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24-EPIBRASSINOLIDE (EBR) REDUCES OXIDATIVE STRESS DAMAGE INDUCED BY CADMIUM TOXICITY BY RESTRICTING CD UPTAKE AND MODULATING SOME KEY ANTIOXIDANT ENZYMES IN MAIZE PLANTS

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Abstract

The current investigation was performed to assess the mitigating role of externally supplemented 24-epibrassinolide (EBR) against cadmium (Cd, 100 and 200 µM) induced stress in maize plants. Cadmium toxicity decreased shoot and root lengths, pigment content, and relative water content, whereas it raised proline content in the maize plants. Cadmium stress also increased the generation of oxidative stress biomarkers like hydrogen peroxide (H_2O_2), lipid peroxidation (determined as malondialdehyde content, MDA) and electrolyte leakage (EL). Highest concentration (200 µM) of Cd showed a maximum enhancement in the oxidative stress biomarkers than 100 µM. The activities of enzymatic antioxidants increased markedly with the Cd stress. The activities of catalase (CAT) and superoxide dismutase (SOD) increased by 42.10% and 23.5% with 200 µM Cd and 64.21% and 54.50% with 200 µM Cd, respectively, whereas the GST activity was decreased by 13.84% with 100 µM and 28.75% with 200 µM Cd. However, EBR application restored the Cd-induced reduction in growth parameters and physio-biochemical attributes EBR supplemented plants also abated the accretion of H_2O_2 and MDA contents and EL. The supplementation of EBR restored the GST activity and it also helped to further enhance the activities of SOD and CAT in the Cd stressed plants. The findings suggest that external supplementation of EBR provides a protection to maize plants against Cd toxicity by modifying growth attributes, and regulating physio-biochemical characteristics and some critical antioxidants.

Key words: Antioxidants; Cd toxicity; Growth; Maize; Oxidative stress biomarkers; Proline.

Introduction

Metal contamination has recently acquired a significant momentum throughout the globe. It has a promising adverse influence on growth of plants. Cadmium (Cd) being a non-essential and noxious heavy metal affects plants as well as animals (Wu *et al.*, 2017). For example, plants exposed to Cd toxicity showed reduced growth because of restricted uptake of mineral elements, reduced activity of metabolic enzymes and oxidative stress-induced injuries to biomolecules (Rahman *et al.*, 2017; Wu *et al.*, 2017). Cadmium toxicity has been shown to affect growth (Liu *et al.*, 2012), reduce photosynthetic activity (Burzyński & Żurek, 2007), and damage protein structures (Kabir *et al.*, 2016). These effects might be due to osmotic as well as oxidative stress of the metal. Plants are known to withstand the osmotic stress by producing osmoprotectants like proline, glycine betaine, soluble sugars and soluble proteins. These osmoprotectants can effectively protect the plant cell from dehydration stress and enhance absorption of nutrients, which is generally hampered by different heavy metals including Cd (Jan *et al.*, 2018). Cadmium stress can significantly genertae abundant amount of reactive oxygen species (ROS) in plants (Ahmad *et al.*, 2011; Andersen & Kupper, 2013; Rahman *et al.*, 2016) and they could react with biomolecules and modify their functions. However, plants are equipped with natural defense system known as antioxidant system which includes: superoxide dismutase (SOD) catalase (CAT) and glutathione *S*-transferase (GST) etc. (Hasanuzzaman *et al.*, 2012; Rahman *et al.*, 2016).

Among the cereal crops, maize (*Zea mays* L.) is an essential food crop. It is cultivated mostly in Asian countries. However, most of the soils of this region are affected with heavy metal pollution, because of urbanization, heavy vehicles and industrial setups and other anthropogenic activities (Faostat, 2015). The growth of crop plants including maize is quite tough in these soils. To keep in mind the progressive growth of human population and the limited cultivated land resources, we have to manage more crop production within these limited resources. So, the strategy was to make a sustainable attempt to strengthen the tolerance of the maize plants against the Cd stress with the external application of phytohormones.

The utilization of phytohormones to improve plant tolerance to environmental stresses has recently gained a ground. Brassinosteroids-induced promotion of growth and alleviation of metal stress have already been documented (Alam *et al.*, 2019). One of the commonly used brassinosteroids is 24-epibrassinolide (EBR), which is documented to boost the growth, photosynthesis and other metabolic activities under normal (non-stress) and stressful cues (Divi *et al.*, 2010; Ahammed *et al.*, 2013; Fariduddin *et al.*, 2013; Kohli *et al.*, 2018). The positive role of EBR might be because of its interacting role with other cellular molecules to mitigate a stress. It may also help up-regulate the defense systems like antioxidant system in plants under stressful cues.

Material and Methods

Viable caryopses of maize (*Zea mays* L.) were sanitized with 0.1% NaOCl solution for 6 min and then washed briefly with double distilled water. The sterilized caryopses were implanted in pots filled with sand, perlite and peat (1:1:1). After 4 days of germination, the seedlings were provided with an aliquot of 200 mL of Hoagland's nutrient solution to each pot daily for 10 days. After 10 days, the seedlings were subjected to varying Cd concentrations (0, 100 and 200 µM) dissolved in Hoagland's solution and were supplied on alternate days for 10 days (20-day old seedlings) and the control plants received Hoagland's solution only. The 24-EBR (10^{-7} mM, 20 mL per pot) with Teepol (0.1 %) as a surfactant was supplied as a foliar spray to the control and Cd treated seedlings on alternate days for 10 days. The plants were allowed to grow under greenhouse conditions with temperature 25/15 day/night, 70-75% relative humidity and 18 h of light period and 6 h of dark period. After 30 days of growth, the seedlings were harvested and measured for various biochemical attributes.

Determination of shoot and root length and pigment content: The root and shoot lengths were appraised with a normal scale. Fresh leaf sample (each 200 mg) was ground in acetone and then centrifuged at 12000x*g* for 5 min. The OD of the supernatant collected was recorded at 480, 645, and 663 nm (Arnon, 1949).

Evaluation of leaf relative water content (LRWC) and proline content: The procedure provided by Yamasaki & Dillenburg (1999) was used for the assessment of LRWC and the following formula was employed to calculate LRWC:

LRWC = Fresh weight−Dry weight /Turgid weight−Dry weight

The spectrophotometric protocol proposed by Bates *et al*., (1973) was pursued for the assessment of proline levels.

Estimation of H_2O_2, MDA content and EL: H_2O_2 content was appraised by the method described by Velikova *et al*., (2000). The OD was noted at 390 nm.

MDA content was analyzed by the protocol of Madhava Rao & Sresty (2000). The OD was noted at 532 and 600 nm.

For the assessment of EL, the method of Dionisio-Sese & Tobita (1998) was used. Following formula was employed to calculate EL:

Electrolyte leakage = (EC1 − EC0)/(EC2 − EC0) × 100
EC0=electrical conductivity (EC) at room temperature
EC1= EC at 50°C
EC2= EC at 100°C

Activities of antioxidant enzymes: A leaf sample (200 mg) was ground in a mortar and pestle containing potassium phosphate buffer (100 mM, pH 7.0) and polyvinyl pyrrolidone. The ground material was centrifuged at 12000x*g* for 30 min at 4°C, and the collected supernatant was utilized for antioxidant assay.

The method of Dhindsa & Matowe (1981) was pursued for the assay of superoxide dismutase (SOD, EC1.15.1.1) activity. The OD of all treated samples was recorded at 560 nm. The sum of protein inhibiting the photochemical reduction of NBT by 50 percent was recorded as one Unit of SOD.

Catalase (CAT, EC1.11.1.6) activity was appraised by the protocol provided by Aebi (1984). The OD was taken at 240 nm and the activity was shown as EU mg^{-1} protein.

The method of Hossain *et al.*, (2006) was utilized for the assay of GST (EC 2.5.1.18) activity and the OD was recorded at 340 nm.

Statistical analysis

All data sets were individually subjected to one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). The data is the mean of five replications. Significant differences among the mean values were worked out using DMRT at the 5% probability.

Results

Growth and pigment content: The lengths of shoot and root were decreased by 40.11% and 24.89%, respectively, with Cd (100 µM) concentration compared to the control plants (Fig. 1A-B). The 200 µM Cd concentration further decreased the shoot length by 56.74% and the root length by 53.04% over the controls. However, supply of EBR boosted the shoot length and root lengths by 21.29% and 23.81%, respectively, with EBR+100 µM Cd compared with Cd alone treated plants. The treatment EBR+200 µM also enhanced the shoot length by 20.52% and root length by 39.17% over those of the Cd-alone treated plants.

Total chlorophyll and carotenoid contents were found to be suppressed by 50.19% and 23.80% with 100 µM Cd and 62.35% and 50.00% with 200 µM Cd, respectively, relative to the controls. The supplementation of EBR raised the total chlorophyll content by 17.32% and 19.79% and carotenoid content by 15.62% and 14.28% with EBR+100 µM and EBR+200 µM, respectively, compared to those in Cd-alone treated counterparts (Fig. 2A-B).

RWC and proline content: Leaf relative water content (LRWC) decreased to 74.88% with 100 µM and 64.72% with 200 µM over the controls. EBR application enhanced the RWC up to 82.15% and 70.39% with EBR+100 µM and EBR+200 µM, respectively (Fig. 2C). The Cd stress applied as 100 and 200 µM enhanced the proline content by 27.71% and 156.02% respectively, compared to the controls. The EBR supplementation decreased the proline content by 16.50% with EBR+100 µM Cd and by 45.41% with 200 µM Cd with respect to those in the Cd-alone treated counterparts (Fig. 2D).

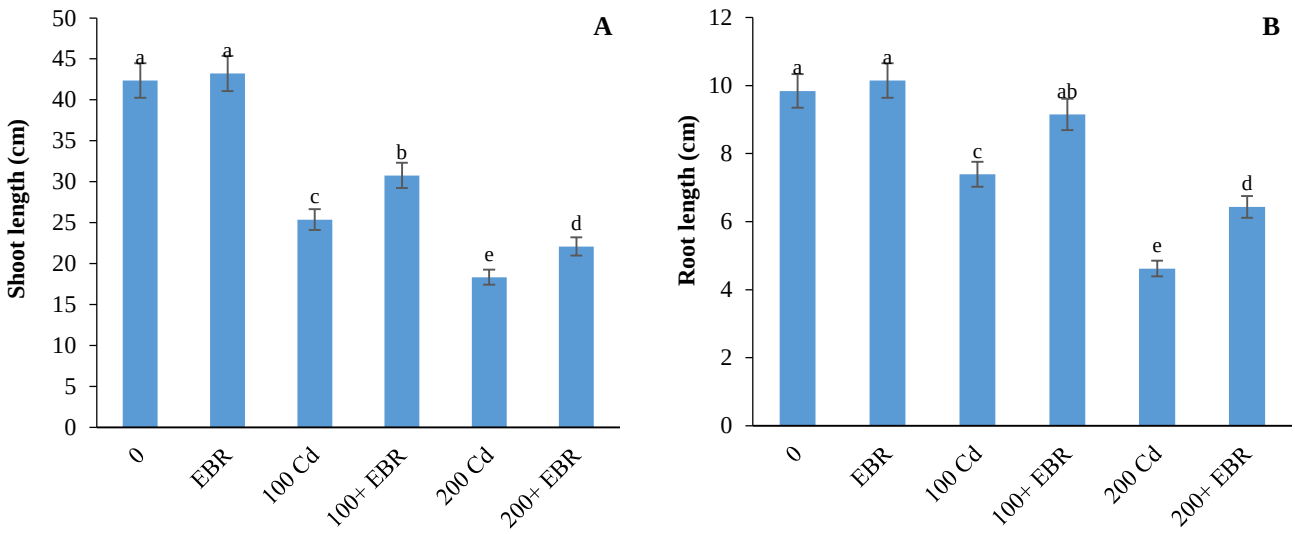


Fig. 1. Effect of external supplementation of 24-epibrassinolide on (A) shoot length and (B) root length in maize plants under Cd toxicity. Each treatment is a mean (±SE) of 5 replicates and different letters on bars denote significant differences among the mean values at $p \leq 0.05$ according to the Duncan's Multiple Range test.

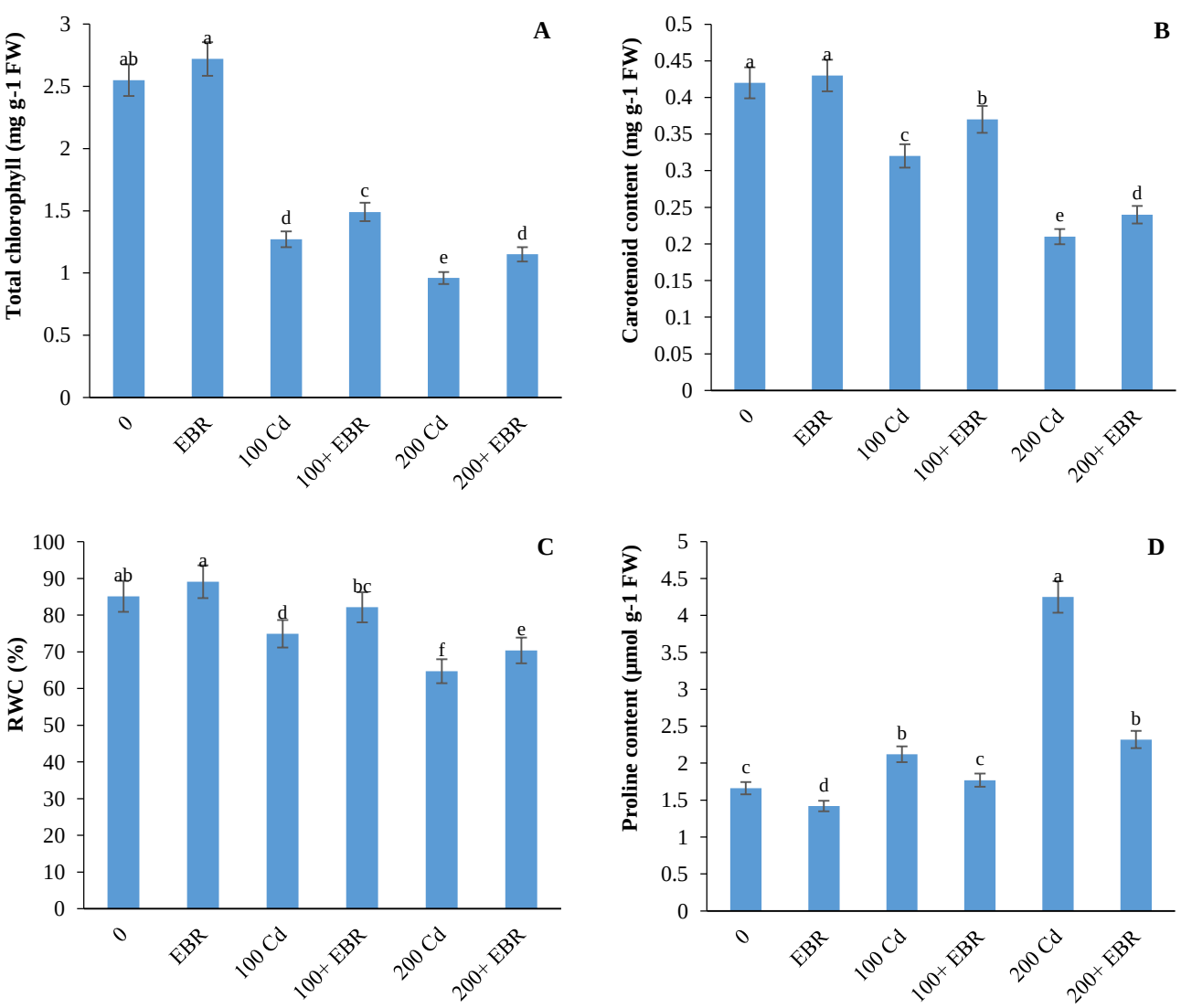
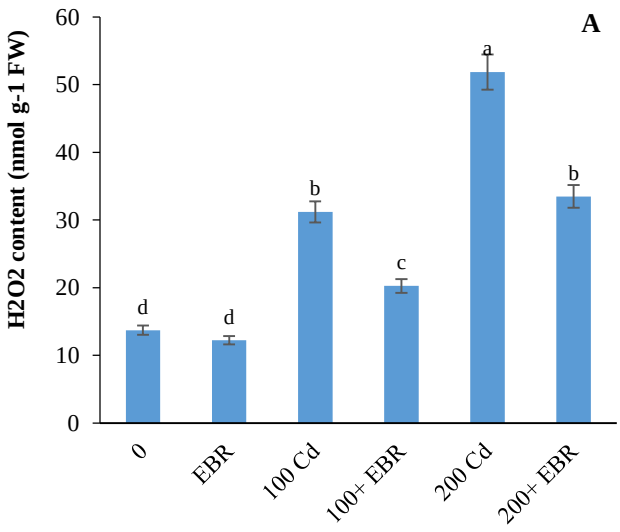
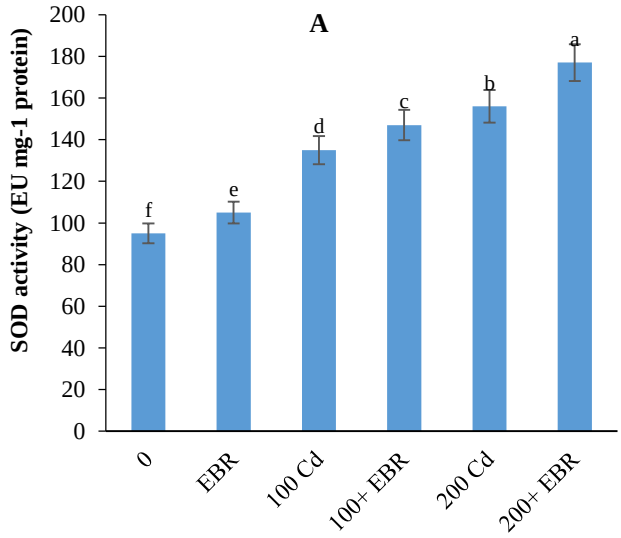
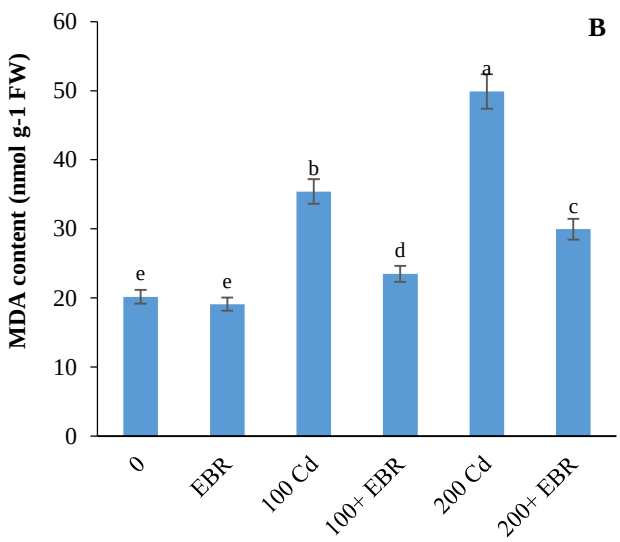
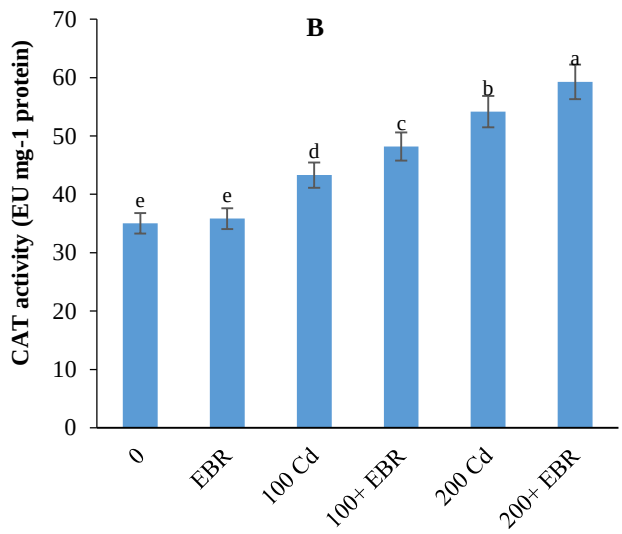


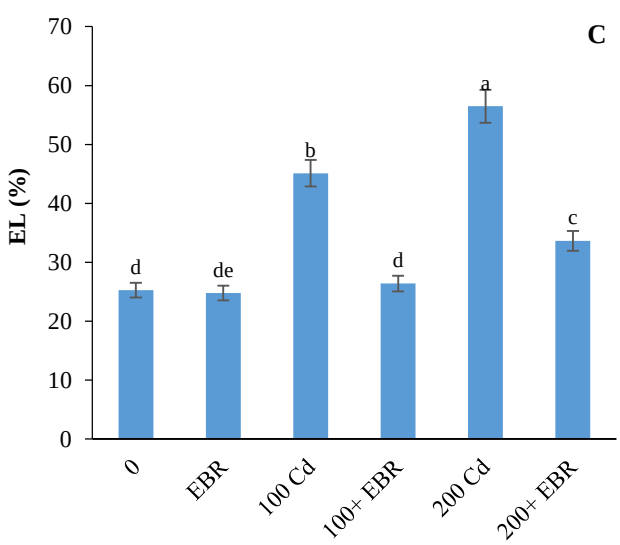
Fig. 2. External supplementation of 24-epibrassinolide enhanced (A) total chlorophyll (B) carotenoid content (C) RWC and (D) proline content in maize plants under Cd toxicity. Each treatment is a mean (±SE) of 5 replicates and different letters on bars denote significant differences among the mean values at $p \leq 0.05$ according to the Duncan's Multiple Range test.











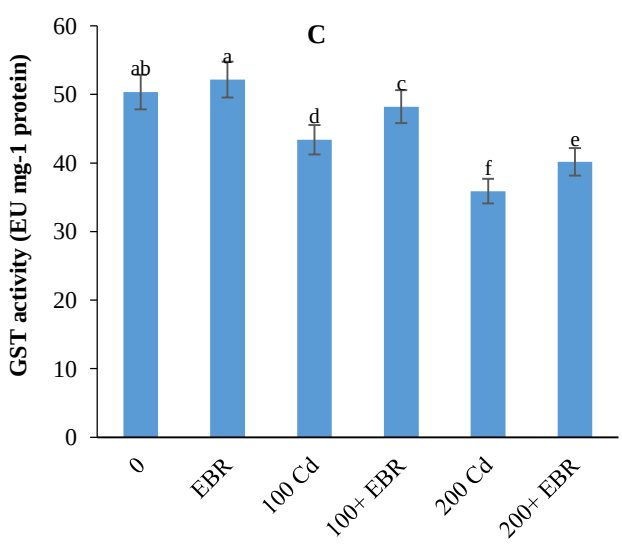


Fig. 3. Application of 24-epibrassinolide declined the production of (A) H_2O_2 content, (B) MDA content and (C) EL in maize plants under Cd toxicity. Each treatment is a mean (±SE) of 5 replicates and different letters on bars denote significant differences among the mean values at $p \leq 0.05$ according to the Duncan's Multiple Range test.

Fig. 4. Supplementation of 24-epibrassinolide enhanced the activities of (A) SOD (B) CAT and (C) GST in maize plants under Cd toxicity. Each treatment is a mean (±SE) of 5 replicates and different letters on bars denote significant differences among the mean values at $p \leq 0.05$ according to the Duncan's Multiple Range test.

H_2O_2 and MDA contents and EL: The contents of H_2O_2 and MDA as well as EL were found to be enhanced by 127.71%, 75.64% and 45.11% with 100 µM Cd treatment, respectively, over the controls (Fig. 3A-C). Further increase by 278.10% in H_2O_2, 147.47% in MDA, and 56.47% in EL was recorded with 200 µM Cd concentration with reference to the controls. However, the EBR supplementation declined the accumulation of H_2O_2, MDA and EL by 35.07%, 33.69% and 42.10% with EBR+100 µM Cd treatments relative to those in the Cd-alone treated plants. The treatment of EBR+200 µM Cd also lowered down the accumulation of H_2O_2 by 35.40% that of MDA by 39.96% and of EL by 13.46% compared to those in the Cd-alone treated plants.

Antioxidant system (Enzymes): The activities of SOD and CAT increased by 42.10% and 23.53% with 100 µM Cd and 64.21% and 54.50% with 200 µM Cd, respectively, compared with controls (Fig. 4A-B). Externally applied EBR further boosted the SOD activity by 8.88% and the CAT activity by 11.33% with EBR+100 µM Cd treatment compared to the Cd –alone treated plants. The treatment EBR+200 µM Cd also showed enhanced activities of SOD and CAT by 13.46% and 9.45% relative to those in the 200 µM Cd stressed plants only. The GST activity decreased by 13.84% and 28.75% with 100 and 200 µM Cd, respectively, over the controls. However, the EBR supplementation elevated the activity of GST by 11.13% with EBR+100 µM Cd and 11.90% with EBR+200 µM Cd relative to that of the Cd-alone treated plants (Fig. 4C).

Discussion

There was a considerable decline in growth and biomass yield in the maize seedlings on exposure to Cd toxicity in the current study. Decrease in lengths of shoot and root, and biomass yield has been also reported by different researchers such as Rady (2011) in *Phaseolus vulgaris*, Tripathi *et al.*, (2012) in *Oryza sativa*, and Ahmad *et al.*, (2011) *Brassica juncea*. The restricted growth due to Cd toxicity could have been due to hindrance in cell division and other processes during cell growth (Irfan *et al.*, 2014). In view of Gomes *et al.*, (2013) high amount of Cd restricts the uptake of nutrients and also hampers sufficient water uptake, so these might be the potential causes of reduced growth and development under Cd stress. However, supplementation of 24-EBL brought revival of the growth attributes under Cd toxicity and these findings coincide with those of Ali *et al.*, (2007). Plants experiencing metal toxicity, when provided with external EBL showed enhanced photosynthesis, so it could be the main reason of enhanced growth with EBL (Ali *et al.*, 2007). Another reason could be the activation of H^+-ATPase which activates cell wall loosening enzymes (Cerana *et al.*, 1983) thereby promoting growth of the cells by cell division and elongation.

Cadmium stress reduced the pigment content in the current study and analogous findings were documented by Khan *et al.*, (2015) in mustard. The reduced pigment content during metal toxicity could be due to modifications in the chlorophyll biosynthetic pathway (Ahanger *et al.*, 2016). The elevated activity of chlorophyllase during a stressful environment could be the main reason for chlorophyll degradation (Fang *et al.*, 1998). Lenti *et al.*, (2002) documented that mercury (Hg) toxicity reduced the biosynthesis of chlorophyll, because it impedes the NADPH protochlorophyllide oxidoreductase activity. Enhanced chlorophyll content due to the supplementation of EBL in the current experiment coincides with the results reported by Jan *et al.*, (2018) in pea plants. EBL promotes the uptake of mineral elements including magnesium (Mg) which is the main constituent of chlorophyll molecule. Choudhary *et al.*, (2012) reported that supplementation of EBL restored the growth and improved the pigment content in *Raphanus sativus* under chromium toxicity. Carotenoids function as antioxidants and they help reduce the accumulation of ROS in the cell, thereby safeguarding the biomolecules from oxidative damage (Sharma *et al.*, 2012). Supplementation of EBL restricts the Cd uptake to upper parts of the plant as has been reported earlier in different plants (Bajguz, 2000; Kanwar *et al.*, 2013). According to Li *et al.*, (2016) BR biosynthetic pathway up-regulates the Calvin cycle enzymes' activities, thus resultantly enhancing photosynthesis.

The reduction in LRWC during Cd toxicity in the current study matches with the reports of Ahmad *et al.*, (2018) in tomato and Rady (2011) in common bean. Cadmium is responsible for the reduction of hydraulic conductivity which reduces cellular turgor, thereby declining LRWC (Ehlert *et al.*, 2009). According to Bayoumi *et al.*, (2008), the reduction in LRWC with Cd stress may occur due to restricted water uptake by the plant roots. The supplementation of EBR enhanced the LRWC in the current investigation. EBR might have restricted the uptake of Cd as is evident from the data presented here, which might have allowed the plants to absorb more water.

Proline content increased due to Cd toxicity in the current study. Such identical findings have been earlier reported by Hashem *et al.*, (2016) in tomato and Ahmad *et al.*, (2015) in mustard. Hayat *et al.*, (2012) also showed the increased content of proline in tomato under Cd stress. Accumulation of proline under a stress has been testified to maintain tissue water potential (Ahanger *et al.*, 2014; Ahanger *et al.*, 2015; Ahmad *et al.*, 2015). Proline accumulation under a stress is attributed to elevated proline synthesizing enzyme activity (Khan *et al.*, 2015). Further enhancement in proline content due to EBR has also been recorded in mustard (Hayat *et al.*, 2007), mung bean (Ali *et al.*, 2008), common bean (Rady, 2011), and peanut (Song *et al.*, 2016) plants. Application of EBR enhanced the proline content under other metal stress elements like chromium (Choudhary *et al.*, 2011), zinc (Ramakrishna & Rao, 2012), and mercury (Ahmad *et al.*, 2018). Enhanced synthesis of proline due to EBR helps the plant to maintain photosynthetic efficiency and growth under stress conditions (Ali *et al.*, 2008). Proline helps in osmoregulation, membrane stability and alleviation of Cd toxicity because it can reinstate enzyme activity hydrating the enzymes (Kishor *et al.*, 2005). EBR enhanced proline content, which could have been due to elevated proline metabolism accompanied with decreased proline catabolism.

The present study showed that the Cd stress enhanced the formation of H_2O_2 and MDA (lipid peroxidation) contents and electrolyte leakage. Analogous findings exhibiting enhanced levels of H_2O_2 and lipid peroxidation have been shown by Ahmad *et al.*, (2011) in *Brassica juncea*, Hashem *et al.*, (2016) in *Solanum lycopersicum*, and Anjum *et al.*, (2011) in *Vigna radiata*. Enhanced H_2O_2 content escalates lipid peroxidation, affecting membrane fluidity and ultimately enhanced electrolyte leakage (Garg & Manchanda, 2009). H_2O_2 being a strong ROS produced as a by-product of photosynthesis and respiration, has high affinity for biomolecules such as DNA and lipids (Tuteja *et al.*, 2009). This H_2O_2 induced lipid peroxidation may also affect enzyme activity and inhibit protein channeling (Garg & Manchanda, 2009). According to Macri *et al.*, (1994) the H_2O_2-induced lipid peroxidation might have been due to elevated lipoxygenase enzyme activity. The supplementation of EBR helped in reducing H_2O_2 and MDA contents and electrolyte leakage. The reason could have been the elevation in antioxidant enzyme activities that quench the H_2O_2 and other oxygen radicals (Raghu *et al.*, 2014; Hashem *et al.*, 2016; Ahmad *et al.*, 2018). The EBR-treated plants showed lower production of H_2O_2 and MDA contents and electrolyte leakage which support the role of EBR in safeguarding the membranes from peroxidation, thus alleviating Cd-induced toxicity. According to Song *et al.*, (2016), supplementation of EBL increases membrane stability due to reduced production of H_2O_2 and MDA contents.

The results for the enhanced activities of SOD and CAT and under Cd stress corroborate with the findings of Melo *et al.*, (2011) in soybean, Shan *et al.*, (2012) in peanut, Ahmad *et al.*, (2011) in mustard, Ahmad *et al.*, (2018) in tomato, and Singh and Prasad (2014) in eggplant. Ahmad *et al.*, (2018) also reported elevated activities of SOD, CAT and other antioxidants in tomato under Hg stress. Analogous findings have been documented in rice (Chen *et al.*, 2012) and wheat (Sahu *et al.*, 2012). SOD helps in the conversion of superoxide radicals to H_2O_2 and then CAT acts on H_2O_2 and breaks it to water and nascent oxygen (Ahmad *et al.*, 2010). Glutathione *S*-transferase (GST) is also involved in oxidative stress management (Conklin and Last, 1995). The endogenous electrophile generation under oxidative stress is quenched by GST in combination with GSH (Kapoor *et al.*, 2014). GST has been reported to function as glutathione peroxidase under stress conditions and it safeguards the plant cell against oxidative damage (Roxas *et al.*, 1997; Cummins *et al.*, 1999). Ezaki *et al.*, (2001) also reported that over-expression of GST in *Arabidopsis* enhanced aluminum (Al) tolerance. The supplementation of EBL boosted the antioxidant enzyme activity in the current study and these findings coincide with those of Hayat *et al*., (2007) in *Brassica juncea*, and Hayat *et al.*, (2012) in *Solanum lycopersicum*. Jin *et al.*, (2015) reported that temperature stress boosts the GST activity which in turn escalates growth in *Ficus concinna*. The EBR supplementation enhanced the GST activity in the current study and parallel findings were recorded in *Raphanus sativus* by the application of EBL during metal stress. The enhanced activity of antioxidants by the application of brassinosteroids was also shown by Fariduddin *et al.*, (2009) and Fariduddin *et al.*, (2011). In the current study, EBR application strengthened the potential of antioxidant enzymes in the maize plants to prevent them from oxidative damage.

Conclusion

Cadmium stress showed its toxic effects in terms of reduced growth, decreased photosynthetic pigments and fluctuations in key physio-biochemical processes. However, EBL supplementation mitigated the harmful impact of Cd and boosted the growth and pigment concentration. Proline accumulation due to EBL supplementation maintained leaf water potential, thereby helping the plants to sustain Cd stress. The oxidative stress markers were also declined due to EBL supplementation. The EBL application up-regulated the antioxidant system that quenched the ROS and provided protection to plants from oxidative damage. Overall, the EBL supplementation enhanced growth performance of maize plants under Cd stress through modulating physio-biochemical attributes and some key antioxidants.

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References

Aebi, H. 1984. Catalase in vitro. In: (Eds.): Packer L. *Methods in Enzymology*. Academic Press, Orlando. 105, pp: 121-126.

Ahammed, G.J., Y.H. Zhou, X.J. Xia, W.H. Mao, K. Shi and J.Q. Yu. 2013. Brassinosteroid regulates secondary metabolism in tomato towards enhanced tolerance to phenanthrene. *Biol. Plant.*, 57(1): 154-158.

Ahanger, M.A., R.M. Agarwal, N.S. Tomar and M. Shrivastava. 2015. Potassium induces positive changes in nitrogen metabolism and antioxidant system of oat (*Avena sativa* L. cultivar Kent). *J. Plant Interact.*, 10(1): 211-223.

Ahanger, M.A., N. Morad-Talab, E.F. Abd-Allah, P. Ahmad and R. Hajiboland. 2016. Plant growth under drought stress. In: Water Stress and Crop Plants. John Wiley & Sons, Ltd. pp: 649-668.

Ahanger, M.A., S.R. Tyagi, M.R. Wani and P. Ahmad. 2014. Drought tolerance: role of organic osmolytes, growth regulators, and mineral nutrients. In: (Eds.): Ahmad, P. and M.R. Wani. *Physiological mechanisms and adaptation strategies in plants under changing environment*. Springer New York: pp: 25-55.

Ahmad, P., M.A. Ahanger, M.N. Alyemeni, L. Wijaya and P. Alam. 2018. Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. *Protoplasma*, 255(1): 79-93.

Ahmad, P., M.A. Ahanger, D. Egamberdieva, P. Alam, M.N. Alyemeni and M. Ashraf. 2018. Modification of psmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea seedlings under mercury (Hg) toxicity. *J. Plant Growth Regul.*, 37(1): 309-322.

Ahmad, P., A. Hashem, E.F. Abd-Allah, A.A. Alqarawi, R. John, D. Egamberdieva and S. Gucel. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Front. Plant Sci.*, 6: 868.

Ahmad, P., C.A. Jaleel, M.A. Salem, G. Nabi and S. Sharma. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotechnol.*, 30(3): 161-175.

Ahmad, P., G. Nabi and M. Ashraf. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *S. Afr. J. Bot.*, 77(1): 36-44.

Alam, P., T.H. Albalawi, F.H. Altalayan, M.A. Bakht, M.A. Ahanger, V. Raja, M. Ashraf and P. Ahmad. 2019. 24-Epibrassinolide (EBR) confers tolerance against NaCl stress in soybean plants by up-regulating antioxidant system, ascorbate-glutathione cycle, and glyoxalase system. *Biomolecules*, 9(11): 640.

Ali, B., S.A. Hasan, S. Hayat, Q. Hayat, S. Yadav, Q. Fariduddin and A. Ahmad. 2008. A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). *Environ. Exp. Bot.*, 62(2): 153-159.

Ali, B., S. Hayat and A. Ahmad. 2007. 28-homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). *Environ. Exp. Bot.*, 59(2): 217-223.

Andersen, E. and H. Kupper. 2013. Cadmium toxicity in plants. In: (Eds.): Sigel, A., H. Sigel and R.K.O. Sigel. Cadmium: From toxicity to essentiality. Series, Metal Ions in Life Sciences, Springer Science+ Business Media, Dordrecht.

Anjum, N.A., S. Umar, M. Iqbal and N.A. Khan. 2011. Cadmium causes oxidative stress in mung bean by affecting the antioxidant enzyme system and ascorbate-glutathione cycle metabolism. *Russ. J. Plant Physiol.*, 58(1): 92-99.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24(1): 1-15.

Bajguz, A. 2000. Effect of brassinosteroids on nucleic acids and protein content in cultured cells of *Chlorella vulgaris*. *Plant Physiol. Biochem.*, 38(3): 209-215.

Bayoumi, T.Y., M.H. Eid and E.M. Metwali. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *Afr. J. Biotechnol.*, 7(14): 2341-2352.

Burzyński, M. and A. Żurek. 2007. Effects of copper and cadmium on photosynthesis in cucumber cotyledons. *Photosynthetica*, 45(2): 239-244.

Cerana, R., A. Bonetti, M.T. Marre, G. Romani, P. Lado and E. Marre. 1983. Effects of a brassinosteroid on growth and electrogenic proton extrusion in Azuki bean epicotyls. *Physiol. Plant.*, 59(1): 23-27.

Chen, Y.A., W.C. Chi, T.L. Huang, C.Y. Lin, T.T. Quynh Nguyeh, Y.C. Hsiung, L.C. Chia and H.J. Huang. 2012. Mercury-induced biochemical and proteomic changes in rice roots. *Plant Physiol. Biochem.*, 55: 23-32.

Choudhary, S.P., M. Kanwar, R. Bhardwaj, B.D. Gupta and R.K. Gupta. 2011. Epibrassinolide ameliorates Cr (VI) stress via influencing the levels of indole-3-acetic acid, abscisic acid, polyamines and antioxidant system of radish seedlings. *Chemosphere*, 84(5): 592-600.

Choudhary, S.P., M. Kanwar, R. Bhardwaj, J.Q. Yu and L.S. Tran. 2012. Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in *Raphanus sativus* L. *PLoS One*, 7(3): e33210.

Conklin, P.L. and R.L. Last. 1995. Differential accumulation of antioxidant mRNAs in *Arabidopsis thaliana* exposed to ozone. *Plant Physiol.*, 109(1): 203-212.

Cummins, I., D.J. Cole and R. Edwards. 1999. A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. *Plant J.*, 18(3): 285-292.

Dhindsa, R.S. and W. Matowe. 1981. Drought tolerance in two mosses: Correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.*, 32(1): 79-91.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.*, 135(1): 1-9.

Divi, U.K., T. Rahman and P. Krishna. 2010. Brassinosteroid-mediated stress tolerance in Arabidopsis shows interactions with abscisic acid, ethylene and salicylic acid pathways. *BMC Plant Biol.*, 10: 151.

Ehlert, C., C. Maurel, F. Tardieu and T. Simonneau. 2009. Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiol.*, 150(2): 1093-1104.

Ezaki, B., M. Katsuhara, M. Kawamura and H. Matsumoto. 2001. Different mechanisms of four aluminum (Al)-resistant transgenes for Al toxicity in Arabidopsis. *Plant Physiol.*, 127(3): 918-927.

Fang, Z.Y., J.C. Bouwkamp and T. Solomos. 1998. Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. *J. Exp. Bot.*, 49(320): 503-510.

Faostat, 2015. Food and Agriculture Organization of the United Nations (FAO). Rome: Organization of the United Nations Statistics Division.

Fariduddin, Q., R.R. Khalil, B.A. Mir, M. Yusuf and A. Ahmad. 2013. 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. *Environ. Monit. Assess.*, 185(9): 7845-7856.

Fariduddin, Q., M. Yusuf, S. Chalkoo, S. Hayat and A. Ahmad. 2011. 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. *Photosynthetica*, 49(1): 55-64.

Fariduddin, Q., M. Yusuf, S. Hayat and A. Ahmad. 2009. Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. *Environ. Exp. Bot.*, 66(3): 418-424.

Garg, N. and G. Manchanda. 2009. ROS generation in plants: Boon or bane? *Plant Biosystems*, 143(1): 81-96.

Gomes, M.P., T.C.L.L.S.E.M. Marques and A.M. Soares. 2013. Cadmium effects on mineral nutrition of the Cd-hyperaccumulator *Pfaffia glomerata*. *Biologia*, 68(2): 223-230.

Hasanuzzaman, M., M.A. Hossain, J.A.T. da Silva and M. Fujita. 2012. Plant response and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: (Eds.): Venkateswarlu, B., A.K. Shanker, C. Shanker and M. Maheswari. *Crop stress and its management: Perspectives and strategies*. Springer Netherlands: pp: 261-315.

Hashem, A., E.F. Abd Allah, A.A. Alqarawi, A.A. Al Huqail, D. Egamberdieva and S. Wirth. 2016. Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. *Saudi J. Biol. Sci.*, 23(2): 272-281.

Hayat, S., B. Ali, S.A. Hasan and A. Ahmad. 2007. Brassinosteroid enhanced the level of antioxidants under cadmium stress in *Brassica juncea*. *Environ. Exp. Bot.*, 60(1): 33-41.

Hayat, S., M.N. Alyemeni and S.A. Hasan. 2012. Foliar spray of brassinosteroid enhances yield and quality of *Solanum lycopersicum* under cadmium stress. *Saudi J. Biol. Sci.*, 19(3): 325-335.

Hossain, M.Z., M.D. Hossain and M. Fujita. 2006. Induction of pumpkin glutathione S-transferases by different stresses and its possible mechanisms. *Biol. Plant.*, 50(2): 210-218.

Irfan, M., A. Ahmad and S. Hayat. 2014. Effect of cadmium on the growth and antioxidant enzymes in two varieties of *Brassica juncea*. *Saudi J. Biol. Sci.*, 21(2): 125-131.

Jan, S., M.N. Alyemeni, L. Wijaya, P. Alam, K.H. Siddique and P. Ahmad. 2018. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in *Pisum sativum* L. seedlings. *BMC Plant Biol.*, 18(1): 146.

Jin, S.H., X.Q. Li, G.G. Wang and X.T. Zhu. 2015. Brassinosteroids alleviate high-temperature injury in *Ficus concinna* seedlings via maintaining higher antioxidant defence and glyoxalase systems. *AoB Plants*, 7: plv009.

Kabir, A.H., M.M. Hossain, M.A. Khatun, A. Mandal and S.A. Haider. 2016. Role of silicon counteracting cadmium toxicity in alfalfa (*Medicago sativa* L.). *Front. Plant Sci.*, 7: 1117.

Kanwar, M.K., R. Bhardwaj, S.P. Chowdhary, P. Arora, P. Sharma and S. Kumar. 2013. Isolation and characterization of 24-Epibrassinolide from *Brassica juncea* L. and its effects on growth, Ni ion uptake, antioxidant defense of Brassica plants and in vitro cytotoxicity. *Acta Physiol. Plant.*, 35(4): 1351-1362.

Kapoor, D., S. Kaur and R. Bhardwaj. 2014. Physiological and biochemical changes in *Brassica juncea* plants under Cd-induced stress. *BioMed. Res. Int.*, 2014: 726070.

Khan, M.I., F. Nazir, M. Asgher, T.S. Per and N.A. Khan. 2015. Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat. *J. Plant Physiol.*, 173: 9-18.

Kishor, P.B.K., S. Sangam, R.N. Amrutha, P.S. Laxmi, K.R. Naidu, K.R.S.S. Rao, S. Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.*, 88(3): 424-438.

Kohli, S.K., N. Handa, A. Sharma, V. Gautam, S. Arora, R. Bhardwaj, M.N. Alyemeni, L. Wijaya and P. Ahmad. 2018. Combined effect of 24-epibrassinolide and salicylic acid mitigates lead (Pb) toxicity by modulating various metabolites in *Brassica juncea* L. seedlings. *Protoplasma*, 255(1): 11-24.

Lenti, K., F. Fodor and B. Boddi. 2002. Mercury inhibits the activity of the NADPH: protochlorophyllide oxidoreductase (POR). *Photosynthetica*, 40(1): 145-151.

Li, X.J., X. Guo, Y.H. Zhou, K. Shi, J. Zhou, J.Q. Yu and X.J. Xia. 2016. Overexpression of a brassinosteroid biosynthetic gene dwarf enhances photosynthetic capacity through activation of Calvin cycle enzymes in tomato. *BMC Plant Biol.*, 16: 33.

Liu, S.J., C.Y. Yang, W.J. Xie, C.H. Xia and P. Fan. 2012. The effects of cadmium on germination and seedling growth of Suaeda salsa. *Seventh International Conference on Waste Management and Technology (Icwmt 7)*, 16: 293-298.

Macri, F., E. Braidot, E. Petrussa and A. Vianello. 1994. Lipoxygenase activity associated to isolated soybean plasma membranes. *Biochim. Biophys. Acta*, 1215(1-2): 109-114.

Melo, L.C.A., L.R.F. Alleoni, G. Carvalho and R.A. Azevedo. 2011. Cadmium- and barium-toxicity effects on growth and antioxidant capacity of soybean (*Glycine max* L.) plants, grown in two soil types with different physicochemical properties. *J. Plant Nutr. Soil Sci.*, 174(5): 847-859.

Rady, M.M. 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Sci. Hort.*, 129(2): 232-237.

Raghu, K., K. Mahesh, N. Divya Sri and S. Rao. 2014. Effect of brassinosteroids on the seed germination and seedling growth of radish (*Raphanus sativus* L) under arsenic toxicity stress. *Int. J.Develop. Res.*, 9: 1929-1933.

Rahman, A., K. Nahar, M. Hasanuzzaman and M. Fujita. 2016. Manganese-induced cadmium stress tolerance in rice seedlings: Coordinated action of antioxidant defense, glyoxalase system and nutrient homeostasis. *C. R. Biol.*, 339(11-12): 462-474.

Rahman, M.F., A. Ghosal, M.F. Alam and A.H. Kabir. 2017. Remediation of cadmium toxicity in field peas (*Pisum sativum* L.) through exogenous silicon. *Ecotoxicol. Environ. Saf.*, 135: 165-172.

Ramakrishna, B. and S.S.R. Rao. 2012. 24-Epibrassinolide alleviated zinc-induced oxidative stress in radish (*Raphanus sativus* L.) seedlings by enhancing antioxidative system. *Plant Growth Regul.*, 68(2): 249-259.

Roxas, V.P., R.K. Smith, Jr., E.R. Allen and R.D. Allen. 1997. Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. *Nat. Biotechnol.*, 15(10): 988-991.

Sahu, G.K., S. Upadhyay and B.B. Sahoo. 2012. Mercury induced phytotoxicity and oxidative stress in wheat (*Triticum aestivum* L.) plants. *Physiol. Mol. Biol. Plants*, 18(1): 21-31.

Shan, S., F. Liu, C. Chunjuan Li and S. Wan. 2012. Effects of cadmium on growth, oxidative stress and antioxidant enzyme activities in peanut (*Arachis hypogaea* L.) seedlings. *J. Agric. Sci.*, 4(6): 142-151.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.*, 2012: 1-26.

Singh, A. and S.M. Prasad. 2014. Effect of agro-industrial waste amendment on Cd uptake in *Amaranthus caudatus* grown under contaminated soil: an oxidative biomarker response. *Ecotoxicol. Environ. Saf.*, 100: 105-113.

Song, Y.L., Y.J. Dong, X.Y. Tian, J. Kong, X.Y. Bai, L.L. Xu and Z.L. He. 2016. Role of foliar application of 24-epibrassinolide in response of peanut seedlings to iron deficiency. *Biol. Plant.*, 60(2): 329-342.

Tripathi, D.K., V.P. Singh, D. Kumar and D.K. Chauhan. 2012. Rice seedlings under cadmium stress: effect of silicon on growth, cadmium uptake, oxidative stress, antioxidant capacity and root and leaf structures. *Chem. Ecol.*, 28(3): 281-291.

Tuteja, N., P. Ahmad, B.B. Panda and R. Tuteja. 2009. Genotoxic stress in plants: shedding light on DNA damage, repair and DNA repair helicases. *Mutat. Res.*, 681(2-3): 134-149.

Wu, Z.C., S. Liu, J. Zhao, F.H. Wang, Y.Q. Du, S.M. Zou, H.M. Li, D. Wen and Y.D. Huang. 2017. Comparative responses to silicon and selenium in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage (*Brassica campestris* L. ssp chinensis var. utilis) under cadmium stress. *Environ. Exp. Bot.*, 133: 1-11.

Yamasaki, S. and L.R. Dillenburg. 1999. Measurements of leaf relative water content in *Araucaria angustifolia*. *Revista Brasilleira de fisiologia vegetal*, 11(2): 69-75.

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