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Differential Sensitivity of Maize to Zinc and High Light Intensity

Nalini Pandey* • Girish Chandra Pathak • Amit Kumar Singh

Plant Nutrition and Stress Physiology Laboratory, Botany Department, University of Lucknow, Lucknow, 226007, India

Corresponding author: * **nalini_pandey@rediffmail.com**

ABSTRACT

The interactive effects of Zinc (Zn) deficiency and light intensity were studied on non-enzymatic and enzymatic components of the antioxidative system in maize lines CM 115 and CM 120 differing in tolerance to Zn deficiency. Compared to plants receiving normal Zn supply (1 μM Zn), plants grown with deficient (0.10 μM Zn) Zn supply showed growth depression, reduced dry matter and visual symptoms of Zn deficiency like white necrotic patches that appeared earlier and were more severe in Zn inefficient line CM 120 than in moderately efficient maize line CM 115. Zinc-deficient plants showed a decrease in carbonic anhydrase (CA) activity which was more pronounced in CM 120 than in CM 115. Accumulation of malondialdehyde and hydrogen peroxide and a decrease in pigments (chlorophyll and carotenoids) was observed in Zn-deficient plants, which was less pronounced in CM 115 than in CM 120. In CM 120 ascorbate (ASA) concentration and glutathione reductase (GR) activity decreased while the levels of ASA, DHA (dehydroascorbate), APX (ascorbate peroxidase) and GR activity increased in CM 115. Although the activity of SOD (superoxide dismutase) and the expression of Cu/Zn SOD were reduced in both CM 115 and CM 120 plants, the decrease was more severe in the latter. Exposure of plants to high intensity of light accentuated these Zn-deficiency effects more so in CM 120. Our results suggest that the efficient utilization of Zn in CM 115 plants contributes to enhanced tolerance to oxidative damage from Zn deficiency and high light intensity by upregulating the antioxidative defense mechanism.

Keywords: antioxidative capacity, high light intensity, maize lines CM 115 and CM 120, Zn deficiency

INTRODUCTION

Multiple stresses are experienced by plants due to the presence of many unfavorable growth conditions in the field such as nutrient disorder, high light intensity, low temperature, salinity, drought and others. Among these adverse environmental factors, high light intensity is a common threat to many crop species, particularly those of arid and semi-arid regions and has been recognized as an important factor limiting their production (Hernández *et al*. 2004, 2006). Zinc (Zn) deficiency is one of the most widespread of all the micronutrient deficiencies in many crop plants, particularly in calcareous soils of arid and semi-arid regions (Singh 2007). As a result, in arid and semi-arid regions where low Zn soils are present, stresses resulting from high light intensity and Zn deficiency are likely to be present simultaneously or sequentially.

Zinc is an essential plant nutrient involved in fundamental metabolic processes. It is a cofactor of over 300 enzymes involved in cell division, nucleic acid metabolism and protein synthesis (Broadley *et al*. 2007). Furthermore, it is a constituent of superoxide dismutase (Cu/Zn SOD), an important antioxidant enzyme, involved in maintaining the balance of the cell redox state (Bowler *et al*. 1994). Zn is known to inhibit NADPH oxidase (Cakmak and Marschner 1988; Cakmak 2000) and Zn deficiency enhances the generation of superoxide radicals ($O_2^{\cdot-}$) by enhancing NADPH-dependent oxidase activity. Zn-deficient plants also inhibit carbonic anhydrase (CA) activity leading to poor intercellular levels of CO_2 and a decrease in photosynthesis (Pandey and Sharma 1989; Fischer *et al*. 1997). This impairs the utilization of electrons and absorbed light energy for CO_2 fixation in plants which accentuates photogeneration of reactive oxygen species (ROS) leading to photo-oxidative damage of chloroplasts in Zn-deficient plants. The severity of Zn deficiency stress on photosynthesis can be particularly exacerbated by simultaneous or sequential exposure to light due to the enhanced risks of photo-oxidative damage (Marschner and Cakmak 1989; Cakmak *et al*. 1995). Data compiled by Cakmak (2000 and references therein) indicated that Zn deficiency elicits an oxidative response in plants. The role of Zn in offering protection against oxidative stress forms a major area of interest in the Zn nutrition of plants (Cakmak and Marshner 1993; Obata *et el*. 2001; Pandey *et al*. 2002b; Pathak *et al*. 2005, 2009; Ozdener and Aydin 2010).

Oxidative stress in plants develops due to enhanced production of ROS such as $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and singlet oxygen ($_1O^2$) formed during the course of metabolic processes chiefly during electron transport in the mitochondria and the photosystems in the chloroplast (Halliwell and Guteridge 2007). Plants have an inherent system of invoking an effective antioxidative mechanism (Mittler 2002; Rouhier *et al*. 2008), which include the non-enzymatic (ascorbate, glutathione, tocopherol and carotene) and the enzymatic components such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and peroxidase (POD)). However, this ability to cope with oxidative stress under different environmental conditions varies to a large extent not only between different plant genera but also within the same species.

A large number of reports exist on oxidative damage in Zn-deficient plants due to inhibition of SOD activity (Cakmak *et al.* 1997; Obata *et al.* 1999; Pandey *et al.* 2002a; López-Milon *et al.* 2005; Pathak *et al*. 2009) leading to excess production of $O_2^{\cdot-}$. The oxidative damage under Zn deficiency could also be aggravated by the reduced activity of H_2O_2-scavenging enzymes CAT, APX and GR (Cakmak and Marschner 1988; Cakmak *et al.* 1993; Pandey *et al.* 2002b; Pathak *et al.* 2005, 2009). High light radiation is another environmental problem which can alter the redox state of plants through the increased production of ROS (Hernández *et al*. 2004, 2006). Under high light intensity photo-

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oxidative damage has been shown to be intensified by adverse environmental conditions such as nutrient deficiencies, chilling and drought (Cakmak and Marshner 1993; Payton *et al*. 2001; Selote and Khanna-Chopra 2006). Zn-deficient plants are known to exhibit enhanced severity of chlorosis and necrosis under high light intensity due to photo-oxidative damage to chloroplasts (Marschner and Cakmak 1989; Cakmak *et al*. 1995).

Under Zn-deficient conditions Zn-efficient wheat cultivars exhibited elevated levels of Zn-requiring enzymes CA and Cu/Zn SOD compared to Zn-inefficient cultivars (Cakmak *et al.* 1997; Hacisalihoglu *et al.* 2003). However, there is little information on whether Zn efficiency can provide protection to plants from high light intensity. In this paper the biochemical utilization of Zn was examined by studying the activity of the Zn requiring enzymes as well as the antioxidative responses of maize plants differing in their susceptibility to Zn deficiency. We hypothesized that: a) the cellular antioxidative defense strategies, used by plants to circumvent the deleterious effects of oxidative damage by the efficient utilization of Zn, could also provide increased tolerance to high light intensity and b) that metabolic adjustments to high light stress would be associated with adjustments of antioxidative components in Zn-efficient plants. To test our hypothesis the interactive effects of Zn deficiency and high light intensity on the activities of antioxidative enzymes and antioxidants in leaves of maize lines differing in their susceptibility to Zn deficiency were examined.

MATERIALS AND METHODS

Plant culture

Six inbred lines of *Zea mays* L. viz CM101, CM 104, CM 105, CM 115, 118 and 120 were screened for Zn efficiency in a sand culture experiment (data not shown) under glasshouse conditions and the optimal concentration of Zn for their growth was determined. Based on this experiment CM 115 was found to be moderately Zn-efficient and CM 120 was Zn-inefficient. Plants of these two lines were raised from seeds in pots containing purified sand and grown in deficient (0.1 μM) and sufficient Zn (1.0 μM) supplied in a nutrient solution containing: 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 μM Cu SO_4, 0.1 μM Na_2MoO_4, 0.1 mM NaCl, 0.1 μM $CoSO_4$ and 0.1 μM $NiSO_4$ (Sharma 1996). The nutrient solution was supplied daily till it leached out from the central drainage hole of the pot. On weekends the pots were thoroughly leached with distilled water to prevent any toxic accumulation of nutrients.

During the period of study, maximum light intensity PFFD at 12:00 noon ranged between 800 to 1000 μmol m^2 sec^{-1}. The temperature during the 24 h ranged between 35 and 40°C (maximum) and 25 to 32°C (minimum), and the relative humidity (RH) at 9:30 am between 68 and 98%. Average daylength was around 12 h. Plants that were grown in 0.1 μM Zn developed visible symptoms of Zn deficiency after 30 days. Leaf discs from the fully expanded comparable top three (young) leaves from Zn-sufficient and Zn-deficient plants of both lines were excised and transferred to Petri dishes. These leaf discs were infiltrated with Zn-sufficient (1.0 μM Zn) and Zn-deficient (0.1 μM Zn) solution respectively and each set was subjected to normal (800 μmol m^{-2} s^{-1}) and high light intensity (1350 μmol m^{-2} s^{-1}) for 12 h. Thus 4 treatments for each maize line were maintained: 1.0 μM Zn + normal light intensity; 1.0 μM Zn + high light intensity; 0.1 μM Zn + normal light intensity; 0.1 μM Zn + high light intensity. The control for each maize line were leaf discs maintained in 1.0 μM Zn and normal light intensity (800 μmol m^{-2} s^{-1}). After 12 h, the leaf discs of both maize lines were washed free of the nutrient solution with distilled water and subjected to biochemical assays. All chemicals used during the investigation were analytical grade chemicals from Merck unless mentioned otherwise.

Biomass yield, Zn concentration

Biomass yield was determined in 30-d old plants of the two maize lines which were uprooted from the sand, separated into leaves, stem and roots and dried in an electric oven at 70°C. The oven-dried samples of the young leaves were ground in pestle and mortar and 1.0 g sub samples were digested in a mixture of HNO_3 and $HClO_4$ (10: 1, v/v). The digests were used for determining Zn concentration by atomic absorption spectrophotometry using a Thermo Jarrel Ash Video 12E.

Chlorophyll, antioxidants, MDA and H_2O_2

Chlorophyll (Chl) *a* and *b* and carotenoids were extracted in 80% acetone (Lichtenthaler 1987) and estimated spectrophotometrically at 645 and 663 nm for Chl and 480 and 510 nm for carotenoids using a Perkin Elmer Lambda Bio 20 UV/VIS Spectrophotometer. Ascorbate (ASA) was assayed according to the method of Law *et al.* (1983) by extracting fresh leaf tissue in TCA (trichloroacetic acid) and determining the reduction of Fe^{+3} to Fe^{2+} by ascorbic acid and formation of the subsequent complex between Fe^{2+} and α,α-bipyridyl, with absorption max at 525 nm. Total ASA was determined after reduction of dehydroascorbate (DHA) to ASA by dithiothreitol. ASA was determined by preparing a standard curve with L-ascorbic acid (Sigma).

Lipid peroxidation was measured in terms of MDA formation (Heath and Parker 1968). Treated leaf discs were homogenized with 0.1% TCA and centrifuged for 10,000 × g for 5 min. The supernatant was treated with 0.5% thiobarbituric acid in 20% TCA and the mixture was incubated in boiling water bath for 30 min. The absorbance was read at 532 nm using the extinction coefficient of 155 (mmol L^{-1} cm^{-1}) and adjusted for non-specific absorbance at 600 nm.

H_2O_2 was estimated after extraction in chilled acetone by method of Brennan and Frenkel (1977). The extract was centrifuged and titanium tetra chloride and chilled liquid ammonia was added to the supernatant. The precipitate was solubilized, centrifuged and after repeated washing with acetone the colorless residue was dissolved in 2N H_2SO_4. The color intensity of the H_2O_2–titanium complex formed was read at 415 nm in Perkin Elmer UV/VIS Lambda Bio 20 spectrophotometer.

Enzymes

Carbonic anhydrase (EC 4.2.1.1) was extracted by grinding fresh leaf tissue in a pre-chilled mixture of 0.1 mM EDTA and β-mercaptoethanol in 0.02 M Veronal (barbituric acid-sodium barbiturate) buffer pH 8.15 and assayed as discussed earlier by Pandey *et al.* (2002a) by noting the difference in time taken for change in color of the reaction mixture (veronal buffer + 0.01% bromothymol blue) from blue to greenish blue after adding CO_2 saturated water.

The activity of CAT (EC 1.11.1.6) and POD (E.C 1.11.1.7) were assayed in fresh leaf tissue extracts prepared by homogenizing fresh tissue samples in ice-cold glass distilled water (1: 10) in a cold mortar and pestle at 4°C and was assayed by the method described earlier by Pandey and Sharma (2002).

For assay of SOD (EC 1.15.1.11), APX (EC 1.11.1.11) and GR (EC 1.6.4.2) leaf samples were homogenized with 150 mM potassium phosphate buffer pH 7.0 containing 1 mM EDTA and 2% PVP. For APX assay 1 mM ascorbate was also included in the extracting medium. The homogenates were centrifuged at 15,000 g for 10 min and the supernatant were used for assay of enzyme activities.

The activity of SOD was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) in a reaction mixture containing 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA and enzyme extract. One unit of SOD represents the amount that inhibits the NBT reduction by 50% (Beauchamp and Fridovich 1971). Cu/Zn SOD was assayed by adding 3 mM KCN prior to the addition of enzyme extract.

The activity of APX was determined by the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer pH 7.0, 0.5 mM ASA and 0.1 mM H_2O_2.

Table 1 Effect of Zn deficiency on dry matter yield, tissue Zn and carbonic anhydrase activity in maize (*Zea mays* L.) lines CM 115 and CM 120. Data are mean ± SE (n = 3).

Plant tissue	CM 115		CM 120	
	Zinc supply			
	1 µM	0.1 µM	1 µM	0.1 µM
Dry matter yield: g plant^{-1}				
Leaf	1.290 ± 0.145 a	0.886 ± 0.105 b	0.982 ± 0.132 a	0.395 ± 0.102 b
Stem	1.055 ± 0.213 a	0.606 ± 0.156 b	0.757 ± 0.128 a	0.177 ± 0.098 b
Roots	0.815 ± 0.115 a	0.644 ± 0.108 b	0.782 ± 0.213 a	0.302 ± 0.122 b
Whole plant	3.110 ± 0.187 a	2.136 ± 0.251 b	2.522 ± 0.193 a	0.875 ± 0.133 b
Leaf tissue Zn: µg g^{-1}dry wt.				
	29 ± 2.21 a	15 ± 3.42 b	31 ± 4.53 a	19 ± 2.35 b
Carbonic anhydrase: Units mg protein^{-1}				
Normal light	89.75 ± 3.21 a	61.12 ± 2.26 b	92.43 ± 2.87 a	46.01 ± 2.87 b
High light	87.34 ± 2.78 a	56.78 ± 4.57 c	90.87 ± 3.64 a	37.98 ± 4.23 c

Different letters indicate significant differences ($P < 0.05$).

Oxidation of ASA was followed as fall in absorbance per min at 290 nm. Assay for GR was carried out in a reaction mixture containing 100 mM phosphate buffer pH 7.0, 1 mM oxidized glutathione (GSSG), 1 mM EDTA, 0.1 mM NADPH and 25 to 50 µl of the enzyme extract. The oxidation of NADPH was followed by monitoring the decrease in absorbance per min at 340 nm (Madamanchi *et al*. 1992)

Non-denaturing polyacrylamide gel electrophoresis was performed on 10% acrylamide gels for SOD and APX, and on 15% gels for GR isoforms. 50-75 µg of protein was loaded in each slot and electrophoretic separation was carried out at 4°C. Activity staining for SOD was performed according to the procedure of Beauchamp and Fridovich (1971) in a staining solution consisting 50 mM potassium phosphate buffer (pH 7.8), 0.03 mM riboflavin, 0.326% (v/v) *N-N-N'–N'*- tetraethylmethylethylene diamine (TEMED) and 1.25 mM of NBT in dark for 30 min. The gels were washed and exposed to light under 15 W fluorescent lamps. Identification of individual isozymes of Mn SOD and Cu/Zn SOD was done by soaking the gels in 5 mM H_2O_2 or 3 mM KCN prior to activity staining. Mn SOD is resistant to both KCN and H_2O_2 while Cu/Zn SOD is sensitive to KCN and Fe SOD is sensitive to H_2O_2 but resistant to KCN.

The activity staining for APX was based on the extent of inhibition of ASA-dependent NBT reduction according to Mittler and Zilinskas (1993). The gels were incubated in 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM ASA for 30 min followed by incubation in phosphate buffer (pH 7.0) containing 4 mM ASA and 2 mM H_2O_2 for 20 min. The gels were washed with phosphate buffer and then soaked in a sodium phosphate buffer (pH 7.8) containing 28 mM TEMED and 2.45 mM NBT with gentle shaking and the APX isoforms were visualized as achromatic bands against a purple-blue background.

GR isoforms were visualized as dark blue bands which appeared after staining gels with 50 mM Tris buffer pH 7.5 containing 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide, 2,6-dichlorophenol indophenol, 3.44 GSSG and 0.4 mM NADPH by the method of Madamanchi *et al*. (1992).

Statistical analysis

All measurements were made on samples drawn in triplicate and the data were statistically analyzed (ANOVA) for significance (LSD at $P < 0.05$) and SE (n=3).

RESULTS

Plants of CM 115 and CM 120 lines, when subjected to Zn deficiency at 0.1 µM, showed inhibited growth and visible symptoms of Zn deficiency but differed in the time duration for manifestation of these effects and their severity. While CM 120 showed perceptible inhibition of growth and deficiency symptoms like chlorosis around 15 days, in CM 115 these were delayed to 25 days. Necrotic lesions and development of bronze coloration on leaves in maize line CM 120 were observed around 35 days and in CM 115 development of these symptoms were less and appeared only slightly after 45 days. Both maize lines receiving a deficient supply of Zn showed a decrease in dry matter production and compared to CM 115 (22%), the decrease in dry matter yield due to Zn deficiency was more pronounced (66%) in CM 120 (**Table 1**).

Zinc concentration in leaves of plants grown with a deficient supply of Zn was lower than in Zn-sufficient plants. Zinc concentration in leaves of plants of maize line CM115 raised under Zn-sufficient and Zn-deficient supply was 29 and 15 µg g^{-1} dry wt, respectively. Compared to Zn-efficient CM115 in Zn-inefficient CM 120, Zn concentration in leaves of plants given Zn-sufficient and Zn-deficient supply was higher, being 31 and 19 µg g^{-1}, respectively (**Table 1**). Subjecting the leaves of both maize lines raised under Zn-sufficient and Zn-deficient supply to high light intensity did not produce any significant change in tissue Zn (data not included). The activity of CA decreased in the leaves of Zn-deficient plants and the decrease was concomitant with tolerance to Zn deficiency in the two maize lines. Thus at normal light intensity the CA activity in Zn-deficient CM 115 was higher than in leaves of Zn-deficient CM 120. At high light intensity, leaves of the two maize lines raised under Zn deficiency showed a more pronounced decrease in CA activity which was as much as 60% in CM 120 and 37% in CM 115 (**Table 1**).

The leaves of Zn-deficient plants especially under high light intensity had a lower concentration of Chl *a*, *b* and total Chl than those exposed to normal intensity of light in both the maize lines. The effect of high light intensity (more so under Zn deficiency) on the decrease in concentration of Chl *a* was more pronounced than on Chl *b* as is evident from the decrease in Chl *a*/*b* ratio (**Table 2**) in both the maize lines. Deficiency of Zn also led to decrease in concentration of carotenoids in Zn-deficient plants of both lines. The decrease in Chl and carotenoids was more marked in leaves of Zn-deficient plants of the two maize lines subjected to high intensity of light than those at normal light especially in CM 120 (**Table 2**).

In both the maize lines Zn deficiency caused increase in the concentration of MDA and H_2O_2 but the magnitude of Zn deficiency effect was less marked in maize line CM 115 (23%) than CM 120 (60%). With increase in the light intensity the increase in MDA concentration was further enhanced in all treatments being more in Zn-deficient CM 120 (**Table 2**).

The two maize lines showed diverse response to Zn deficiency in terms of ASA accumulation. Under Zn deficiency, maize line CM 115 showed an increase (20%) and maize line CM 120 showed decrease (30%) in ASA concentration. The Zn deficiency response on ASA concentration in leaves of both the maize lines was accentuated under high intensity of light. It was particularly so in maize line CM 120. DHA concentration also showed a similar trend in both the maize lines. The ratio of the DHA/ASA couple did not vary much in Zn sufficient and deficient plants of CM 115 plants under both light regimes but was appreciably

Table 2 Effect of Zn deficiency and high light intensity on the concentration of total Chl, Chl *a*/*b* ratio, carotenoids, ASA, DHA, DHA/ASA ratio, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in two maize lines CM 115 and CM120. Data are mean ± SE (n = 3).

Light intensity	CM 115		CM 120	
	Zinc supply			
	1 µM	0.1 µM	1 µM	0.1 µM
Total Chlorophyll: mg g^{-1} fr. Wt.				
Normal	1.622 ± 0.054 a	1.269 ± 0.017 b	1.521 ± 0.009 a	0.774 ± 0.018 b
High	1.452 ± 0.065 c	1.067 ± 0.007d	1.314 ± 0.018 c	0.703 ± 0.003 b
Carotenoids: mg g^{-1} fr. wt.				
Normal	0.357 ± 0.009 a	0.297 ± 0.013 b	0.268 ± 0.012 a	0.192 ± 0.005 b
High	0.327 ± 0.007 a	0.260 ± 0.010 b	0.250 ± 0.003 a	0.162 ± 0.001 c
Chl *a*/Chl *b*				
Normal	2.19 ± 0.081 a	2.01 ± 0.101 b	2.52 ± 0.086 a	1.80 ± 0.037 b
High	2.08 ± 0.086 b	1.72 ± 0.037 c	2.34 ± 0.086 a	1.36 ± 0.028 c
Ascorbate: µmol g^{-1} fr. wt.				
Normal	5.89 ± 0.075 a	7.08 ± 0.034 b	6.75 ± 0.132 a	4.70 ± 0.071 b
High	6.55 ± 0.034 c	7.51 ± 0.069 d	6.62 ± 0.003 a	3.73 ± 0.024 c
Dehydroascorbate: µmol g^{-1} fr. wt.				
Normal	0.51 ± 0.031 a	0.65 ± 0.008 b	0.54 ± 0.003 a	0.74 ± 0.024 b
High	0.60 ± 0.008 b	0.69 ± 0.041 c	0.65 ± 0.034 c	0.71 ± 0.028 b
DHA/AS				
Normal	0.086 ± 0.006 a	0.91 ± 0.012 b	0.080 ± 0.005 a	0.157 ± 0.008 b
High	0.091 ± 0.026 b	0.93 ± 0.016 b	0.098 ± 0.011 c	0.190 ± 0.013 d
MDA: nmol g^{-1} fr. wt.				
Normal	4.95 ± 0.083 a	18.44 ± 0.098 b	20.11 ± 0.155 a	32.06 ± 0.310 b
High	6.14 ± 0.026 c	20.48 ± 0.075 d	28.24 ± 0.028 c	38.92 ± 0.420 d
H_2O_2: µmol g^{-1} fr. Wt.				
Normal	1.02 ± 0.005 a	1.55 ± 0.012 b	1.08 ± 0.005 a	2.17 ± 0.014 b
High	1.13 ± 0.009 c	1.78 ± 0.013 d	1.51 ± 0.003 c	2.49 ± 0.011 d

Different letters indicate significant differences within maize lines ($P < 0.05$).

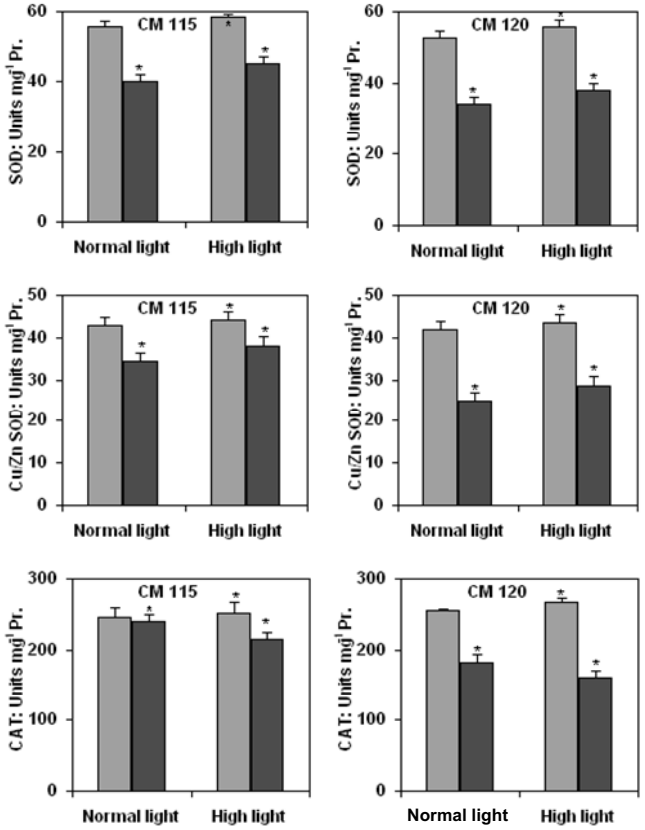

Fig. 1 Effect of Zn deficiency and high light intensity on the activity of SOD, Cu/Zn SOD, and CAT in two maize lines CM 115 and CM120. Empty bars indicate sufficient Zn supply and filled bars indicate Zn-deficient supply. Asterisks denote significant differences at $P < 0.05$. Data are mean ± SE (n = 3).

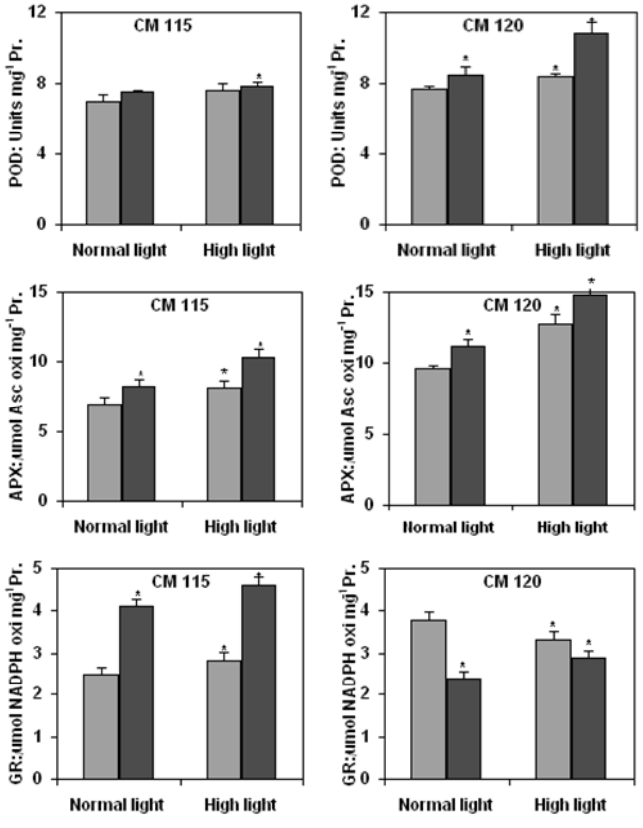

Fig. 2 Effect of Zn deficiency and high light intensity on the activity of POD, APX and GR in two maize lines CM 115 and CM120. Empty bars indicate sufficient Zn supply and filled bars indicate Zn-deficient supply. Asterisks denote significant differences at $P < 0.05$. Data are mean ± SE (n = 3).

high in plants of line CM 120 especially when subjected to high light intensity (**Table 2**).

The total as well as Cu/Zn SOD activity was decreased in leaves of CM 115 and CM 120 subjected to Zn deficiency under both light regimes. The decrease in the activity was less pronounced in leaves of Zn-deficient CM115 line than CM 120. However, exposure to high light intensity slightly alleviated their activities, the increase being more in

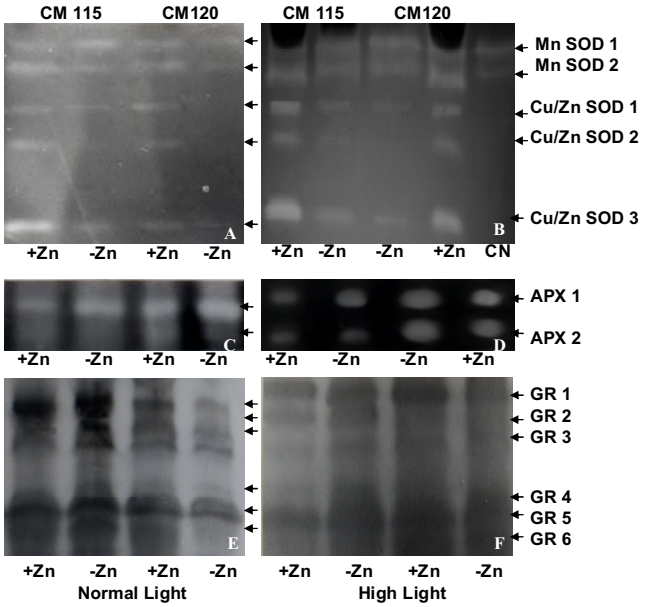

Fig. 3 Effect of Zn deficiency on the isoforms of SOD (A, B), APX (C, D) and GR (E, F) at normal and high light intensity in two maize lines CM 115 and CM120.

CM 115 especially in Cu/Zn SOD (**Fig. 1**). PAGE separation and activity staining revealed 5 isoforms of SOD, two of Mn SOD and three of Cu/Zn SOD. The decrease in activity of Cu/Zn SOD in leaves of Zn-deficient CM 120 plants was more marked than in Zn-deficient CM 115. This was reflected by the absence of bands of Cu/Zn SOD 1 and 2 and a very faint band of Cu/Zn SOD 3 in CM 120 under normal light and Zn deficiency (**Fig. 3A**). Under high light intensity expression of all the isoforms were enhanced except Cu/Zn SOD 2 which was not detected in Zn-deficient plants of CM 120 (**Fig. 3B**).

A decrease in the activity of CAT and a marginal increase in POD were observed in plants of CM 115 and CM 120 subjected to Zn deficiency at normal light intensity. The decrease in CAT activity was further enhanced under high light intensity (**Fig. 1**), more so in CM 120 than in CM 115. The increase in POD activity under normal light was same in Zn-deficient plants of both the lines but was enhanced to 21% in CM 115 and 40% in CM 120 on exposure to high intensity of light (**Fig. 2**).

In both the maize lines CM 115 and CM 120, normal as well as high intensity of light increased the activity of APX in Zn-sufficient as well as Zn-deficient plants. The increase in the activity of APX was most pronounced in Zn-deficient plants of CM 120 exposed to high intensity of light (**Fig. 2**). PAGE separation and activity staining of APX showed two isoforms APX 1 and APX 2, which were more prominent in Zn-deficient plants of line CM 120 than in CM 115, under both normal and high light intensity (**Fig. 3C, 3D**).

Zinc deficiency led to enhancement in GR activity in maize line CM 115 but decrease in the activity of the enzyme in maize line CM 120 (**Fig. 2**). Six isoforms of GR were visible on activity staining. Zinc deficiency enhanced the expression of almost all the isoforms in maize line CM 115 and inhibited their expression in maize line CM 120 (**Fig 3E**). High light intensity enhanced the effect of Zn deficiency on the expression of the GR isoforms in both maize lines (**Fig. 3F**).

DISCUSSION

The maize lines CM 120 and CM 115 differed in their response to deficient Zn supply. The decrease in dry matter yield due to Zn deficiency was less in CM 115 as compared to CM 120. The appearance and symptoms like necrotic bronze patches on leaves were earlier and severe in maize line CM 120 but appeared slightly and quite late in CM 115. These results indicate that CM 120 was highly sensitive to Zn deficiency and that CM 115 possessed more tolerance to Zn deficiency and was moderately Zn efficient. The differential sensitivity of maize lines to Zn deficiency and the severity of Zn deficiency symptoms did not relate to the concentration of Zn in the leaves indicating that the status of Zn in plants cannot be used as a parameter in screening Zn efficiency of genotypes. A higher concentration of leaf tissue Zn in Zn-deficient plants of maize line CM 120 as compared to that in CM 115 also suggested that the severity of Zn deficiency symptoms in the two maize lines was not concomitant to decrease in the Zn concentration due to limitation in Zn supply and that the difference in susceptibility of the two maize lines was not a function of the tissue concentration of Zn. Similar findings have been reported earlier (Cakmak *et al*. 1997; Erenoglu *et al.* 1999; Hacisalihoglu *et al*. 2001) wherein the workers did not find any correlation between total leaf Zn concentration and Zn efficiency.

Genotypic differences in physiological mechanisms have been attributed to differential capacity of plants to take up and utilize Zn efficiently. An efficient utilization of Zn at cellular levels and the status of the physiological active Zn in leaves have been shown to be a better indicator for determining expression of Zn efficiency in plants. In the present case also the severity of deficiency symptoms in CM 120 in spite of higher Zn concentration in leaves than in CM 115 seemed to be due to the differences in the status of the physiologically active Zn. Thus although Zn deficiency decreased the activity of CA and Cu/Zn SOD in both the maize line the decrease was less in the moderately Zn-efficient CM 115 even under high light intensity. Such a positive correlation between the expression and activity of the Zn-requiring enzymes, CA and Cu/Zn SOD and Zn efficiency within cereals have been reported previously (Rengel 1995; Cakmak *et al*. 1997; Hacisalihoglu *et al.* 2003). But it is for the first time that protection from high light intensity by Zn efficiency is being reported.

Fischer *et al*. (1997) reported that Zn-efficient pea cultivar showed higher photosynthesis than the Zn-inefficient cultivar and the higher photosynthesis in the former was related to higher activity of CA which facilitates CO_2 transfer for photosynthetic fixation. Impaired photosynthetic CO_2 fixation due to lower CA activity along with reduced Cu/Zn SOD in Zn-deficient leaves would accentuate the generation of ROS which cause photo-oxidative damage to the chloroplast, In the present study degradation of chlorophyll and carotenoids was observed under Zn deficiency and high light intensity in the two maize lines. The photo-oxidative damage of the chloroplastic pigments was less pronounced in Zn-efficient maize line CM 115 than in maize line CM 120 and this was also visibly apparent by the severe necrosis and bronzing of the leaves in the later. Earlier, Obata *et al*. (1997) had reported that shading of leaves resulted in alleviation of the Zn deficiency symptoms. In the present study we observed that leaves of Zn-deficient plants showed a decrease in Chl *a*/*b* ratio especially when subjected to high light intensity. This preferential degradation of Chl *a* over Chl *b* was probably because the former is in close association to the reaction centers of PSI and PSII and therefore more susceptible to damage by $^1O_2$ which are formed when the transfer of electrons along the photosynthetic electron chain are not at par with excitation of the pigment molecule in the pigment bed (Demmig-Adams and Adams 1996; Li *et al.* 2009). This occurs when photon flux density is higher than required to saturate the photosynthetic reaction, as in the present case. A decrease in the Car in the Zn-deficient plants more so under high light intensity, also implicates the inability of these plants to protect the photosynthetic apparatus from photo-oxidative damage by dissipation of excess light energy by the carotenoids (violixanthin, anteroxanthin and zeaxanthin) via the xanthophylls cycle within the chlorophyll pigment bed. The development of thermal energy dissipation via the xanthophyll cycle is

further facilitated by ASA which is a co-substrate for formation of zeaxanthin (Baroli and Niyogi 2000). The observed increase in the ASA concentration in leaves of Zn-deficient maize line CM 115 as against the maize line CM 120 in which a decrease was observed probably provides the former an effective protection from oxidative damage. The capacity of ASA to eliminate different ROS directly through APX or indirectly has been widely reported (Mittler and Zilinskas 1993; Smirnoff 1995). In stressful conditions ASA oxidation is enhanced (Hernández *et al*. 2004) which results in increase in DHA and an enhanced DHA/ASA ratio which was also observed by us in the present case.

Oxidative damage was more severe in CM 120, not only in terms of the loss of chloroplastic pigments but also due to enhanced H_2O_2 concentration and lipid peroxidation. An increase in the level of H_2O_2 under high light intensity has been observed in detached pea leaves (Hernández *et al*. 2004). Loss of membrane integrity as a consequence of ROS is among the primary effects of Zn deficiency (Cakmak and Marschner 1988) and an increase in the concentration of lipid peroxidation products like malondialdehyde has been reported earlier (Obata *et al*. 2001; Pandey *et al*. 2002b; Pathak *et al*. 2005, 2009). In view of the reported increased accumulation of Fe in Zn-deficient plants, the increased lipid peroxidation under Zn deficiency may be attributed to Fe-catalyzed generation of $OH^{\cdot}$ radical in presence of increased H_2O_2 (Cakmak 2000; Asada 2006).

SOD is the key enzyme involved in providing protection against oxidative damage from $O_2^{\cdot-}$ radicals. The SOD activity has been shown to be well correlated to the Zn concentration in plants (Pathak *et al*. 2009). There are three SOD in plants with metal cofactors MnSOD, Cu/Zn SOD and FeSOD. In maize three Cu/Zn SOD isoforms were observed and were found to be weakly expressed in Zn-deficient plants. The decrease in Cu/Zn SOD due to Zn deficiency was highly critical for the Zn-sensitive CM 120 more so under high light intensity. The Cu/Zn SOD has been shown to be highly inducible under conditions of oxidative stress such as low temperature and high light intensity (Lee and Lee 2000; Hernández *et al*. 2004, 2006). In the Zn-deficient plants we found that Cu/Zn SOD isoforms were up-regulated in both maize lines under high light intensity but the increase was more in CM 115. Thus under high light intensity the higher expression of Cu/Zn SOD isoforms in Zn-efficient CM 115 made it less vulnerable to oxidative damage as is evident from the lower H_2O_2 and higher MDA concentration in this line as compared to CM 120. Cakmak *et al*. (1996) had shown that Zn-inefficient cultivars of durum wheat were highly sensitive to light stress as compared to the Zn-efficient bread wheat and that the decrease in Cu/Zn SOD was more pronounced in the former than the later. Earlier, Sen Gupta *et al*. (1993) showed that tobacco plants overexpressing chloroplastic Cu/Zn SOD were found to be less sensitive to photoinhibition than the non-transgenic controls.

A decrease in CAT and an increase in the POD and APX in Zn-deficient plants of both lines was observed. The concurrent functioning of these enzymes which are involved in breakdown of H_2O_2 was reported earlier under stressful conditions (Slomka *et al*. 2008; Ozdener and Aydin 2010). The importance of enhanced APX in determining stress tolerance under chilling and high light intensity has been observed earlier (Sato *et al*. 2001; Hernández *et al*. 2006). Recent studies have focused on changes in activity and gene expression for APX isozymes which are highly responsive to environmental stresses, such as drought, ozone, high light and extreme temperatures (Mittler and Zilinskas 1994; Yoshimura *et al*. 2000; Shigeoka *et al*. 2002). In the present study we observed only two isoforms of APX both of which were overexpressed under Zn deficiency and high light intensity. However after exposure to high light APX was further enhanced in Zn-deficient CM 120 resulting in a disturbed redox status of the ASA. This was apparent by the decrease in the ASA and an appreciable increase in the ratio of redox couple DHA/ASA observed in leaves of Zn-deficient CM 120 plants. This disturbed cellular redox environment could also account for the higher oxidative damage in CM 120 than in the CM 115 plants.

One of the important antioxidant responses of plants is the regeneration of ASA by GR and MDHAR and DHAR (Hernández *et al*. 2006). The increase in the activity of GR in the Zn deficient CM 115 was enhanced by preferential induction of almost all isoforms especially under high light intensity and is also indicative of an enhanced glutathione–ascorbate cycle in these plants. Although we have not measured dehydroascorbate reductase (DHAR) and monodehydratase reductase (MDHAR) activities, the increase in GR in CM 115 plants suggests regeneration of ASA. In contrast CM 120 plants exhibit decrease in GR activity. Earlier GR has been suggested to be regulated in response to photoinhibition and various environmental stresses and to contribute to stress tolerance in GR-overexpressing plants (Foyer *et al*. 1995; Lee and Lee 2000). Thus the increase in GR activity and ASA in the leaves of Zn-deficient plants of CM 115 exposed to high light intensity appeared to be a protective adaptation against enhanced ROS contributing to better tolerance to oxidative damage under high light intensity.

CONCLUSIONS

In conclusion Zn deficiency alone and in combination with high light intensity disturbs the redox state of the cytosol in the Zn-inefficient maize line CM 120 as was evident from the increase in DHA/ ASA ratio. It appears that compared to CM 115 the lower activity of CA and Cu/Zn SOD in Zn-inefficient CM 120 made the later more vulnerable to oxidative stress especially under high light intensity. The increase in the GR activity and ASA concentration also helped to efficiently regulate the antioxidative defense system in the moderately efficient CM 115. The data also reiterates that tissue Zn is not a good indicator of Zn efficiency, and that higher activity of SOD and CA in Zn-efficient maize line contributes to efficient biochemical utilization of Zn under normal as well as high light intensity. Our results support the hypothesis that Zn efficiency is closely related to efficient physiological utilization of Zn which provides cross protection from photoinhibition and oxidative damage under Zn deficiency and high light intensity.

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