *Plant Cell* **33:** 957-961.

Pathak, G.C., Pandey, N. and Sharma, C.P. (2005). Zinc regulation of antioxidant defense in green gram (*Vigna radiata* L.). *J. Plant Biol.* **32:** 211-216.

Prasad, K.V.S.K., Saradhi, P.P. and Sharmila, P. (1999). Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. *Environ. Exp. Bot.* **42:** 1-10.

Rao, K.V.M. and Sresty, T.V.S. (2000). Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* **157:** 113-128.

Rickli, E.E., Ghazanfor, A.S., Gibbons, B.H. and Edsall, J.T. (1964). Carbonic anhydrase from human erythrocytes: preparation and properties of two enzymes. *J. Biol. Chem.* **239:** 1065-1078.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiol.* **101:** 7-12.

Sharma, C.P. (1996). Deficiency Symptoms and Critical Concentration of Micronutrient in Crop Plants. Micronutrients Project (ICAR) Bulletin 1, pp. 1-12. Lucknow University Center, Lucknow.

Sharma, C.P. (2006). Plant Micronutrients. Science Publishers, New Hampshire, USA.

Sharma, P.N., Bisht, S.S., Kumar, P. and Mishra, M.K. (1999). Induction of oxidative stress by deficiency and toxicity of zinc in wheat plants grown in solution culture. *Indian J. Agric. Biochem.* **12:** 10-13.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y. and Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.* **53:** 1305-1319.

Shimizu, N., Kobayashi, K. and Hayashi, K. (1984). The reaction of superoxide radical with catalase. *J. Biol. Chem.* **259:** 4414-4418.

Weckx, J.E.J. and Clijsters, H.M.M. (1997). Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. *Plant Physiol. Biochem.* **35:** 405-410.

Wenzel, A.A. and Mehlhorn, H. (1995). Zinc deficiency enhances ozone toxicity in bush beans. (*Phaseolus vulgaris* L. cv. Saxa). *J. Exp. Bot.* **46:** 867-872.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., In: Z.D. and Van Camp, W. (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defense in C_3 plants. *EMBO J.* **16:** 4806-4816.

Yu, Q. and Rengel, Z. (1999). Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. *Ann. Bot.* **83:** 175-182.