The effect of brassinosteroids on radish (*Raphanus sativus* L.) seedlings growing under cadmium stress

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ABSTRACT

The effect of 24-epibrassinolide and 28-homobrassinolide on seed germination and seedling growth of radish (*Raphanus sativus* L.) was studied under cadmium toxicity. The impact of brassinosteroids (BRs) on free proline levels and the activity of antioxidant enzymes catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1), ascorbic peroxidase (APOX; EC 1.11.1.11) and guaiacol peroxidase (GPX; EC 1.11.1.7) in radish seedlings under Cd toxicity was evaluated. The effect of BRs on the activity of ascorbic acid oxidase (AAO; EC 1.10.3.3) and lipid peroxidation in radish seedlings challenged with Cd stress was also investigated. BRs supplementation alleviated the toxic effect of the heavy metal and increased the percentage of seed germination and seedling growth. Out of the two substances, HBL was found to be more effective than EBL in stress alleviation. HBL (3μM) alleviated the toxic effect of the heavy metal and increased the percentage of seed germination by 57% over Cd and 20% over unstressed control. Similarly supplementation of HBL (3μM) caused an increase of 156%, 78% and 91% in length, fresh weight and dry weight of seedling, respectively, over Cd treatment alone. The amelioration of seedling growth by BRs under metal toxicity was associated with enhanced levels of free proline. The activities of antioxidant enzymes CAT, SOD, APOX and GPX were increased in the seedlings from treatments with Cd along with BRs. Brassinosteroid treatment reduced the activity of POD and AAO in heavy metal stressed seedlings. Lipid peroxidation induced by Cd was found reduced with the supplementation of BRs. The results obtained in the study clearly indicated the ameliorative influence of brassinosteroids on the inhibitory effect of Cd toxicity.

Keywords: brassinosteroids; radish; cadmium; seedling growth; catalase; peroxidase; proline; superoxide dismutase

Cadmium (Cd) is a heavy metal and extremely toxic to plants. Major anthropogenic sources of cadmium are Cd-containing phosphate fertilizers, sewage sludge and industrial emissions (Adriano 1986). Cd toxicity in plants causes leaf rolls; chlorosis and reduced growth of both root and stem (Choudhury and Panda 2004). Cd alters the levels of enzyme activities disturbing the normal physiological process in plants (Mattioni et al. 1976). Cd induces oxidative stress in plants by generating reactive oxygen species (ROS) like superoxide (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), alkoxy radical (RO); the latter causes oxidative damage in cellular macromolecules of plants. There are few studies on the potential of growth regulators in mitigating the harmful effects of abiotic stress in plants (Davies and Cury 1991). Brassinosteroids (BRs) are steroidal sixth group of phytohormones with significant growth promoting effects and are essential for many processes in plant growth and development (Rao et al. 2002, Sasse 2003). Besides growth stimulation they have an ability to confer resistance to plants against various abiotic stresses (Priti 2003). Epibrassinolide was found to increase drought tolerance in wheat (Nilovskaya et al. 2001). BRs developed resistance to low temperature in maize (He et al. 1991) and rice (Kamuro and Takaysuto 1991), and increased tolerance to high temperature in wheat (Kulaeva et al. 1991). The increase in thermotolerance in *Brassica napus* seedling by epibrassinolide was associated with accumulation of heat shock proteins (Dhaubhadel et al. 1999). The ability of BRs to reduce the inhibitory effect of water stress on sorghum seedling growth was also reported (Vardhini and Rao 2003).

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They also reduced the inhibitory effect of salt stress in rice plants, and improved the pigment levels and nitrate reductase activity (Anuradha and Rao 2003). Though there are valuable information on stress alleviation by brassinosteroids, the importance of this new group of substances in the metal stress did not draw much attention.

Radish is considered to be a model crop and is widely used for studies related to heavy metal pollution (Khan and Frankland 1983, Kostka-Rick and Manning 1993). The advantage of using radish and other members of cabbage (*Brassicaceae*) family for heavy metal studies are well described by Mathe-Gaspar and Anton (2002).

The present study examines the influence of BRs on seed germination and seedling growth under Cd toxicity in *Raphanus sativus* seedlings. The study also aims at determining the levels of free proline, activity of antioxidant enzymes and lipid peroxidation in radish seedlings in response to Cd stress.

MATERIAL AND METHODS

Chemicals and plant material. 24-epibrassinolide and 28-homobrassinolide were procured from the CID Technologies Inc., Brampton, Ontario, Canada. Seeds of radish (*Raphanus sativus* L. cv. Pusa Rashmi) were obtained from the National Seeds Corporation, Hyderabad, India.

Seed germination. Seeds were surface disinfested with dilute sodium hypochlorite solution (with 0.02% available chlorine content) and washed thoroughly with several changes of sterile distilled water. They were soaked for 24 h in: (i) distilled water (control), (ii) 1.0mM Cd^{2+} ($CdCl_2$), and (iii) 1.0mM Cd^{2+} supplemented with 1.0, 2.0 and 3.0µM brassinosteroids (BRs). For each treatment 20 seeds were placed in 90 mm sterile Petri dishes over Whatman No. 1 filter paper. For every treatment 5 replicates (Petri dishes), each with 20 seeds was maintained. The seeds were allowed to germinate in dark at 20 ± 1°C, and the number of germinated seeds was recorded. After 48 h, five seedlings were retained in each plate, and 5 ml of treatment solution was added to respective Petri dishes. On the 5th day 5 seedlings were selected randomly one each from each Petri dish and the length (hypocotyl), fresh weight and dry weight were recorded. On the 6th day, proline estimation was done and various antioxidant enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APOX), guaiacol peroxidase (GPX) and ascorbic acid oxidase (AAO) together with lipid peroxidation were assayed.

Free proline. Proline was extracted with 3% sulphosalicylic acid, and determined according to Bates et al. (1973). It was determined in the supernatant by measuring the absorbance of the proline ninhydrin product formed at 520 nm on LKB Biochrom Ultrospec 4050, spectrophotometer (LKB Biochrom, Cambridge, England) using toluene as a solvent.

Extraction and assay of antioxidant enzymes. The seedlings were ground in sodium phosphate buffer at pH 7.0 for CAT, POD, APOX, GPX and at pH 7.8 for SOD. The supernatant was used to measure the activity of the enzymes, and the protein content in the supernatant was determined according to Lowry et al. (1951).

Catalase (EC 1.11.1.6). Catalase activity was assayed by the method of Barber (1980). Enzyme extract (0.5 ml) was added to 2.0 ml of hydrogen peroxide and 3.5 ml of phosphate buffer (pH 7.0). The reaction was stopped by adding 10.0 ml of 2% (v/v) concentrated sulphuric acid, and the residual hydrogen peroxide was titrated against 0.01M $KMnO_4$ until a faint purple colour persisted for at least 15 sec. The activity of the enzyme was expressed as enzyme units.

Peroxidase (EC 1.11.1.7). Peroxidase activity was assayed adopting the method of Kar and Mishra (1976). To 0.5 ml of enzyme extract, 2.5 ml of 0.1M phosphate buffer (pH 7.0), 1.0 ml of 0.01M pyrogallol and 1.0 ml of 0.005M H_2O_2 were added. After incubation, the reaction was stopped by adding 1.0 ml of 2.5N H_2SO_4. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm. The enzyme activity was expressed in absorbance units.

Ascorbate peroxidase (EC 1.11.1.11). The reaction mixture contained 50mM phosphate buffer (pH 7.0), 0.2mM EDTA, 0.5mM ascorbic acid, 250mM H_2O_2 and 50 µg of protein. The activity of APOX was measured spectrophotometrically by measuring the rate of ascorbate oxidation at 290 nm for 1 min. The amount of ascorbate was calculated from the extinction coefficient of 2.6mM^{-1} cm^{-1} by the method of Nakano and Asada (1981).

Guaiacol peroxidase (EC 1.11.1.7). GPX was measured by the method of Mazhoudi et al. (1997). The reaction mixture contained 50mM phosphate buffer, 0.2mM guaiacol, 10mM H_2O_2 and distilled water in the total volume of 3.0 ml. The reaction was started by adding 50 µg of protein. The change in absorbance of one unit per min at

470 nm (extinction coefficient of 26.6mM^{-1} cm^{-1}) gave the activity of GPX, and it was expressed in enzyme units.

Superoxide dismutase (EC 1.15.1.1). SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium adopting the method of Beauchamp and Fridovich (1971). The 3.0 ml reaction mixture contained 40mM phosphate buffer (pH 7.8), 13mM methionine, 75mM NBT, 0.1mM EDTA, 0.1 ml of enzyme extract and 2μM riboflavin, which was added at the end. After mixing the contents, test tubes were shaken and placed 30 cm below light source consisting of two 20 W fluorescent lamps for 15 min. A tube with protein kept in the dark served as a blank, while the control tube was without the enzyme and kept in the light. The absorbance was measured at 540 nm. The activity of SOD is the measure of NBT reduction in light without protein minus NBT reduction in light with protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

Ascorbic acid oxidase (EC 1.10.3.3). Ascorbic acid oxidase was extracted and assayed following the method of Povoskya and Sedenka (1956) as modified by Gopalachari (1963). Seedlings (200 mg) were homogenised with cold phosphate citrate buffer (pH 5.0) and filtered through glass wool. The filtrate was taken as the enzyme extract for the assay of the ascorbic acid oxidase. To 4.0 ml of the enzyme extract, 2.5 ml of phosphate citrate buffer (pH 5.0) and 0.2% ascorbic acid were added, and they were kept for 30 min with occasional shaking; 1.0 ml of 10% (v/v) trichloroacetic acid was added to stop the enzyme activity after 30 min. 1.0 ml of the solution diluted with 5.0 ml of distilled water was titrated against 0.001N 2,6-dichlorophenol indophenol till a permanent pink colour was obtained. The ascorbic acid oxidase activity was calculated using the formula:

$$A = V/W \times 0.088 \times 10 \times 25/4 = 5.5\ V/W$$

where: A = ascorbic acid oxidase activity; V = difference in titre values between the control and treatment in 1.0 ml; W = fresh weight (g) of plant material

Lipid peroxidation. Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). Seedlings (1.0 g) were homogenized with 3 ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA, v/v). The homogenate was incubated at 95°C for 30 min and the reaction was stopped in ice. The samples were centrifuged at 10 000 × g for 5 min, the absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of malondialdehyde was calculated by using the extinction coefficient of 155mM^{-1} cm^{-1}.

Statistical analyses. The data represent the mean values of 5 replicates. The data were analyzed by one-way ANOVA, followed by Post Hoc Test (Multiple Comparisons). The differences were considered significant if P was at least ≤ 0.05. The mean values were compared and lower case letters are used in the table to highlight the significant differences between the treatments.

RESULTS AND DISCUSSION

The results showed that Cd toxicity inhibited the seed germination in radish (Table 1). Brassinosteroids (BRs) reduced the toxic effect of Cd on seed germination. The percentage of seed germination increased with an increase in brassinosteroid concentration. In heavy metal treatments supplemented with BRs, the percentage of seed germination approached that of unstressed control treatments, indicating the stress alleviation capability of BRs. It is well known that heavy

Table 1. Effect of brassinosteroids on the percentage of seed germination of radish under cadmium stresses

Treatment	12 h	24 h
Control	40.8 ± 4.05^{b}	72.0 ± 2.76^{a}
Cd 1.0mM	21.6 ± 2.61^{d}	43.2 ± 2.90^{e}
Cd 1.0mM + 1.0μM EBL	25.6 ± 3.85^{d}	59.2 ± 3.23^{c}
Cd 1.0mM + 2.0μM EBL	34.0 ± 4.65^{c}	65.2 ± 2.38^{b}
Cd 1.0mM + 3.0μM EBL	48.8 ± 6.95^{a}	66.4 ± 3.85^{b}
Cd 1.0mM + 1.0μM HBL	29.6 ± 3.58^{cd}	49.2 ± 2.86^{d}
Cd 1.0mM + 2.0μM HBL	32.6 ± 3.86^{c}	57.6 ± 2.71^{c}
Cd 1.0mM + 3.0μM HBL	50.4 ± 6.06^{a}	65.6 ± 3.85^{b}

The data presented above are mean ± SE (n = 5). Cd – cadmium; EBL – 24-epibrassinolide; HBL – 28 homobrassinolide. Data represent an average of replicate and the values given are mean ± SE. Mean followed by the same alphabet in a column is not significantly different at P = 0.05 level

metals cause several toxic effects on plants such as inhibition of seed germination (Al-Helal 1995) and metabolic disturbances by altering the essential biochemical reactions (Krupa et al. 1993).

In the present study, Cd toxicity also resulted in a substantial reduction in seedling growth, and their inhibitory effects on seedling growth were ameliorated by the application of brassinosteroids (Table 2). Both 24-epibrassinolide and 28-homobrassinolide improved the seedling growth in terms of seedling length, seedling fresh weight and dry weight. The two BRs at 3.0μM concentration caused a considerable increase in seedling growth even under stress and restored the growth to the level of unstressed control seedlings. It was shown that brassinosteroids relieved the salinity-induced inhibition of seed germination and seedling growth in barley (Kulaeva et al. 1991) and rice (Anuradha and Rao 2001). Similarly, brassinosteroids improved the percentage of seed germination and seedling growth in sorghum under osmotic stress (Vardhini and Rao 2003). The present study demonstrated the ability of brassinosteroids to counter the toxic effects of Cd on seed germination and seedling growth.

In response to Cd stress radish seedlings accumulated proline, and supplementation of brassinosteroids further enhanced the proline contents (Table 3). Though a minor constituent of the amino acid pool, proline accumulates in large quantities in plants when subjected to abiotic stresses, and relieves the enzymes and cellular structures from stress factors. It was suggested that proline synthesized during stress condition might serve as an organic nitrogen reserve that can be utilized during recovery (Trotel et al. 1989). The chilling tolerance in rice caused by epibrassinolide was also found to be associated with elevated levels of free proline (Wang and Zang 1993). Similarly, Bouchereau et al. (1999) demonstrated that exogenous putrescine (polyamine) supplied at low concentrations stimulated the proline accumulation.

Cd toxicity decreased the CAT activity in radish seedlings and addition of BRs increased its activity (Table 3). CAT is an important oxidizing enzyme that helps in the removal of H_2O_2 and helps in detoxifying harmful metabolic products; its activity appears to be positively correlated with an increase in growth. A decrease in CAT activity due to heavy metals can be attributed to inhibition of the CAT synthesis and other oxidase proteins (Das et al. 1978). A similar increase in CAT activity of sorghum seedlings under water stress caused by the application of brassinosteroids was reported previously (Vardhini and Rao 2003). In contrast to CAT activity, Cd toxicity increased the POD activity in radish seedlings (Table 3). An increase in POD activity is a common response to oxidative and abiotic stresses. In plants, POD protects cells against harmful concentrations of hydroperoxides and helps in a variety of cellular functions (Beauchamp and Fridovich 1971). Increased total peroxidase activities in response to salinity were reported by Sancho et al. (1996). A similar increase in POD was also observed after the application of Ni to the leaves of *Silene italica* (Gabbrielli et al. 1987). The high POD activity in radish seedlings treated with Cd observed in the

Table 2. Effect of brassinosteroids on the seedling growth of radish under cadmium stresses

Treatment	Seedling length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Control	6.10 ± 0.28^{a}	377.0 ± 7.17^{b}	31.01 ± 0.04^{c}
Cd 1.0mM	2.64 ± 0.39^{c}	217.6 ± 14.17^{e}	23.66 ± 0.03^{e}
Cd 1.0mM + 1.0μM EBL	3.72 ± 0.35^{c}	311.4 ± 4.13^{d}	24.16 ± 0.07^{e}
Cd 1.0mM + 2.0μM EBL	6.00 ± 0.37^{a}	340.0 ± 3.34^{c}	28.63 ± 0.04^{d}
Cd 1.0mM + 3.0μM EBL	5.76 ± 0.77^{b}	364.8 ± 4.17^{b}	33.20 ± 0.08^{c}
Cd 1.0mM + 1.0μM HBL	5.80 ± 0.39^{b}	343.2 ± 3.38^{c}	29.41 ± 0.04^{d}
Cd 1.0mM + 2.0μM HBL	6.08 ± 0.26^{a}	372.2 ± 3.49^{b}	38.28 ± 0.05^{b}
Cd 1.0mM + 3.0μM HBL	6.76 ± 0.51^{a}	391.2 ± 3.45^{a}	45.26 ± 0.06^{a}

The values are mean ± SE (n = 5). One-way ANOVA indicates a significant difference at $P \leq 0.05$ level. Cd – cadmium; EBL – 24-epibrassinolide; HBL – 28-homobrassinolide. Data represent an average of replicate and the values given are mean ± SE. Mean followed by the same alphabet in a column is not significantly different at $P = 0.05$ level

Table 3. Effect of brassinosteroids on free proline, catalase and peroxidase activity in radish seedlings under cadmium stresses

Treatment	Free proline (mg/g fresh weight)	CAT	POD
Control	1.84 ± 0.04^{d}	54.59 ± 2.85^{c}	0.680 ± 0.05^{d}
Cd 1.0mM	2.74 ± 0.39^{c}	26.60 ± 1.69^{f}	0.810 ± 0.01^{a}
Cd 1.0mM + 1.0μM EBL	2.90 ± 0.35^{c}	37.11 ± 4.54^{e}	0.789 ± 0.02^{a}
Cd 1.0mM + 2.0μM EBL	3.17 ± 0.37^{bc}	44.58 ± 2.65^{d}	0.768 ± 0.02^{b}
Cd 1.0mM + 3.0μM EBL	3.62 ± 0.77^{bc}	52.04 ± 2.86^{c}	0.733 ± 0.04^{c}
Cd 1.0mM + 1.0μM HBL	3.82 ± 0.39^{b}	55.90 ± 1.92^{c}	0.772 ± 0.03^{b}
Cd 1.0mM + 2.0μM HBL	3.98 ± 0.26^{b}	67.81 ± 2.16^{b}	0.754 ± 0.01^{bc}
Cd 1.0mM + 3.0μM HBL	4.52 ± 0.51^{a}	73.66 ± 2.54^{a}	0.694 ± 0.04^{d}

The values are mean ± SE (n = 5). One-way ANOVA indicates a significant difference at $P \leq 0.05$ level. Cd – cadmium; EBL – 24-epibrassinolide; HBL – 28-homobrassinolide. CAT – catalase activity is expressed in terms of U/mg protein. POD – peroxidase activity is expressed in terms of U/mg protein. Data represent an average of replicate and the values given are mean ± SE. Mean followed by the same alphabet in a column is not significantly different at P = 0.05 level

present study may indicate an initiation of disruption in the biochemical processes. However, we observed that brassinosteroids applied to heavy metal-stressed radish seedlings reduced the POD activity. Similarly, a reduction in POD activity in putrescine-alleviated salt stress in spinach leaves was reported (Ozturk and Demir 2003).

APOX and GPX activities showed an increase with heavy metal stress as well (Table 4). However, BRs supplementation further enhanced the activity of radish seedlings. With the increase in BRs concentration, corresponding increase in the activity of the enzyme was found. Like CAT, APOX and GPX break down H_2O_2 to H_2O and O_2. A comparable increase in APOX activity was induced by salt stress in *Raphanus sativus* plants (Lopez et al. 1996) and in cotton callus tissue (Bellaire et al. 2000). Overexpression of APOX enhanced the tolerance to salt stress and water deficit in tobacco chloroplast was observed (Badawi et al. 2004). The amelioration of salt induced oxidative damage by polyamines was found to be associated with an increase in APOX activity in *Virginia pine* (Tang and Newton 2005).

Cd stress increased the SOD activity of radish seedlings (Table 4). The supplementation of brassinosteroids resulted in further enhanced SOD enzyme activity. The increase in SOD activity by brassinosteroids might be due to an increase in efficiently eliminating superoxide radicals (O_2^-) formed during stress. The obtained results are consistent with those reported by Mazorra et al. (2002) with brassinosteroids enhancing SOD activity in tomato under temperature stress.

The activity of AAO increased under heavy metal stress, while the supplementation of brassinosteroids to heavy metals resulted in its decrease (Table 5). The lowered activity of AAO was shown to be an adaptive feature found in flooding-tolerant cultivars of rice, which show higher quantities of ascorbic acid, and the increase of ascorbic acid is very important to develop defense system against abiotic stress (Sarkar and Das 2000).

An increase in malondialdehyde content in radish seedlings grown under Cd stress was observed (Table 5), indicating a high level of lipid peroxidation. Malondialdehyde is a product of peroxidation of unsaturated fatty acids in phospholipids, and lipid peroxidation is responsible for cell membrane damage (Halliwell and Gutteridge 1985). A high level of lipid peroxidation was reported in the case of higher plants under Cr and other heavy metals (Panda and Choudhury 2004). Brassinosteroid supplementation to Cd treatments reduced lipid peroxidation levels. The counteracting effects of BRs on heavy metal-induced lipid peroxidation were particularly more prominent at high concentrations used in the study. Zeatin riboside (a cytokinin) inhibited the accumulation of malondialdehyde under high temperatures. Similarly, benzyladenine played the same role as antioxidants in scavenging active oxygen and protecting mem-

Table 4. Effect of brassinosteroids on the activity of ascorbate peroxidase, guaiacol peroxidase and superoxide dismutase in radish seedlings under cadmium stresses

Treatment	APOX	GPX	SOD
Control	19.57 ± 1.58^{d}	8.92 ± 0.43^{e}	9.28 ± 1.28^{d}
Cd 1.0mM	15.08 ± 0.40^{e}	26.63 ± 0.31^{d}	3.75 ± 2.44^{e}
Cd 1.0mM + 1.0μM EBL	17.82 ± 1.31^{e}	29.26 ± 0.06^{c}	9.68 ± 2.76^{d}
Cd 1.0mM + 2.0μM EBL	20.46 ± 1.98^{cd}	34.44 ± 0.15^{bc}	12.99 ± 2.18^{c}
Cd 1.0mM + 3.0μM EBL	26.37 ± 1.66^{b}	39.11 ± 0.11^{b}	16.86 ± 2.59^{b}
Cd 1.0mM + 1.0μM HBL	22.49 ± 2.54^{c}	26.50 ± 0.09^{d}	11.10 ± 2.78^{c}
Cd 1.0mM + 2.0μM HBL	24.22 ± 0.88^{bc}	39.53 ± 0.06^{b}	14.70 ± 2.85^{bc}
Cd 1.0mM + 3.0μM HBL	33.43 ± 1.51^{a}	43.84 ± 0.14^{a}	20.74 ± 2.30^{a}

The values are mean ± SE (n = 5). One-way ANOVA indicates a significant difference at $P \leq 0.05$ level. Cd – cadmium; EBL – 24-epibrassinolide; HBL – 28-homobrassinolide. APOX – the amount of ascorbate oxidized was calculated from extinction coefficient of 2.6mM^{-1} cm^{-1} and the enzyme activity was expressed in mg/g fresh weight protein. GPX – the change in absorbance of one unit per min was calculated using extinction coefficient of 26.6mM^{-1} cm^{-1} and the enzyme activity was expressed in mg/g fresh weight protein. SOD – superoxide dismutase activity is expressed in mg/g fresh weight protein. Data represent an average of replicate and the values given are mean ± SE. Mean followed by the same alphabet in a column is not significantly different at P = 0.05 level

Table 5. Effect of brassinosteroids on the activity of ascorbic acid oxidase and lipid peroxidation in radish seedlings under cadmium stresses

Treatment	Ascorbic acid oxidase activity	Lipid peroxidation
Control	233 ± 0.05^{d}	58.03 ± 0.10^{d}
Cd 1.0mM	386 ± 0 .02^{a}	83.54 ± 0.06^{a}
Cd 1.0mM + 1.0μM EBL	374 ± 0.07^{a}	77.91 ± 0.11^{a}
Cd 1.0mM + 2.0μM EBL	348 ± 0.12^{b}	70.22 ± 0.09^{b}
Cd 1.0mM + 3.0μM EBL	309 ± 0.04^{c}	64.84 ± 0.06^{c}
Cd 1.0mM + 1.0μM HBL	376 ± 0.09^{a}	72.57 ± 0.09^{b}
Cd 1.0mM + 2.0μM HBL	236 ± 0.02^{c}	65.56 ± 0.08^{c}
Cd 1.0mM + 3.0μM HBL	221 ± 0.06^{c}	56.89 ± 0.06^{d}

The values are mean ± SE (n = 5). One-way ANOVA indicates a significant difference at $P \leq 0.05$ level. Cd – cadmium; EBL – 24-epibrassinolide; HBL – 28 homobrassinolide. AAO – ascorbic acid oxidase activity is expressed as μg of ascorbic acid oxidized in 30 min/g seedling fresh weight. Lipid peroxidation – the amount of malondialdehyde was calculated from extinction coefficient of 155mM^{-1} cm^{-1} and the enzyme activity was expressed in mg/g fresh weight protein. Data represent an average of replicate and the values given are mean ± SE. Mean followed by the same alphabet in a column is not significantly different at P = 0.05 level

branes in *Phaseolus vulgaris* from ozone damage (Pauls and Thompson 1982). Polyamines reduced the salt induced oxidative damage by decreasing the lipid peroxidation in *Pinus virginiana* Mill. (Tang and Newton 2005).

Perfus-Barbeoch et al. (2002) suggested that Cd phytotoxicity is associated with altered water status. Poschenrieder and Barcelo (2004) attributed alteration of water relations as the secondary effect, which further enhanced heavy metal induced growth reduction. One of the causative factors in structural changes in the cells of seedling of *Raphanus sativus* induced by Cd were due to a decrease in water uptake (Vitoria et al. 2003). Earlier Vardhini and Rao (2003) reported the water stress alleviation in sorghum seedlings by brassinosteroids. Thus there exists a possibility of brassinosteroids reversing the heavy metal induced decline in water uptake by radish seedlings.

The results obtained in the present study clearly indicated the ameliorative effect of brassinosteroids on Cd toxicity stress. With the increase in BRs concentration, the protection against metal damage was increased. The stress alleviation effect of brassinosteroids was associated with enhanced levels of proline. Brassinosteroids strongly protects radish seedling from Cd induced oxidative stress by minimizing the impact of reactive oxygen species by increasing antioxidant enzyme activity, which

may represent a secondary defensive mechanism against oxidative stresses. The finding further indicated the ability of brassinosteroids to protect the membrane integrity as observed in the case of radish seedlings challenged with Cd stress. Hence, it can be concluded that the supplementation of brassinosteroids proved to be beneficial for the plant system in combating metal toxicity.

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