RESEARCH ARTICLE

Epibrassinolide regulated synthesis of polyamines and auxins in *Raphanus sativus* L. seedlings under Cu metal stress

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ABSTRACT

The effects of various conc of (10^{-11}, 10^{-9} and 10^{-7} M) 24-epibrassinolide (24-epiBL) on polyamines and auxins contents was analyzed in 7days old seedlings of *Raphanus sativus* L. cv. Pusa chetki under copper metal stress. Cu metal (0.2mM) treatment significantly increased endogenous levels of putrescine, spermidine and spermine when compared with control values. Cadaverine content got decreased under metal stress. Endogenous polyamines showed varying levels in seedlings when applying 24-epiBL treatment only. Maximum increase was found in putrescine, cadaverine and spermidine in 10^{-11}, 10^{-9} and 10^{-7} M conc of 24-epiBL respectively when applied alone to *R. sativus* seedlings. However, metal treatment supplemented with 24-epiBL revealed small increase in polyamines. Putrescine and cadaverine got enhanced in combination of Cu metal and 10^{-7} M (24-epiBL) in comparison to metal treatment alone, whereas decrease in spermidine and spermine levels were recorded in combinations of Cu metal and 10^{-9} and 10^{-7} M 24-epiBL respectively. Endogenous contents of Indole-3-acetic acid (IAA) and Naphthalene acetic acid (NAA) were significantly affected by 24-epiBL when applied alone or along with Cu metal. Both free and bound forms of NAA were not detected in untreated seedlings, but were recorded in seedlings treated with metal stress. Decreased IAA contents observed in metal treatment got enhanced significantly in seedlings with 24-epiBL supplementation. To the best of our knowledge the present investigation is the first one to explain the effects of 24-epiBL on endogenous contents of polyamines, indole acetic acid and naphthalene acetic acid under copper metal stress.

Keywords: *Raphanus sativus*, polyamines, auxins, brassinosteriods, oxidative stress, heavy metal stress.

INTRODUCTION

Human interference has significantly contributed in adding various stress factors on the ecosystems, hence causing species extinction and depletion of biodiversity. Heavy metal contamination of the biosphere has increased substantially during the last few years and poses a major environmental and human problems worldwide (Ensley, 2000). Main sources of heavy metal pollution are the industrial wastes of mining, chemical and metal processing industries. Metals such as Cd, Cu, Pb and Cr appear in natural and agricultural areas and water bodies resulting in their penetrance in the food chain (Chary et al., 2008). Heavy metals are detrimental to living organisms even at low concentrations (Benavides et al., 2005). Among metals however, copper is an essential metal required for normal plant growth and development (Mengel and Kirkby, 1987), but toxic at higher concentrations (Chatterjee et al., 2006; Berenguer et al., 2008). Harmful effects of Cu metal are mediated via formation of free radicals (Luna et al., 1994) and by the catalysis of the Haber-Weiss reaction (Halliwel and Gutteridge, 1984). These free radicals are very toxic and oxidize biological macromolecules such as nucleic acids, proteins and lipids, thereby disturbing the cell stability and membrane permeability (Lovaas, 1997; Schutzendubel and Polle*,* 2002).

Therefore heavy metals are implicated in the generation of oxidative stress in plant cells. To counteract harmful effects of oxidative stresses, plants have antioxidants and antioxidative enzymes (POD, SOD, GR, CAT and APOX). Phytohormones, e.g. auxins (Dimpka et al., 2008), cytokinins (Zhang and Ervin, 2008), ethylene (Tamaoki et al., 2008), ABA (Kurepin et al., 2008) are also known to confer stress protection in plants. Additionally, brassinosteriods (Clouse, 2008) and polyamines (Tassoni et al., 2008) are actively involved in stress management.

Brassinosteriods (BRs) are polyhydroxylated derivatives of cholestane, which are widely distributed in the plant kingdom. Their pleiotropic involvement in plant cell elongation, cell division, vascular differentiation, growth and reproductive development (Sasse, 2003; Clouse, 2008; Haubrick and Assman, 2006) demonstrated their importance as indispensable component of plant metabolism. BRs have been reported to protect plants from various stresses like salt (Ozdemir et al., 2004), heat (Dhaubhadel et al., 2002) and heavy metals (Khripach et al., 1996; Kaur and Bhardwaj, 2004; Janeczko et al., 2005; Zhang et al., 2005). BRs also interact with other plant hormones to modulate various plant stress responses. Interactions of BRs with auxins (Katsumi, 1985; Gaudinova et al., 1995; Halliday, 2004), gibberellins (Mayumi and Shiboaka, 1995), ABA (Kurepin et al., 2008) and cytokinins (Gaudinova et al., 1995) have been recorded to regulate a variety of physiological and plant stress responses.

Polyamines (PAs) are small aliphatic nitrogenous compounds present in all living organisms (Tassoni et al., 2008). Putrescine (Put), Cadaverine (Cad), Spermidine (Spd) and Spermine (Spm) are major PAs involved in the regulation of a large number of physiological activities such as embryogenesis, cell division, morphogenesis, development, ethylene production and senescence (Tiburcio et al., 1997; Bagni and Tassoni, 2001; Groppa and Benavides, 2008). Stress protective properties indicate, that PAs are an integral component of plant stress management. Variations in PA contents have been associated with several types of stresses, e.g. osmotic stress (Aziz et al., 1997), salinity (Pirintsos et al., 2004; Tang et al., 2005; Shevyakova et al., 2006; Liu et al., 2007; Wen et al., 2008) and heavy metals (Shevyakova et al., 2006; Liu and Moriguchi, 2007; Zapata et al., 2008; Vladimir et al., 2009). The strong antioxidant character of PAs is proven (Ha et al., 1998). Enhanced accumulation of putrescine under copper stress had been reported in rice leaves (Lin and Kao, 1999).

Partial restoration of cambial activity in *Luffa cylindrica* L. under mercury stress on application of auxins was reported by Khan and Chaudhary (2006). Similarly, Dimpka et al. (2008) recorded the phytoremediation role of auxins and siderophores in *Streptomyces spp.* against heavy metals (Cu, Ni and Cd). Brassinosteriods showed synergistic effects with auxins. Their co-application increased hypocotyls elongation in *Arabidopsis* seedlings (Tanaka et al., 2003). However, the influence of BRs on the endogenous levels of PAs and auxins under metal stress remains poorly understood. Therefore, the aim of the present investigation was to study the effect of 24-epibrassinolide (24-epiBL) (a potent BRs) on the endogenous contents of free polyamines and auxins in the seedlings of *Raphanus sativus* L. under copper metal stress.

MATERIAL AND METHODS

Plant material and treatments: Seeds of *Raphanus sativus* L. cv. Pusa chetki procured from Punjab Agriculture University, Ludhiana, India were used as study material. They were surface sterilized with 0.01% sodium hypochlorite and then rinsed three to four times with distilled water. Seeds were grown in autoclaved Petriplates lined with *Whatman No*.1 filter paper at 25 ± 2^{o} C with a photoperiod of 16 h under fluorescent white light (175 μmol/m^2/s) in a controlled environmental growth chamber maintained at 70-80% relative humidity. Seeds were exposed to 24-epiBL (10^{-11}, 10^{-9} and 10^{-7}M) and 0.2 mM of Cu metal alone and in combination with 24-epiBL (10^{-11}, 10^{-9} and 10^{-7}M). Controls were raised in distilled water only. Seedlings were harvested on the 7th day, (3-3.5 cm hypocotyls length) to quantify PAs (Put, Cad, Spd and Spm) and auxins (Indole acetic acid and Naphthalene acetic acid) and seedling growth. The concentration of Cu metal was based on its inhibitory concentration (IC_{50}).

Morphological parameters: The effects of 24-epiBL treatments on 7 days old *Raphanus* seedlings under Cu metal stress were determined. The growth was quantified in the units shoot length, root length and percentage of germination for both, treated and untreated seedlings.

Extraction and measurement of polyamines: PAs were extracted and quantified according to the method of Fontaniella et al. (2001). For extraction of PAs, 2 grams of seedlings after various treatments with Cu metal and 24-epiBL were homogenized with 5% Perchloric Acid (PCA) (v/v) in a pre-chilled pestle and mortar at 4° C. Homogenized extracts were kept at 4° C for 1 h, and then centrifuged at 15.000 r.p.m. for 25 min at 4° C. Supernatant was dried *in vacuo* at 45° C and residues were redissolved in 5% PCA and were stirred with Polyvinylpolypyrrolidone (PVPP) (50mg/ml) for the removal of impurities like polyphenolics.

Derivatization of polyamines: PAs were derivatized with dansyl chloride in 5 ml vials and adjusted to pH 8.0 (using NaOH solution). The reaction mixture consisted of 1 ml sample, 1 ml of saturated Na_2CO_3 and 1 ml of dansyl chloride (75 mM in acetone). The constituent parts were mixed vigorously and left undisturbed for 18 hours at room temperature. Dansylated PAs were extracted with 3.0 x 3.0 ml of benzene (HPLC grade) after vortexing for 1 min. Benzene phase was air dried at 40° C and samples were cleaned by adding 1.2 ml of 5 mM KOH in methanol (Seiler and Knodgen, 1979). Mixtures were left to stand for 1 hour at 45° C and then treated with aqueous mixture of 300 mg of KH_2PO_4 and 300 mg of Na_2HPO_4. PAs were extracted using 3 x 3.0 ml of benzene (HPLC grade) and benzene phase was dried *in vacuo*. Then residue was redissolved in 1 ml of methanol (HPLC grade) for the characterization and quantification of PAs.

Analysis of polyamines: PAs were analyzed on Waters 515 (USA) Chromatograph HPLC equipped with Waters (717) Plus Autosampler and Photodiode Array Detector (2996). They were eluted in a RP-C-18 column (15 cm x 4 mm i.d.), (5 μM particle sizes) reversed phased column at 30° C using methanol: water gradient. Gradient elution was carried out using Methanol-Milli-Q water in a linear gradient from 50:50 (v/v) to 80:20 (v/v) for 30 minutes. The last proportion was maintained for 15 minutes until the end of the analysis. Mobile phase was maintained at a flow rate of 1 ml/min. Fluorescence intensities of the extracted PAs were compared, peaks and retention times of samples were compared to those of standard polyamines (Put, Cad, Spd and Spm, purchased from Sigma Aldrich Ltd. India).

Extraction and measurement of auxins: Auxins were extracted and quantified according to the method of Nagar and Sood (2006). Two gram of seedlings after various treatments with 24-epiBL, Cu and their combinations were homogenized using 80% methanol (20 ml/g) containing butylated hydroxytoulene (BHT) at 100 mg/l. Homogenates were kept overnight at 4° C in the dark, filtered, and re-extracted (4 x) with methanol 80%. Resulting solution was frozen at -20° C, thawed and centrifuged at 9000 r.p.m. for 30 min at 4° C to remove suspended impurities. Supernatants were dried *in vacuo* and taken up in 0.1 M potassium phosphate buffer (pH 8.1) and then applied to polyvinylpyrrolidone (PVP) column. Eluate obtained was evaporated to dryness, taken up in water and pH adjusted to 2.5 with 1 N HCl and then partitioned (4 x) with diethyl ether. Combined ether phases were evaporated to dryness in *vacuo* and the residue was dissolved in methanol for the estimation of free auxins up to 80%. The remaining aqueous phase was hydrolyzed at pH 7.0 for 3 h at 100° C (Sundberg et al., 1994). The hydrolysate was cooled, neutralized with 1 N HCl up to pH 3.0 and finally partitioned against diethyl ether. The combined ether phases were evaporated in *vacuo*, and taken up in methanol (HPLC grade) for the estimation of bound auxins.

Analysis of auxins: For the estimation of endogenous contents of auxins, methanolic extracts ready for HPLC analysis were filtered through 0.45 μM Millipore filters. Elution was carried out with Methanol (40%) in 30 mM acetic acid (HPLC grade) at a flow rate of 1 ml/min. Column eluants were passed through a UV detector (2996 PDA detector) at 280 nm and auxins were characterized and quantified. Standard auxins were used as reference (Indole acetic acid and Naphthalene acetic acid, purchased from Sigma Aldrich Ltd. India).

Statistical analysis: Three repetitions were designed for each experiment. Data was expressed as mean ± standard error (SE). One way analysis of variance (ANOVA) was carried and data were considered significant at $p \leq 0.05$.

RESULTS

Seedling growth: The shoot and root length of *Raphanus* seedlings was markedly reduced under Cu metal stress (Table

1). Metal stress reduced shoot length to 2.21 cm and root length to 3.41 cm when compared with shoot length (3.53 cm) and root length (6.50 cm) in controls. However 24-epiBL treatment increased the shoot and root length significantly at 10^{-11}, 10^{-9} and 10^{-7} M 24-epiBL concentration alone and in combination with copper. A maximal increase was found in 10^{-11} M 24-epiBL alone with 4.71 cm and 7.32 cm enhancement in shoot and root length respectively. Whereas combination of copper with 10^{-11} M 24-epiBL showed minimal increases in shoot (3.45 cm) and root (6.32 cm) length as compared to metal treated seedlings. The percentage of germination decreased to 81% under copper treatment, whereas 24-epiBL treatment exogenously enhanced the percentage of germination to 98%. In addition to these effects copper stress also resulted in poor root formation, interveinal chlorosis and development of necrotic spots on the leaf lamina.

Table 1. Morphological parameters of 7days old *Raphanus* seedlings given Cu-metal and 24-epibrassinolide treatment.

S.No.	Root length (cm)	Shoot length (cm)	Percent germination (%)
Control	6.50 ± 0.099^{b}	3.53 ± 0.186^{b}	98
0.2mM Cu	3.41 ± 0.071^{a}	2.21 ± 0.121^{a}	81
10^{-7} M epiBL	4.93 ± 0.093^{a,b}	3.75 ± 0.169^{b}	98
10^{-9} M epiBL	6.93 ± 0.109^{b}	3.82 ± 0.196^{b}	98
10^{-11} M epiBL	7.32 ± 0.098^{a,b}	4.71 ± 0.188^{a,b}	98
Cu + 10^{-7} M epiBL	4.12 ± 0.038^{a,b}	2.51 ± 0.107^{a}	92
Cu + 10^{-9} M epiBL	4.98 ± 0.044^{a,b}	3.39 ± 0.061^{b}	94
Cu + 10^{-11} M epiBL	6.32 ± 0.174^{b}	3.45 ± 0.096^{b}	96

$^{a,\ b}$ indicate statistically significant differences from control and metal treatment at $p \leq 0.05$.

Polyamine contents: Copper treatment enhanced Put (52.84 μg g^{-1} f.w.) and Spd (404.99 μg g^{-1} f.w.) contents in *R. sativus* seedlings when compared to Put (38.25 μg g^{-1} f.w.) and Spd (184.92 μg g^{-1} f.w.) levels recorded in untreated control seedlings. Spm (1288.75 μg g^{-1} f.w.) recorded in metal treated seedlings was not detected in control treatments. Cad (57.24 μg g^{-1} f.w.) found in controls showed significant decrease (5.96 μg g^{-1} f.w.) after metal treatment. Seedlings treated with 24-epiBL (10^{-7}, 10^{-9} and 10^{-11} M) alone showed varied pattern in the endogenous levels of PAs (Table 2). Put level got increased to a lesser extent (73.70 μg g^{-1} f.w.) in 10^{-9} and (47.56 μg g^{-1} f.w.) in 10^{-11} M than 10^{-7} M (105.26 μg g^{-1} f.w.) 24-epiBL treated seedlings. Combinations of copper and 24-epiBL (10^{-11}, 10^{-9} and 10^{-7} M) showed decrease in the synthesis of Put level. Cu-metal and 10^{-11} M 24-epiBL combination showed a maximal decrease in Put (14.01μg g^{-1} f.w.) over combinations of 10^{-9} M (44.72 μg g^{-1} f.w.) and 10^{-7} M (60.37 μg g^{-1} f.w.) (Table 2).

Table 2. Endogenous Polyamines content (μg/g f.w.) in 7 days old seedlings of *Raphanus* subjected to Cu-metal and 24-epibrassinolide treatments.

S.No.	Putrescine	Cadaverine	Spermidine	Spermine	Total (μg/g f.w.)
Control	38.25 ± 2.20^{b}	57.24 ± 4.03	184.92 ± 9.21^{b}	n.d.	280.41
0.2mM Cu	52.84±3.90^{a}	5.96 ± 0.75^{a}	404.99 ± 6.96^{a,b}	1288.75±17.22	1752.54
10^{-7} M epiBL	105.26±7.5^{a,b}	0.358±0.34^{a,b}	7.21 ± 0.98^{a,b}	n.d.	112.568
10^{-9} M epiBL	73.70 ± 3.34^{a}	13.82 ± 0.99^{a}	63.25 ± 5.42^{a,b}	n.d.	150.77
10^{-11} M epiBL	47.56 ± 2.09	7.07 ± 0.87^{a}	193.56 ± 5.55^{b}	n.d.	248.19
Cu + 10^{-7} M epiBL	60.37± 3.14^{a}	8.65 ± 0.98^{a}	242.24 ±12.12^{a,b}	2.98 ± 0.54^{b}	314.24
Cu + 10^{-9} M epiBL	44.72 ± 2.67	0.361 ± 0.03^{a}	7.30 ± 1.1^{a,b}	899.22 ± 21.15^{b}	951.601
Cu + 10^{-11} M epiBL	14.01 ± 0.92^{a}	2.24 ± 0.21a	16.24 ± 1.3^{a,b}	1127.25±28.93^{b}	1159.74

($^{a,\ b}$ indicate statistically significant differences from control and metal treatment at $p \leq 0.05$)

A significant decrease in Cad level 5.96 µg g^{-1} fw under metal stress was observed in comparison to 57.24 µg g^{-1} f.w. in control. Reduced levels of Cad were recorded in seedlings given treatments of 24-epiBL alone, more significantly at 10^{-7} M 24-epiBL (0.358 µg g^{-1} f.w.). Significant reduction in Cad contents was also recorded in seedlings, given combinations of Cu metal and 24-epiBL, with maximum decrease (0.361 µg g^{-1} f.w.) found in Cu metal and 10^{-9} M 24-epiBL treatment. Enhanced contents of Spd were recorded in seedlings exposed to 0.2 mM copper treatment (404.99 µg g^{-1} f.w.) in comparison to control (184 µg g^{-1} f.w.) seedlings (Table2). The treatments of 24-epiBL alone revealed a decrease in Spd levels from 190.34 µg g^{-1} f.w. in 10^{-11} M 24-epiBL to 63.25 µg g^{-1} f.w (10^{-9} M) and 7.21 µg g^{-1} f.w. in 10^{-7} M. Combinations of copper with different concentrations of 24-epiBL indicated still lower contents of Spd, with a significant decrease in Cu-metal and 10^{-9} (7.30 µg g^{-1} f.w.) and 10^{-11}(16.24 µg g^{-1} f.w.) M 24-epiBL combination. Enhancement in Spd level to 242.24 µg g^{-1} f.w. was observed in combination of copper metal with 10^{-7} M 24-epiBL (Table 2).

The Spm which was not detected in control seedlings was reported in seedlings exposed to copper (1288.75 µg g^{-1} f.w.). No Spm was recorded in seedlings treated with 24-epiBL alone (10^{-11}, 10^{-9} and 10^{-7} M). Significant decrease in Spm content was observed in seedlings where copper was supplemented with 24-epiBL (10^{-11}, 10^{-9} and 10^{-7} M), with maximum reduction (2.98 µg g^{-1} f.w.) found in 10^{-7} M 24-epiBL over Copper treatment alone.

Endogenous contents of auxins: Indole-3-acetic acid (IAA) and Naphthalene acetic acid (NAA) levels showed varied pattern under the influence of 24-epiBL treatment, Cu metal and their combinations (Table 3). Free IAA (7.06 µg/g f.w.) and bound IAA (2.03 µg/g f.w.) contents recorded in control seedlings got decreased to (2.56 µg/g f.w.) and (1.40 µg/g f.w.) under Cu metal stress (Table 3). Significant increases in the contents of both, free and bound auxins were recorded in seedlings given 24-epiBL treatment alone. Maximum rise in free IAA (12.40 µg/g f.w.) and bound IAA (13.81 µg/g f.w.) recorded in 10^{-7} M 24-epiBL treatment (Table 3) in comparison to free IAA (9.05 µg/g f.w.) and bound IAA (12.99 µg/g f.w.) in 10^{-9} M; free IAA (5.92 µg/g f.w.) and bound IAA (5.42 µg/g f.w.) in 10^{-11} M 24-epiBL treatment. Cu-metal treatment supplemented with 24-epiBL (10^{-7} M) showed maximum decrease in free IAA (0.53 µg/g f.w.), whereas lowest concentration of bound IAA was detected in seedlings given Cu metal supplemented with 10^{-9} M 24-epiBL. Endogenous levels of NAA showed significant changes under metal stress and 24-epiBL treatments. Both, free and bound NAA were not detected in untreated control seedlings and seedlings given 24-epiBL treatment alone (Table 3). Cu metal stress alone significantly enhanced the levels of free (34.71 µg/g f.w) and bound (22.27 µg/g f.w.) NAA (Table 3). Free and bound NAA levels were found to decrease in seedlings, where Cu metal treatment was supplemented with 24-epiBL. Maximum decrease in free (4.41 µg/g f.w.) and bound (4.81 µg/g f.w.) NAA was recorded in combination of Cu metal treatment with (10^{-7}M) 24-epiBL.

Table 3. Endogenous auxins content (µg/g f.w.) in 7days old seedlings of Raphanus subjected to Cu-metal and 24-epibrassinolide treatments. a, b indicate statistically significant differences from control and metal treatment at $p \leq 0.05$.

S.NO.	Auxins content (µg/g f.w.)				
	Indole acetic acid		Naphthalene acetic acid		Total (µg/g f.w.)
	Free	Bound	Free	Bound	
Control	7.06 ± 0.99	2.03 ± 0.14	n.d.	n.d.	9.09
0.2mM Cu	2.56 ± 0.39^{a}	1.40 ± 0.21	34.71 ± 4.2	22.27 ± 3.9	60.94
10^{-7} M epiBL	$12.40 \pm 0.473^{a,b}$	$13.81 \pm 0.638^{a,b}$	n.d.	n.d.	26.21
10^{-9} M epiBL	9.05 ± 0.460^{a}	$12.99 \pm 0.318^{a,b}$	n.d.	n.d.	22.04
10^{-11} M epiBL	5.92 ± 0.231^{b}	$5.42 \pm 0.202^{a,b}$	n.d.	n.d.	11.34
Cu + 10^{-7} M epiBL	$0.53 \pm 0.029^{a,b}$	n.d.	4.41 ± 0.207^{b}	4.81 ± 0.039^{b}	9.75
Cu + 10^{-9} M epiBL	6.7 ± 0.115^{b}	3.8 ± 0.275^{b}	6.1 ± 0.577^{b}	5.28 ± 0.111^{b}	21.88
Cu + 10^{-11} M epiBL	$9.60 \pm 0.240^{a,b}$	$12.99 \pm 0.260^{a,b}$	4.75 ± 0.220^{b}	5.14 ± 0.142^{b}	32.48

DISCUSSION

To the best of our knowledge, this is the first report dealing with the influence of 24-epiBL on the endogenous contents of PAs and auxins under heavy metal stress. In the present investigation exogenous application of 24-epiBL to *R. sativus* seedlings alone and under Cu metal stress revealed its stress ameliorative property. The seedling growth which was severely affected by metal treatment returned to almost normal values after 24-epiBL application. Maximum improvement in shoot length (4.71 cm) and root length (7.32) was recorded in seedlings given 10^{-11} M 24-epiBL treatment alone and in combination with Cu metal (6.32 and 3.45 cm) when compared to metal treated seedlings. Improvement in seedling growth on 24-epiBL application might be due to blockade of Cu uptake (Sharma and Bhardwaj, 2007). Changes in the endogenous contents of PAs have been associated with environmental stresses. Cu metal treatment increased levels of Put by 3.8 folds and Spd by 11.9 folds. Only Cad content reduced under metal treatment by 8.9 folds when compared to control. Metal treatment supplemented with 24-epiBL showed reduction in PAs contents. 24-epiBL treatment alone decreased levels of Cad and Spd, with maximum decrease in combination of Cu metal and 10^{-9} M 24-epiBL. Our findings are in agreement with early reports of PAs synthesis under stress. Groppa et al. (2003) reported enhanced contents of PAs in leaf discs of wheat or sunflower, subjected to Cd^{2+} or Cu^{2+} stress. Similarly significant increase in the levels of free PAs has been reported in Tobacco BY-2 cells exposed to 0.05mM $CdCl_2$ (Kuthanova et al., 2004). The synergistic interaction of BRs and auxins has been widely studied (Tanaka et al., 2003; Nemhauser et al., 2004). Reduced contents of free IAA by 6.3 folds and bound IAA by 3.1 folds were recorded in seedlings treated with Cu metal, when compared with controls. Supplementation of metal treatment with 24-epiBL further recorded decrease in free and bound IAA, with maximum decrease observed in metal and 10^{-7} M 24-epiBL combination. Free and bound IAA showed maximum increase in 24-epiBL treatment alone, while maximum NAA levels (free and bound) were recorded in metal treated seedlings only. In controls and 24-epiBL treated seedlings free and bound NAA was not detected. Moreover, a decrease in both forms of NAA was recorded in seedlings given treatments of Cu metal and 24-epiBL combinations. Enhanced contents of free and bound forms of NAA under metal treatment may suggest its effectiveness in oxidative stress management than IAA. However, supplementation of 24-epiBL with metal treatment reduced free NAA level by 8.72 folds and bound by 7.84 folds more significantly in 10^{-7} M conc, when compared with metal treated seedlings. Both forms of NAA were not detected in seedlings subjected to 24-epiBL treatment alone. This may suggest their production and involvement in stress management under metal treatment. Vert et al. (2008) studied synergistic interactions of BRs and auxins and reported integration of auxins and brassinosteriod pathways by auxins response factors responsible for synergism between BRs and auxins. Elevated expression of *TaIAA1* gene of Aux/IAA gene family on application of 24-epiBL in wheat was also recorded (Singla et al., 2006). Similarly, the stress protective role of auxins against heavy metals was also observed by Dimpka et al. (2008) in *Streptomyces* spp. Enhanced expression of *Ospdr9*, which encodes PDR type ABC transporter by naphthalene acid under heavy metals, hypoxic stress and redox perturbations, was reported by Moons (2003) in rice shoots Results of the present investigation suggest significant effects of 24-epiBL on endogenous contents of PAs and auxins under heavy metal stress, thereby indicating the modulation of stress management by BRs *via* regulating the contents of PAs and auxins.

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