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RESEARCH ARTICLE

24-epibrassinolide induced antioxidative defense system of *Brassica juncea* L. under Zn metal stress

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Abstract The present study deals with the effects of 24-epibrassinolide on growth, lipid peroxidation, antioxidative enzyme activities, non-enzymatic antioxidants and protein content in 30 days old leaves of *Brassica juncea* (var. PBR 91) under zinc metal stress in field conditions. Surface sterilized seeds of *B. juncea* were given pre-soaking treatments of 24-EBL (10^{-10}, 10^{-8} and 10^{-6} M) for 8 h. Different concentrations of zinc metal in the form of $ZnSO_4.7H_2O$ (0, 0.5, 1.0, 1.5 and 2.0 mM) were added in the soil kept in experimental pots. Seeds soaked in 24-EBL for 8 h were sown in the earthern pots containing different concentrations of Zn metal. After 30 days of sowing, the plants were analyzed for growth parameters in terms of shoot length and number of leaves. Thereafter, leaves were excised and content of proteins, non-enzymatic antioxidants, malondialdehyde (MDA) and the activities of antioxidative enzymes (superoxide dismutase (SOD) (EC 1.15.1.1) catalase (CAT) (EC 1.11.1.6), ascorbate peroxidase (APOX) (EC 1.11.1.11), guaiacol peroxidase (POD) (EC 1.11.1.7) glutathione reductase (GR) (EC 1.6.4.2), monodehydroascorbate reductase (MDHAR) (EC 1.1.5.4) and dehydroascorbate reductase (DHAR) (EC 1.8.5.1)) were analyzed. It was observed that the growth of plants was inhibited under Zn metal stress. However, 24-EBL seed-presoaking treatment improved the plant growth in terms of increase in shoot length. 24-EBL also mitigated the toxicity of Zn metal by increasing the number of leaves. The activities of antioxidative enzymes (SOD, CAT, POD, GR, APOX, MDHAR and DHAR) and contents of proteins and glutathione were also enhanced in leaves of plants treated with 24-EBL alone, 10^{-8} M concentration being the most effective. The activities of antioxidative enzymes also increased in leaves of *B. juncea* plants by the application 24-EBL supplemented Zn metal solutions. Similarly, the content of proteins and glutathione increased considerably in leaves of *B. juncea* plants treated with 24-EBL, whereas the level of MDA content decreased in 24-EBL treated plants as compared to untreated control plants thereby revealing stress-protective properties of the brassinolide.

Keywords Antioxidative enzymes · *Brassica juncea* · 24-epibrassinolide · Zn toxicity

Abbreviations

ANOVA	Analysis of variance
APOX	Ascorbate peroxidase
CAT	Catalase
Cont.	Control
DHAR	Dehydroascorbate reductase
24-EBL	24-epibrassinolide
FW	Fresh weight
GR	Glutathione reductase
28-HBL	28-homobrassinolide
MDHAR	Monodehydroascorbate reductase
POD	Guaiacol peroxidase
ROS	Reactive Oxygen Species
SA	Specific activity
SOD	Superoxide dismutase
UA	Unit activity
Zn	Zinc
$Zn_{0.5}$	0.5 mM of Zn
$Zn_{1.0}$	1.0 mM of Zn
$Zn_{1.5}$	1.5 mM of Zn
$Zn_{2.0}$	2.0 mM of Zn

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Introduction

Brassica juncea L., an amphidiploid species, is grown as an oilseed crop in India. Although, oilseed *Brassicas* are grown over 15% arable land in India but their productivity is considerably hindered by various biotic and abiotic stresses (Shah 2002), like drought, chilling, pesticide and heavy metals etc. The soil in which plants grow may contain phytotoxic levels of the heavy metals including Cr, Cu, Hg, Ni and Zn etc. Zinc is a vital component of many important enzymes, a structural stabilizer for proteins, membrane and DNA-binding proteins (Zn-fingers) (Vallee and Auld 1992). Zn toxicity is indicated by a decrease in growth and development, metabolic activity and an induction of oxidative damage in various plant species (Panda et al. 2003). Although considered tolerant, uptake of these heavy metals in excess to nutritional requirements by plants may initiate a variety of metabolic responses which can cause damage at the cellular level or possibly lead to wider phytotoxic responses (Vangronsveld and Clijsters 1994). Due to this phytotoxic response, the plant stimulates the formation of reactive oxygen species (ROS) at various sites of respiratory and photosynthetic electron transport chain (Arora et al. 2002) and thus creates oxidative stress in cellular systems. The ROS such as hydrogen peroxide, superoxide radical and hydroxyl radical are highly reactive and induce lipid peroxidation, thereby affecting the structural integrity and permeability of cellular membranes. ROS can also cause protein denaturation and DNA damage (Savoure et al. 1997). To prevent accumulation of these reactive molecules, plants have developed a highly efficient antioxidative defense system, including low-molecular-weight antioxidants, such as ascorbate and glutathione and protective enzymes, such as SOD, CAT and the enzymes of the ascorbate-glutathione cycle. A common adaptive response of plants to oxidative stress is the increase of antioxidative compounds, as well as the increase in the activity and/or expression of one or more antioxidant enzymes (Hernandez et al. 2000). Several hormones have been implicated in modulating plant responses to oxidative stress, including ethylene (Vahala et al. 2003), salicylic acid (SA) (Metwally et al. 2003), abscisic acid (Kovtun et al. 2000) and brassinosteroids (BRs) (Cao et al. 2005).

Brassinosteroids (BRs) are potent plant growth regulators of steroidal nature, of which first compound isolated from a natural source was brassinolide. They are widely distributed in the plant kingdom and are active at very low concentrations ranging from nanomolar to micromolar. These are involved in multiple plant growth and development processes, such as cell elongation, vascular development, senescence, photomorphogenesis, flowering time control, and stress responses. An important feature of BRs is their ability to increase not only the yield, but also the quality of crops (Prusakova et al. 1999a). Many results suggest that BRs are required for optimal productivity and resistance to unfavourable influences of the environmental stresses (Khripach et al. 2000). The potential applications of BRs in agriculture and horticulture are based not only on their ability to increase crop yield, but also to ameliorate stress. Although innumerable works have confirmed the potential of the plant hormones to synergistically improve crop performance under normal conditions, very little light has been thrown on the influence of BRs under heavy metal stress. Keeping in mind the stress-ameliorative properties of BRs, the purpose of the present study was to increase our understanding of the effects of 24-epibrassinolide (24-EBL) on antioxidative defense system of *Brassica juncea* L. plants under zinc metal stress.

Materials & methods

The seeds of *B. juncea* L. cv. PBR 91 (certified) used in the present experiment were obtained from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. They were surface sterilized with 0.01% $HgCl_2$ followed by three rinses in double distilled water. These surface sterilized seeds were soaked for 8 h in different concentrations of 24-EBL (0, 10^{-10}, 10^{-8} and 10^{-6} M). The earthern pots to be used for the experiment were arranged in triplicates in the Botanical Garden of the University. Different concentrations of zinc metal in the form of $ZnSO_4.7H_2O$ (0, 0.5, 1.0, 1.5 and 2.0 mM) were added in the pots containing approximately 5 Kg soil per pot. The soil used for the present study was prepared using garden soil, silt and cow dung manure in the ratio of 2: 1: 1. The seeds treated (8 h) with 24-EBL were sown (12 seeds) in the earthern pots (24 cm diameter) up to 3–4 cm deep that contained different concentrations of Zn metal. The earthern pots were kept in natural seasonal conditions. Irrigation was applied every 2 days to achieve soil water field capacity level. Thirty plants from three replicate (10 plants from each of the three replicates) were analyzed for shoot length and number of leaves per plant after 30 days of sowing. Thereafter, the leaves were excised from each plant.

For estimation of antioxidative enzyme activities and protein content, 0.5 g leaves of 30-day old *B. juncea* L. plants were homogenized in 5.0 ml of 100 mM potassium phosphate buffer (pH, 7.0). The homogenate was centrifuged at 4°C for 20 min at 15,000 *g*. The supernatant was used for assays of the activities of SOD, POD, CAT, GR, APOX, MDHAR and DHAR.

Antioxidative enzymes assays

Superoxide dismutase (EC 1. 15. 1. 1)

The activity of SOD was estimated according to Kono (1978) by monitoring its potential to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) dye by superoxide radicals, which are produced by the autooxidation of hydroxylamine hydrochloride

Catalase (EC 1. 11. 1. 6)

Catalase activity was determined as per the method of Aebi (1974).

Guaiacol peroxidase (EC 1. 11. 1. 7)

The activity of peroxidase was estimated according to the method proposed by Putter (1974).

Ascorbate peroxidase (EC 1. 11. 1. 11)

The activity of ascorbate peroxidase was estimated according to the method proposed by Nakano and Asada (1981).

Glutathione reductase (EC 1. 6. 4. 2)

Glutathione reductase activity was measured using the method given by Carlberg and Mannervik (1975).

Monodehydroascorbate reductase (EC 1.1.5.4)

Monodehydroascorbate reductase activity was determined according to the method proposed by Hossain et al. (1984).

Dehydroascorbate reductase (EC 1.8.5.1)

Activity of dehydroascorbate reductase was measured following the method given by Dalton et al. (1986).

Non-enzymatic antioxidant

Glutathione (GSH)

The reduced glutathione content was determined by the method proposed by Sedlak and Lindsay (1968).

Lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the method of Heath and Packer (1968).

Protein estimation

Protein content was determined following the method of Lowry et al. (1951).

Results

1. Growth parameters
   30 DAS (Days After Sowing)
   Morphological parameters
   The observation made on various morphological parameters revealed that the treatment of 24-EBL to plants under Zn stress reduced the toxicity of metal by showing improved growth. The effects of seed presoaking treatments of 24-EBL on morphological parameters (shoot length and number of leaves) under Zn metal stress in field experiment are presented in Fig. 1(a).The studies revealed that the shoot length was reduced considerably from 6.1 cm in untreated control plants to 2.9 cm under increasing concentrations of Zn stress. However, 24-EBL treatment markedly increased the shoot length at 10^{-10}, 10^{-8} and 10^{-6} M concentrations. Supplementation of Zn metal solution with 24-EBL considerably reduced the inhibitory effect of Zn on plant growth. The shoot length of plants treated with 10^{-6} M of 24-EBL supplemented with 0.5 mM of Zn metal solution (7.82 cm) was maximum in comparison to metal treated plants (5.68 cm), whereas the number of leaves did not reveal significant changes with Zn treatment as compared to untreated and unstressed control (or distilled water treated) leaves of *B. juncea*. The number of leaves of plants revealed very less decrease as the concentration of metal increased and was observed in case of plants treated with 1.0 mM of Cr (4.33) when compared to untreated and unstressed control plants (4.66). However, treatment with 24-EBL further improved the number of leaves at 10^{-10}, 10^{-8} and 10^{-6} M alone and in combination with Zn metal (Fig. 1a).
2. Biochemical studies
   I. Protein content and lipid peroxidation:
      Protein content: Different concentrations of zinc viz., $Zn_{0.5}$, $Zn_{1.0}$, $Zn_{1.5}$ and $Zn_{2.0}$mM lowered the protein content (24.11 mg/g FW in $Zn_{0.5}$) compared to untreated control (42.42 mg/g FW) in leaves of 30 days old *B. juncea* plants (Fig. 1b). But, seed-presoaking treatment with 24-EBL considerably increased protein content in *B. juncea* leaves under Zn metal stress. Plants treated with 10^{-8} M of 24-EBL under the stress of 2.0 mM Zn depicted

maximum protein content (81.24 mg/g FW) in comparison to metal treated plants (32.07 mg/g FW).

Lipid peroxidation: The leaves of *B. juncea* showed an increase in MDA (malondialdehyde) content with increasing concentrations of Zn metal (Fig. 1b). The increase in MDA content was maximum at 2.0 mM (27.62 μmol/g FW) in comparison to untreated control (4.19 μmol/g FW). However, 24-EBL treatment lowered the MDA content in leaves of *B. juncea* plants. 10^{-6} M concentration of 24-epiBL caused a maximum decrease in MDA content (2.27 μmol/g FW). Minimum content of MDA (3.76 μmol/g FW) was observed in 10^{-6} M of 24-EBL supplemented with 2.0 mM of Zn solution as compared to 2.0 mM of Zn metal treated plants (27.62 μmol/g FW).

II. Antioxidant

Glutathione content

The glutathione content of leaves of 30 days old plants increased considerably in all treatments of 24-EBL in comparison to untreated control (Fig. 1b). It was maximum in leaves of plants treated with 10^{-8} M of 24-EBL (0.26 mg/g FW) when compared to untreated control plants (0.11 mg/g FW). It was remarkably higher (0.47 mg/g FW) in the leaves of plants treated with 10^{-6} M of 24-EBL supplemented with 2.0 mM of Zn when compared to $Zn_{2.0}$ metal treated plants (0.33 mg/g FW).

III. Antioxidative enzyme activities

The activities of antioxidative enzymes (SOD, CAT, POD, GR, APOX, MDHAR and DHAR) were also enhanced in leaves of plants treated with 24-EBL alone, 10^{-8} M concentration being the most effective. The activities of antioxidative enzymes were also enhanced in leaves of *B. juncea* by the application 24-EBL supplemented Zn solutions.

Enzymes involved in detoxification of oxygen radical

(a) Detoxification of superoxide (O_2^-) radical

SOD activity got enhanced from 5.75 mol UA/mg protein in untreated control leaves of *B. juncea* plants to 8.13 mol UA/mg protein in Zn metal treated plants (2.0 mM). 24-EBL alone further boosted the SOD activity, with maximum rise at 10^{-8} M concentration of 24-EBL. The activity of SOD revealed considerable increase at 10^{-6} M of 24-EBL supplemented with $Zn_{2.0}$ solution (13.58 mol UA/mg protein) when compared to $Zn_{2.0}$ concentration (8.13 mol UA/mg protein) treated plants.

(b) Detoxification of hydrogen peroxide

CAT and POD: The activities of CAT and POD did not increase significantly under zinc metal stress (Figs. 1b & c for CAT and POD respectively). 10^{-10} M of 24-EBL showed maximum activity of CAT (24.59 mol UA/mg protein) whereas 10^{-8} M showed highest POD activity (0.048 m mol UA/mg protein) in comparison to untreated control (5.22 mol UA/mg protein and 0.041 mmol UA/mg protein respectively for CAT and POD). The activities of these hydrogen peroxide detoxifying enzymes were enhanced by 24-EBL under Zn metal stress. Maximum increase in activity of CAT (22.0 mol UA/mg protein) and POD (0.11 mmol UA/mg protein) was observed in leaves of plants treated with 10^{-10} and10^{-8} M respectively of 24-EBL in combination with 2.0 and 1.5 mM of Zn respectively as compared to $Zn_{2.0}$ for CAT (5.93 mol UA/mg protein) and $Zn_{1.5}$ for POD (0.06 mmol UA/mg protein).

(c) Ascorbate-glutathione cycle

APOX and GR: During the present investigation, there was an increase in the activity of APOX but there wasn't considerable increase in the activity of GR with increasing concentrations of Zn metal (Fig. 1c). The APOX activity got increased from 0.43 mmol UA/mg protein (untreated control) to 1.08 mmol UA/mg protein (2.0 mM of Zn metal stress). Whereas GR activity merely revealed an increase from 0.141 mmol UA/mg protein in untreated control plants to 0.147 mmol UA/mg protein in 2.0 mM of Zn metal treated plants. Seed presoaking treatment at a concentration of 10^{-6} M of 24-EBL showed an increment in the APOX activity (2.49 mmol UA/mg protein) whereas 10^{-8} M of 24-EBL treatment increased the activity of GR (0.16 mmol UA/mg protein) in comparison to untreated control (0.43 mmol UA/mg protein and 0.14 mmol UA/mg protein for APOX and GR). To overcome the stress, maximum activity of APOX was observed at 10^{-8} M of 24-EBL in combination with 2.0 mM of Zn metal solution (1.089 mmol UA/mg protein) whereas maximum GR activity was observed at 10^{-8} M of 24-EBL in combination with 1.5 mM of Zn solution (0.306 mmol UA/mg protein) as compared to leaves of 2.0 mM of Zn metal treated plants (1.081 mmol UA/mg protein) for APOX and 1.5 mM of Zn (0.132 mmol UA/mg protein) for GR.

MDHAR and DHAR: The results of the studies carried out to ascertain the involvement of other enzymes (MDHAR and DHAR) of the ascorbate-

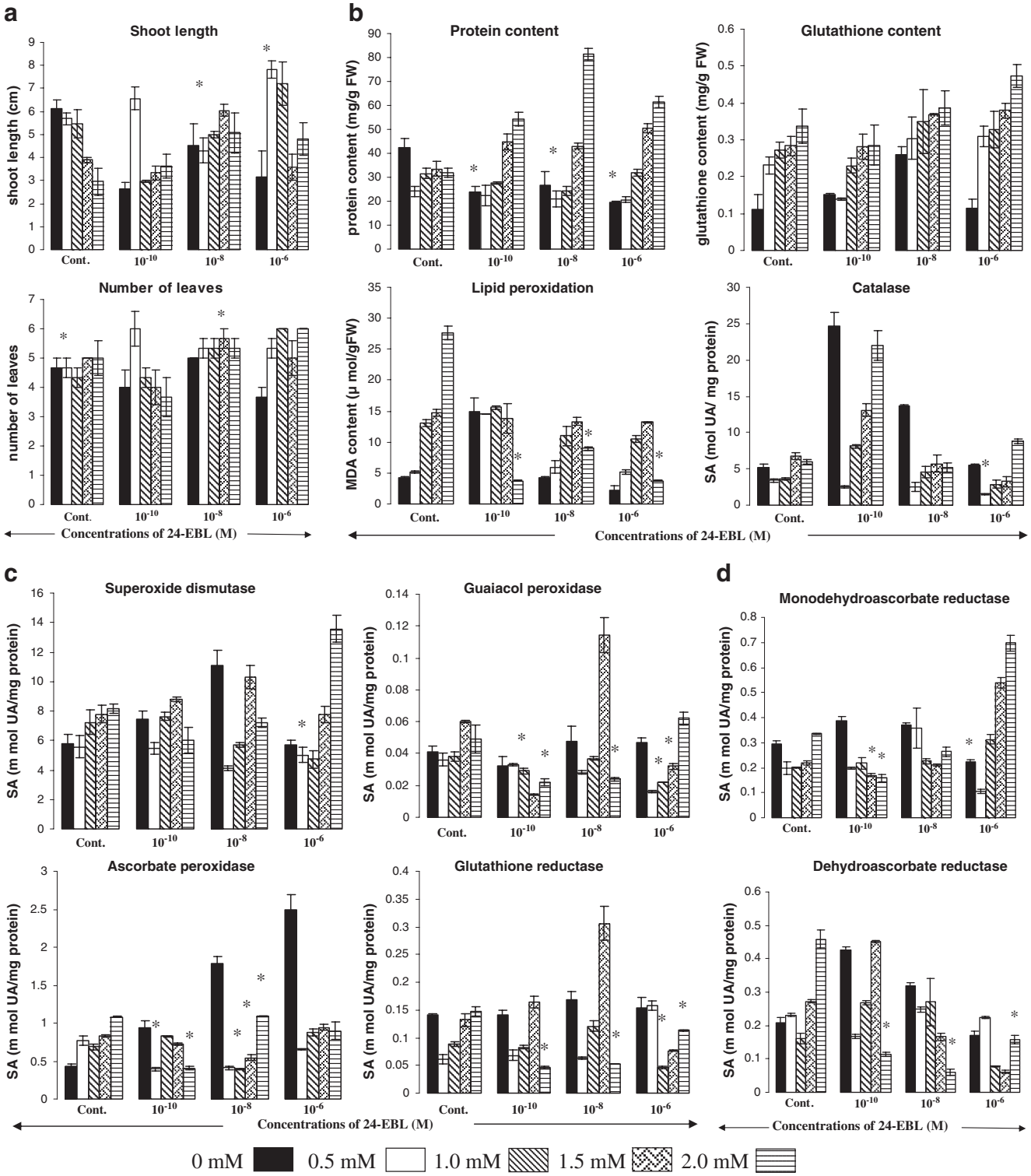

Fig. 1 **a** Effect of 24-EBL on shoot length and number of leaves of 30-days old leaves of *B. juncea* under Zn metal stress. Bars represent the SE (n=3) and asterisks indicate statistically significant differences from control treatments at P<0.05. **b** Effect of 24-EBL on protein content, glutathione content, malondialdehyde content and specific activity of catalase on 30-days old leaves of *B. juncea* L. under Zn metal stress. *Bars* represent the SE (n=3) and *asterisks* indicate statistically significant differences from control treatments at P<0.05. **c** Effect of 24-EBL on specific activities of superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase on 30-days old leaves of *B. juncea* L. under Zn metal stress. *Bars* represent the SE (n=3) and *asterisks* indicate statistically significant differences from control treatments at P<0.05. **d** Effect of 24-EBL on specific activities of monodehydroascorbate reductase and dehydroascorbate reductase on 30-days old leaves of *B. juncea* L. under Zn metal stress. *Bars* represent the SE (n=3) and *asterisks* indicate statistically significant differences from control treatments at P<0.05

glutathione cycle in detoxification of toxic oxygen species are depicted in Fig. 1d. The activity of MDHAR did not reveal significant increase whereas DHAR activity was enhanced considerably over the control leaves under zinc metal stress. MDHAR activity revealed a mere increase from 0.294 mmol UA/mg protein (untreated control) to 0.33 mmol UA/mg protein ($Zn_{2.0}$) whereas DHAR activity showed a tremendous increase from 0.207 mmol UA/mg protein (untreated control) to 0.459 mmol UA/mg protein ($Zn_{2.0}$). MDHAR and DHAR activity further got enhanced by the treatment of 24-EBL alone, with maximum rise at 10^{-10} M concentration of 24-EBL (0.38 mol UA/mg protein for MDHAR and 0.42 mol UA/mg protein for DHAR). MDHAR activity revealed a maximum increase at 10^{-6} M of 24-EBL in combination with 2.0 mM concentration of Zn solution (0.69 mol UA/mg protein) whereas DHAR activity showed a maximum increase at 10^{-10} M of 24-EBL in combination with 1.5 mM Zn solution (0.45 mol UA/mg protein) as compared to $Zn_{2.0}$ (0.33 mol UAmg protein) and $Zn_{1.5}$ (0.27 mol UA/mg protein) metal treated plants respectively.

Discussion

India ranks second in the world with respect to production of *Brassicas* (Afroz et al. 2005) and supplies about 7% of the world's edible oil (Khan et al. 2002). However, Indian mustard production still remains inadequate to meet even the daily requirement of its people (Khan et al. 2002). This low economic yield can be attributed to the crop's susceptibility to a number of biotic and abiotic stresses, among which of alarming concern is the heavy metal. The role of an agronomist is, therefore, to manipulate the crop in order to counteract the influence of this stress, and boost performance even under metal stressed conditions. In this regard, attention has now been paid to the use of plant growth regulators, such as gibberellic acid (GA_3), cytokinins, salicylic acid, BRs etc. which are known to be prominently concerned in the regulation of plant responses. BRs are endogenous plant polyhydroxysteroids eliciting remarkable growth-promoting and developmental effects at nanomolar to micromolar concentrations (Ali et al. 2008; Hasan et al. 2008). They are involved in regulatory processes, which are more specific to plant growth, including photomorphogenesis and skotomorphogenesis (Cevahir et al. 2008; Li and Jin 2007; Vert and Chory 2006). The enthusiasm of BR research stemmed from exciting early observations showing that brassinolide treatment significantly improved growth rate, yield, stress tolerance, and disease resistance of the major crops, ornamentals, vegetables and trees (Szekeres et al. 1996). In the present study, it was observed that application of 24-EBL improved the growth of 30-d old Zn-stressed *B. juncea* plants (Fig. 1a). Plant growth is dependent upon the synthesis of nucleic acids and proteins. BRs participate in the growth of plant tissue in the processes of transcription and translation. Bajguz (2000) demonstrated that the activation of the growth of plant tissue and higher levels of RNA and DNA polymerase is manifested by the increase of the DNA, RNA and protein contents. Enhancement of these enzyme activities may be the result of regulation of gene expression by BRs and may be concerned directly, or indirectly, with growth promotion induced by BRs. Earlier studies also indicated stress-protective properties of BRs. Ali et al. (2007) also observed that 24-EBL and 28-HBL 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). Similarly, Vardhini and Rao (2003) reported that BRs application resulted in enhancement of seedling growth, which was evident in terms of seedling length, seedling fresh and dry weights of all the three varieties of sorghum (*Sorghum vulgare*) viz. CSH-14 and ICSV-745 (susceptible to water stress) and M-35-1 (resistant towater stress) under osmotic stress.

24-EBL further improved the plant growth by increasing the protein content and by decreasing the levels of lipid peroxidation under Zn metal stress (Fig. 1b). 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). Kartal et al. (2009) reported that 28-HBL treatment significantly increased protein content in barley seedlings. Kulaeva et al. (1991) reported that BRs can protect cereal leaf cells from heat shock stress. Pre-treatment with both 22*S*, 23*S*-homoBL and 24-EBL activated total protein synthesis and de novo synthesis of different polypeptides when wheat leaves were heat-shocked by subjecting them to 40°C as well as normal temperatures. In addition, 22*S*, 23*S*-homoBL stimulated the formation of heat-shock granules in the cytoplasm and increased thermo tolerance of total protein

synthesis under heat shock. 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; Arora et al. 2008; Bhardwaj et al. 2008). As membrane destruction results from ROS induced oxidative damage (Mittler 2002), the 24-EBL treated plants might be scavenging ROS more effectively than the plants treated with metal alone. The observations are in consistence with the results of Ozdemeir et al. (2004), who reported that lipid peroxidation level induced by NaCl was significantly lowered in rice seedlings when treated with 24-EBL. In the present study, it was also observed that the root length got decreased in plants treated with 24-epibrassinolide (24-EBL) which in turn might have reduced the protein content. 24-EBL has also been found to retard root growth in maize, mungbean, wheat and soybean (Roddick and Ikekawa 1992; Hunter 2001). The alteration of root growth may be an expression of the cascade of the altered pattern of biochemical reactions either by directly involving the genes or through extra-genetic route (Hayat and Ahmad 2003). The greater increase in malondialdehyde (MDA) content at 10^{-10} M of 24-EBL treated plants may be a reflection of changes in the activities of antioxidative enzymes. It might be possible that different mechanisms may co-operate in order to strengthen the antioxidative defense system of plants (Rizhsky et al. 2002).

In the present study, there was an increase in glutathione content by the application of 24-EBL in leaves of 30-d old *B. juncea* plants (Fig. 1b). The results are in coherence with Bajguz (2000). He reported that BRs and its derivatives at a concentration of 10^{-4} mol/L under lead metal stress in *Chlorella vulgaris* acted as a stimulator of phytochelatins which are derived from glutathione pool thus proving the role of glutathione in lead metal detoxification.

The present investigation also reveals that treatment of 24-EBL significantly enhanced the activities of all antioxidative enzymes (e.g. SOD, CAT, POD, APOX, GR, MDHAR and DHAR) when compared to untreated plants (treated with Zn alone) (Fig. 1c and d). 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). 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). Besides, SOD, CAT and POD represent the endogenous defense of plant cells. These enzymes are present in different isoforms in numerous cell compartments and their expression is genetically controlled and regulated both by developmental (as in the present study i.e. 30 days after sowing) and environmental stimuli and also according to the necessity for removing ROS (reactive oxygen species) produced in cells (Mittler et al. 2004). Apart from this, the regulation of number of processes such as alteration of antioxidative enzyme activities, assimilate partitioning etc. varies with the type of plant, the cultivar used for study, the type of hormones and the mode of application (Fariduddin et al. 2008).

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, APOX, GR, MDHAR and DHAR becomes very important.

In the present study it was observed that application of 24-EBL to stressed plants further boosted and strengthened the antioxidative defense system of the plant by stimulating the activities of various antioxidative enzymes viz. SOD, CAT, POD, APOX, GR, MDHAR and DHAR. Earlier reports also showed that exogenous application of BRs modified antioxidant enzyme activity (Arora et al. 2008; Ali et al. 2007; Hayat et al. 2009). Nunez et al. (2003) observed higher activity of antioxidative enzymes in rice, grown under salinity and supplemented with polyhydroxylated spirostanic brassinosteroid analogue (BB-16). 24-EBL also ameliorated the cadmium toxicity in *B. juncea* and *Cicer arietinum* plants by increasing the activities of peroxidase, catalase and superoxide dismutase (Hayat et al. 2007; Hasan et al. 2008). The present study therefore reveals the anti-stress properties of BRs in the *B. juncea*, an important oilseed crop of edible and medicinal importance, exposed to heavy metal (Zn) stress. 24-EBL improved the vigor in terms of increased shoot length and number of leaves, decreased lipid peroxidation, increased protein and glutathione content and enhanced activities of Asada-Halliwell pathway regulated key antioxidative enzymes.

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