SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 3, pp. 503-509, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

AMELIORATION OF ZINC TOXICITY BY 28-HOMOBRASSINOLIDE IN *ZEA MAYS* L.

Nitika Arora, *Renu Bhardwaj, Priyanka Sharma, Hardesh Kumar Arora and Priya Arora
Department of Botanical and Environmental Sciences
Guru Nanak Dev University, Amritsar – 143005, Punjab, India.

ABSTRACT

The effects of 28-homobrassinolide (28-homoBL) on growth, lipid peroxidation and antioxidative enzyme activities in the *Zea mays* L. (var. Partap-1) seedlings exposed to zinc (Zn) metal were studied. The surface sterilized seeds of *Zea mays* were given treatments of different concentrations of Zn metal (0.5, 1.0, 1.5 and 2.0 mM) alone or in combination with 28-homoBL (10^{-4}, 10^{-6} and 10^{-8} mM) for seven days. The activities of antioxidative enzymes (superoxide dismutase (EC 1.15.1.1) catalase (EC 1.11.1.6), ascorbate peroxidase (EC 1.11.1.11), guaiacol peroxidase (EC 1.11.1.7) and glutathione reductase (EC 1.6.4.2)), protein and malondialdehyde (MDA) content were analyzed in seven days old seedlings. It was observed that 28-homoBL treatments stimulated the activities of antioxidative enzymes and the level of MDA content was decreased, thus indicating the stress-ameliorative effects of 28-homoBL.

Keywords: Antioxidative enzymes, 28-Homobrassinolide, lipid peroxidation, maize, Zinc.

INTRODUCTION

Zinc is one of the trace elements that is required for healthy growth and reproduction of plants (Welch, 1995). It plays an important role in several metabolic processes such as nitrogen metabolism, cell multiplication, photosynthesis, auxin synthesis and carbohydrate, nucleic acid and lipid metabolism in plants (Marshner, 1986; Pahlsson, 1989; Shier, 1994). It is also an important constituent of metalloenzymes and a cofactor for several enzymes such as anhydrases, dehydrogenases and peroxidases. However enhanced levels of Zn, cause alterations in physiological responses like photosynthesis and chlorophyll biosynthesis (Rout and Das, 2003, Atici *et al.,* 2005; Doncheva *et al.,* 2001) and membrane integrity (Devos *et al.,* 1991). An excess of Zn has been reported to induce chlorosis of young leaves (Shen *et al.,* 1997), inhibit root growth and Fe translocation (Pearson and Rengel, 1995), disintegration of cell organelles, disruption of membranes and condensation of chromatin material and increase in number of nucleoli of root tip cells (Sresty and Madhava, 1999). In addition to these effects, the toxic levels of Zn have a negative effect on mineral nutrition (Stoyanova and Doncheva, 2002). The toxic level of Zn stimulates the formation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), singlet oxygen (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) resulting in oxidative stress in plants (Dietz *et al.,* 1999, Hall and Williams, 2003). The scavenging system controlling ROS comprises both non-enzymatic antioxidants (e.g. glutathione, ascorbate, tocopherols, carotenoids and proline) and an enzymatic antioxidative system (e.g. Superoxide Dismutase (SOD), Guaiacol Peroxidase (POD), Ascorbate Peroxidase (APOX), Catalase (CAT) and Glutathione Reductase (GR) (Elstner, 1982).

Various plant metabolites including hormones are involved in protection against oxidative stress generated by ROS. Among plant hormones abscisic acid, salicylic acid, ethylene, auxins and brassinosteroids (BRs) have been reported to play a significant role in stress protection (Cao *et al.,* 2005). BRs, a new class of polyhydroxysteroidal phytohormones has been found to influence the wide spectrum of physiological processes in plants (Khripach *et al.,* 2000; Rao *et al.,* 2002; Bajguz and Tretyn 2003; Bhardwaj *et al.,* 2006, 2007). In addition to their role in plant development, BRs have been reported recently to ameliorate various abiotic/biotic stresses (Krishna, 2003; Kagle *et al.,* 2007). The exogenous application of BRs improved the antioxidant system in plants subjected to salt stress (Nunez *et al.,* 2003; Özdemir *et al.,* 2004) and heavy metal stress (Hayat *et al.,* 2007; Ali *et al.,* 2007; Alam *et al.,* 2007). Our earlier studies also indicated stress-protective properties of BRs (Kaur and Bhardwaj, 2003; Sharma and Bhardwaj, 2007; Bhardwaj *et al.,* 2008). In continuation to previous studies, the present study was aimed to study the stress-protective properties of 28-homobrassinolide (28-homoBL) in the seedlings of *Zea mays* L. subjected to Zn metal-stress by determining various parameters of seedlings like growth, activities of antioxidative enzymes and MDA content.

MATERIALS AND METHODS

Seeds of *Zea mays* L. (var. Partap-1) were procured from Punjab Agricultural University, Ludhiana (Punjab), India.

*Corresponding author email: renubhardwaj82@gmail.com

Healthy and uniform-sized seeds of maize were surface sterilized with 0.05% mercuric chloride for 5 min followed by repeated rinses in sterile distilled water. The surface sterilized seeds were germinated in *Whatman No.1* filter paper lined glass Petriplates (10 cm diameter, 10 seeds per Petriplate) containing different concentrations of Zn (0.5,1.0,1.5 and 2.0 mM) alone, 28-homoBL (10^{-4},10^{-6} and 10^{-8} mM) alone and Zn (0.5,1.0,1.5 and 2.0 mM) supplemented with 28-homoBL (10^{-4},10^{-6} and 10^{-8} mM). The Zn metal treatment was given in the form of $ZnSO_4.7H_2O$. Each Petriplate was supplied with 4ml of test solution on first day and 2 ml of test solution on alternate days, up to 7 days. Control seedlings were supplied with distilled water. Each treatment was replicated 5 times. The experiment was conducted under controlled conditions (24.5 ± 0.5°C, 16 h photoperiod). On 7th day, seedlings were harvested and shoot and roots of seedlings were separated. Seedling growth in terms of length and fresh weight was recorded. Forty seedlings per treatment were used for measurement of morphological parameters (root/shoot length and root/shoot fresh weight). Experiment was repeated twice.

Using leaf extracts biochemical analysis was carried out. For estimation of antioxidative enzyme activities and protein content, 1 g leaves of 7 day old seedlings were homogenized in 3 ml of ice-cold 100 mM potassium phosphate buffer (pH=7). The homogenate was

Table 1. Effect of 28-homoBL on morphological parameters (length and fresh weight of roots and shoots) of 7-days old *Zea mays* seedlings.

Treatments of 28-homoBL	Root length (cm)	Shoot length (cm)	Fresh weight Root (g)	Fresh weight Shoot (g)
0	12.5±0.19	4.26±0.145	0.23±0.004	0.154±0.004
10^{-4} mM	13.06±0.41	4.96±0.12	0.24±0.01	0.159±0.005
10^{-6} mM	14.88±0.42	6.0±0.28	0.286±0.007	0.189±0.007
10^{-8} mM	13.64±0.087	4.92±0.03	0.259±0.012	0.156±0.002
F-ratio	10.29 (1.43)*	17.04 (0.78)*	7.04 (0.039)*	10.73 (0.022)*

*Indicate significant at $P \leq 0.05$ (Inside bracket is HSD value)

Table 2. Effect of 28-homoBL on morphological parameters (length and fresh weight of roots and shoots) of 7-days old Zn-stressed *Zea mays* seedlings.

Treatments	Root length (cm)	Shoot length (cm)	Fresh weight Root (g)	Fresh weight Shoot (g)
Zn (0.5 mM)	12.65±0.65	4.2±0.25	0.218±0.04	0.171±.009
Zn(0.5mM)+28-homoBL(10^{-4} mM)	12.66±0.75	4.29±0.17	0.226±0.003	0.187±.007
Zn(0.5mM)+28-homoBL(10^{-6} mM)	14.53±0.49	4.96±0.22	0.253±0.013	0.195±0.003
Zn(0.5mM)+28-homoBL(10^{-8} mM)	12.73±0.32	4.65±0.12	0.284±0.013	0.203±0.008
F-ratio	2.55 (2.62)	3.01(0.90)	7.39(0.049)*	3.25(0.03)
Zn (1.0 mM)	12.31±0.55	3.96±0.12	0.199±0.011	0.143±0.006
Zn(1.0mM)+28-homoBL(10^{-4} mM)	13.33±0.62	4.87±0.24	0.251±0.011	0.186±0.004
Zn(1.0mM)+28-homoBL(10^{-6} mM)	13.87±0.40	4.82±0.27	0.238±0.02	0.151±0.008
Zn(1.0mM)+28-homoBL(10^{-8} mM)	14.97±0.20	5.21±0.15	0.269±0.009	0.186±0.003
F-ratio	5.39 (2.15)*	6.49(0.94)*	4.92(0.06)*	17.87(0.024)*
Zn(1.5mM)	10.63±0.18	3.76±0.14	0.174±0.008	0.138±.007
Zn(1.5mM)+28-homoBL(10^{-4} mM)	11.33±0.85	4.45±0.11	0.207±0.01	0.172±0.005
Zn(1.5mM)+28-homoBL(10^{-6} mM)	13.72±0.47	4.48±0.26	0.192±0.009	0.154±.004
Zn(1.5mM)+28-homoBL(10^{-8} mM)	12.4±0.50	4.78±0.14	0.251±0.013	0.194±0.007
F-ratio	5.84(2.52)*	6.19(0.78)*	10.8(0.045)*	30.23(0.023)*
Zn(2.0mM)	9.90±0.16	2.93±0.14	0.159±.006	0.13±0.009
Zn(2.0mM)+28-homoBL(10^{-4} mM)	13.3±0.49	4.54±0.15	0.167±0.01	0.195±0.008
Zn(2.0mM)+28-homoBL(10^{-6} mM)	11.21±0.69	4.25±0.16	0.191±0.018	0.133±0.008
Zn(2.0mM)+28-homoBL(10^{-8} mM)	12.24±0.17	4.17±0.18	0.203±.008	0.171±0.009
F-ratio	10.77(2.00)*	19.15(0.73)*	3.30(0.051)	3.58(0.038)*

*Indicate significant at $P \leq 0.05$ (Inside bracket is HSD value)

Table 3. Effect of 28-homoBL on protein content, malondialdehyde(MDA) content and specific activities of antioxidative enzymes (superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) of 7-days old *Zea mays* seedlings. Mean±SE.

Treatments of 28-homoBL	Protein content (mg g^{-1} FW)	MDA content (μ mol g^{-1} FW)	SOD (mol U mg^{-1} protein)	POD (m mol U mg^{-1} protein)	CAT (mol U mg^{-1} protein)	APOX (m mol U mg^{-1} protein)	GR (m mol U mg^{-1} protein)
0	16.33±0.50	3.78±0.07	1.68±0.014	4.03±0.095	1.04±.007	1.28±0.05	2.85±0.11
10^{-4} mM	28.74±1.15	2.99±0.09	2.03±0.059	4.14±0.15	1.34±0.05	1.41±0.042	1.029±0.006
10^{-6} mM	28.18±1.09	2.79±0.11	1.83±0.017	4.46±0.10	2.10±0.11	1.64±0.032	1.082±0.005
10^{-8} mM	24.92±0.56	3.18±0.01	2.48±0.032	6.59±0.18	4.25±0.20	2.84±0.062	1.158±0.011
F-ratio	42.38(3.98)*	25.91(0.37)*	96.13(0.15)*	35.84(0.96)*	149.9(0.53)*	212.4(0.22)*	237.6(0.25)*

*Indicate significant at $P \leq 0.05$ (Inside bracket is HSD value)

centrifuged at 4°C for 20 min at 15,000 *g*. The supernatant was used for the estimation of the activities of SOD, POD, CAT, GR and APOX. The activity of SOD was determined by monitoring its ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT) at 540 nm (Kono, 1978). POD activity was determined according to Putter (1974). CAT activity was determined by following the initial rate of disappearance of H_2O_2 at 240 nm (Aebi, 1983). The activities of APOX and GR were measured by the methods of Nakano and Asada (1981) and Carlberg and Mannervik (1975) respectively. Protein content was determined following the method of Lowry *et al.* (1951) using the bovine serum albumin as a standard. The level of lipid peroxidation was determined as the content of malondialdehyde (MDA) using leaf homogenates prepared in 0.1% trichloroacetic acid (TCA) and thiobarbituric acid (TBA) as described by Heath and Packer (1968). Data was analyzed statistically by using one-way analysis of variance (ANOVA) and comparisons with *P*-values ≤0.05 were considered significantly different. Data was presented as mean ± SE.

RESULTS

The observations made on seedling growth indicated that the treatments of 28-homoBL significantly effected seedlings growth. Application of 28-homoBL alone increased length and fresh weight of root/shoot of seedlings when compared with seedlings treated with distilled water alone. 10^{-6} mM concentration of 28-homoBL alone was found to be most effective in increasing all morphological parameters of seedlings. It was noted that growth of seedlings decreased with the increase in concentration of Zn and maximum decline in growth was observed in 2.0 mM of Zn treated seedlings. However, supplementation of Zn solution with 28-homoBL considerably reduced the inhibitory effect of Zn to seedling growth thereby showing better seedling growth. Maximum increase in root/shoot length under the influence of brassinosteroids treatments was observed in seedlings treated with 10^{-8} mM 28-homoBL supplemented with 1.0 mM of Zn concentration as compared to seedlings treated with Zn alone. In seedlings given 0.5 mM of Zn metal solution supplemented with 10^{-8} mM of 28-homoBL, maximum fresh weight of root/shoot was observed (Table 1 and 2).

The studies done on biochemical constituents also revealed significant effects of BRs treatments. The seedlings treated with 28-homoBL alone showed, an increase in soluble protein content and decrease in MDA content in comparison to untreated seedlings. The treatment of 10^{-4} mM of 28-HomoBL resulted in maximum protein content (28.74 mg g^{-1} FW) of seedlings while 10^{-6} mM of 28-homoBL treatment caused maximum decrease in MDA content (2.79 μ mol g^{-1} FW) in seedlings. The protein content of seedlings under heavy metal stress was found to increase significantly in all treatments of 28-homoBL. Maximum protein content (27.94 mg g^{-1} FW) was observed in case of seedlings treated with 10^{-6} mM of 28-homoBL supplemented with 2.0 mM of Zn as compared to 2.0 mM Zn treated seedlings (24.65 mg g^{-1} FW). The concentration of MDA got increased by Zn treatments but decreased with 28-homoBL supplementations. Minimum content of MDA (2.09 μ mol g^{-1} FW) was observed in 10^{-8} mM 28-homoBL supplemented with 0.5 mM of Zn solution as compared to 0.5 mM Zn treated seedlings (2.432 μ mol g^{-1} FW) (Table 3 and 4).

The activities of antioxidative enzymes (SOD, POD, CAT and APOX) were also enhanced in seedlings treated with 28-homoBL alone, 10^{-8} mM concentration being the most effective. The activities of antioxidative enzymes were also enhanced in seedlings by the application 28-homoBL supplemented Zn solutions. The activity of SOD was alleviated by 10^{-6} mM of 28-homoBL supplemented with 2 mM of Zn (6.11 mol U mg^{-1} protein) when compared to 2.0 mM of Zn concentration (5.23 mol U mg^{-1} protein). The activity of POD in metal treated seedlings was observed highest in 2.0 mM Zn concentration (2.92 m mol U mg^{-1} protein). 28-HomoBL treatments to salt stressed seedlings further enhanced the POD activity that was maximum (4.06 m mol U mg^{-1} protein) in seedlings

Table 4. Effect of 28-homoBL on protein content, malondialdehyde(MDA) content and specific activities of antioxidative enzymes (superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) of of 7-days old Zn-stressed *Zea mays* seedlings. Mean±SE.

Treatments	Protein content	MDA Content	SOD	POD	CAT	APOX	GR
Zn(0.5mM)	19.69±0.39	2.43±0.08	3.83±0.057	2.10±0.043	1.09±0.021	1.67±0.056	0.99±0.026
Zn(0.5mM)+28-homoBL(10^{-4} mM)	24.13±0.76	2.10±0.058	3.94±0.066	3.00±0.039	1.63±0.065	2.69±0.092	1.09±0.047
Zn(0.5mM)+28-homoBL(10^{-6} mM)	21.63±0.83	2.11±0.103	4.00±0.092	4.06±0.048	2.65±0.057	3.02±0.091	1.30±0.014
Zn(0.5mM)+28-homoBL(10^{-8} mM)	24.22±0.90	2.09±0.092	4.03±0.095	3.22±0.193	2.99±0.181	3.29±0.2	1.07±0.015
F-ratio	8.45(3.39)*	3.63(0.39)	1.16(0.35)	60.86(0.46)*	76.47(0.45)*	33.55(0.55)*	21.09(0.13)*
Zn(1.0mM)	20.48±0.36	2.78±0.071	4.22±0.11	2.45±0.07	1.26±0.01	1.91±0.026	1.01±0.002
Zn(1.0mM)+28-homoBL(10^{-4} mM)	23.73±0.76	2.33±0.053	4.29±0.079	2.85±0.11	1.86±0.059	2.70±0.051	1.38±0.064
Zn(1.0mM)+28-homoBL(10^{-6} mM)	20.4±1.11	2.19±0.034	4.43±0.103	3.34±0.064	1.79±0.152	3.46±0.128	1.31±0.002
Zn(1.0mM)+28-homoBL(10^{-8} mM)	24.76±0.54	2.55±0.07	4.3±0.159	2.80±0.093	3.98±0.24	4.60±0.344	2.17±0.008
F-ratio	7.52(3.58)*	19.15(0.26)*	0.596(0.527)	17.39(0.39)*	8.27(1.89)*	38.11(0.84)*	235.9(0.14)*
Zn(1.5mM)	22.42±0.32	3.23±0.096	4.19±0.208	2.61±0.076	1.35±0.035	2.30±0.52	1.04±0.019
Zn(1.5mM)+28-homoBL(10^{-4} mM)	24.61±0.717	2.80±0.118	4.99±0.15	3.37±0.085	2.089±0.074	3.55±0.186	1.19±0.012
Zn(1.5mM)+28-homoBL(10^{-6} mM)	23.9±0.51	2.20±0.059	5.03±0.12	3.58±0.082	2.082±0.131	3.89±0.175	1.20±0.006
Zn(1.5mM)+28-homoBL(10^{-8} mM)	26.91±0.91	3.23±0.106	4.56±0.21	3.101±0.017	4.30±0.37	3.24±0.203	1.05±0.027
F-ratio	8.09(2.98)*	4.95(0.44)*	4.97(0.81)*	35.23(0.32)*	40.20(0.91)*	17.16(0.74)*	25.3(0.08)*
Zn(2.0mM)	24.65±0.63	3.49±0.112	5.23±0.14	2.92±0.055	1.57±0.026	2.58±0.05	1.29±0.006
Zn(2.0mM)+28-homoBL(10^{-4} mM)	25.07±0.64	3.06±0.129	5.88±0.11	3.86±0.103	2.29±0.061	2.20±0.16	1.39±0.032
Zn(2.0mM)+28-homoBL(10^{-6} mM)	27.94±0.08	3.30±0.082	6.11±0.15	2.468±0.044	2.55±0.15	4.05±0.104	1.24±0.058
Zn(2.0mM)+28-homoBL(10^{-8} mM)	27.69±0.82	3.24±0.11	5.93±0.21	3.02±0.095	3.21±0.118	1.84±0.126	1.66±0.048
F-ratio	7.83(2.77)*	2.55(0.50)	5.54(0.73)*	57.40(0.34)*	44.06(0.46)*	6.67(0.53)*	21.12(0.18)*

*Indicate significant at $P \leq 0.05$ (Inside bracket is HSD value)

treated with 10^{-6} mM 28-homoBL supplemented with 0.5 mM of Zn concentration. The CAT activity increased with the Zn treatments to maize seedlings and maximum increase was observed in case of 2.0 mM Zn treated seedlings (1.57 mol U mg^{-1} protein). CAT activity showed maximum enhancement (4.30mol U mg^{-1} protein) in case of seedlings treated with 10^{-8} mM 28-homoBL supplemented with 1.5mM of Zn treatment. The activity of APOX and GR was found to increase with increase in concentrations of Zn. However, the treatments of 28-homoBL to Zn stressed seedlings further enhanced the APOX and GR activities. Maximum enhancement in the activity of APOX (4.60 m mol U mg^{-1} protein) and GR (2.17 m mol U mg^{-1} protein) was observed in case of seedlings, treated with 10^{-8} mM 28-homoBL

supplemented with 1.0 mM of Zn concentration (Table 3 and 4).

DISCUSSION

It was observed that application of 28-homoBL improved the growth of Zn-stressed maize seedlings (Table 1 and 2). Earlier studies also indicated stress-protective properties of BRs. Brassinolide (BL) ameliorates the Al toxicity and promoted the growth of mungbean seedlings (Bilkisu *et al.* 2003). Ali *et al.* (2007) also observed that 24-epiBL and 28-homoBL improved the growth of Al-stressed mung bean seedlings by increasing the rate of photosynthesis and activity of carbonic anhydrase. The studies on BRs report that they regulate cell elongation and divisional activities by activating the cell wall loosening enzymes, which increase the synthesis of cell wall and membrane materials (Khripach *et al.,* 2000). The cell wall loosening enzymes get activated by H^+- ATPases which acidifies the apoplast. The involvement of BRs in enhancing the growth of seedlings might be taking place through activation of H^+-ATPase (Cerana *et al.,* 1983; Haubrick and Assmann, 2006).

The 28-homoBL further improved the seedling growth by increasing the protein content and by decreasing the level of lipid peroxidation (Table 3 and 4). BRs are found to affect the transcription and translation processes of specific genes related to stress tolerance (Bajguz, 2000; Dhaubhadel *et al.,* 2002; Kagale *et al.,* 2007). Kagale *et al.* (2007) reported that 24-epiBL treated *Arabidopsis thaliana* seedlings tolerate the drought stress by accumulating *rd29A*, *ERD10* and *rd22* mRNAs at higher level. The *rd29A* and *ERD10* genes encode a class of proteins that have molecular chaperone like functions, preventing protein aggregation during drought stress (Goyal *et al.,* 2005). The increase in the transcript levels of these genes suggests that 24-epiBL treated *Arabidopsis thaliana* seedlings tolerate the drought stress better than untreated seedlings. Further Sam *et al.* (2001) observed in tomato leaf discs that BB6 (brassinosteroids analogue with spirostanic structure as active ingredient) increased the rate of production of heat shock proteins, which protected mRNA from stress-induced degradation. Our earlier studies also reported that BRs confer tolerance against heavy metals (Ni, Cu, Mn) either by reducing their uptake or by activating the antioxidative enzymes in case of *B. juncea*, *B. campestris* and *Zea mays*. (Kaur and Bhardwaj, 2003; Sharma and Bhardwaj, 2007; Bhardwaj *et al.,* 2007; Sharma *et al.,* 2007; Bhardwaj *et al.,* 2008). As membrane destruction results from ROS induced oxidative damage (Mittler, 2002), the 28-homoBL treated seedlings might be scavenging ROS more effectively than the seedlings treated with metal alone. The observations are in consistence with the results of Özdemir *et al.* (2004), who reported that lipid peroxidation level induced by NaCl was significantly lowered in rice seedlings when treated with 24-epiBL.

The present investigation also reveals that treatment of 28-homoBL significantly enhanced the activities of all antioxidative enzymes (e.g. superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase) as compared to untreated seedlings (treated with Zn alone) (Table 3 and 4). The increase in activities of these antioxidative enzymes is a general response to ROS produced by various biotic and abiotic stresses including heavy metal stress (Arora *et al.,* 2002; Mithöfer *et al.,* 2004). Among ROS, superoxide radical ($O_2^{\cdot -}$) is dismutated by SOD into H_2O_2 and is further removed by CAT in the peroxisomes or by APOX of the ascorbate-glutathione antioxidant cycle in the chloroplast or by membrane bounded POD (Foyer *et al.,* 1997). GR activity maintains the pool of glutathione in the reduced state, which in turn reduces dehydroscorbate to ascorbate through the ascorbate-glutathione cycle (Noctor and Foyer, 1998). It is widely accepted that ROS are responsible for various stress-induced damages to macromolecules and ultimately to cellular structures (Halliwell and Gutteridge, 1999). Consequently, the role of antioxidative enzymes, such as SOD, CAT, POD, GR and APOX becomes very important.

In the present study it was observed that application of 28-homoBL to stressed seedlings further strengthen the antioxidative defence system of the plant by stimulating the activities of enzymes (SOD, POD, CAT, APOX and GR). Previous reports also showed that exogenous application of BRs modified antioxidant enzyme activity. Nunez *et al.* (2003) observed higher activity of antioxidative enzymes in rice, grown under salinity and supplemented with polyhydroxylated spirostanic brassinosteroid analogue (BB-16). 28-HomoBL also ameliorated the cadmium toxicity in *Brassica juncea* and *Cicer arietinum* plants by increasing the activities of peroxidase, catalase and superoxide dismutase (Hayat *et al.,* 2007; Hasan *et al.,* 2007). The present study therefore reveals the stress ameliorative properties of BRs in the maize seedlings exposed to heavy metal (Zn) stress by decreasing the extent of lipid peroxidation and by further stimulating the activities of key antioxidative enzymes.

ACKNOWLEDGEMENT

Financial assistance from University Grants Commission, New Delhi, India is duly acknowledged.

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