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CONTRASTING TOLERANCE AMONG SOYBEAN GENOTYPES SUBJECTED TO DIFFERENT LEVELS OF CADMIUM STRESS

MOHAMMED NASSER ALYEMENI[1], MOHAMMAD ABASS AHANGER[2], LEONARD WIJAYA[1], PRAVEJ ALAM[3] AND PARVAIZ AHMAD[1,4*]

[1]Botany and Microbiology Department, College of Science, King Saud University, P. O. Box. 2460 Riyadh 11451, Saudi Arabia
[2]School of Studies in Botany, Jiwaji University, Gwalior MP-474011, India
[3]Biology Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University (PSAU), Alkharj, Kingdom of Saudi Arabia (KSA)
[4]Department of Botany, S.P. College, Srinagar, 190001, Jammu and Kashmir, India
**Corresponding author's email: parvaizbot@yahoo.com*

Abstract

The present study investigated the effects of cadmium stress on the growth, physio-biochemical attributes, and enzyme activity of five soybean genotypes. Cadmium stress significantly reduced growth attributes, such as the length of plant shoots and roots and the fresh and dry weight of plant shoots, but enhanced hydrogen peroxide (H_2O_2) production, lipid peroxidation (MDA), and electrolyte leakage, especially in the PK-416 and Pusa-24 genotypes. Cadmium stress also enhanced leaf proline content and the activity of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, especially in the Pusa-37 and Pusa-16 genotypes. Efficient antioxidant systems determine the stress tolerance potential of specific genotypes. Cadmium accumulated to higher levels in roots than in shoots, which indicated that cadmium was selectively absorbed to upper sensitive plant parts. The present study may provide a sustainable approach for identifying soybean genotypes that can be cultivated at heavy metal-polluted sites.

Key words: Growth, Proline, Lipid peroxidation, Antioxidants, Cadmium, Soybean, Cd uptake.

Introduction

In plants, normal growth and development depend on optimal biotic and abiotic conditions, and heavy metal pollution is one of the main causes of reduced crop growth and yield (Singh & Prasad, 2014). Although a few heavy metals are essential for plant growth, cadmium is non-essential and differs from other heavy metals in its high solubility and absorption by plants (Pagani *et al.*, 2012), and the high mobility of cadmium in soil-plant systems is the main reason for its toxicity (DalCorso *et al.*, 2008; Groppa *et al.*, 2012; Hassan *et al.*, 2016). Cadmium pollution occurs as a result of both natural and anthropogenic causes, including the weathering of metal-rich rocks, mining, power station activities, the application of mineral fertilizers (especially phosphate fertilizer), and the excessive use of waste water and sewage sludge for agricultural purposes (McLauglin *et al.*, 2000; Zoffoli *et al.*, 2013). The consumption of cadmium-containing food causes renal disorders and the development of weak bones (Horiguchi *et al.*, 2010).

When cadmium concentrations surpass species-specific threshold levels (Lagriffoul *et al.*, 1998), the symptoms of cadmium toxicity, which include necrosis, reduced growth, and, hence, phytotoxicity, become apparent (Dias *et al.*, 2013). The high affinity of cadmium to thiol compounds is one of the main causes of cadmium toxicity since the binding of cadmium to the cysteine sulfhydryl groups results in enzyme inactivation (Mendoza-Cozatl *et al.*, 2005). Cadmium also impedes key physiological and biochemical processes, including electron transport, and, moreover, severely disrupts mineral nutrition and water uptake (Ahmad *et al.*, 2011; Groppa *et al.*, 2012). Furthermore, cadmium also induces the production of reactive oxygen species (ROS), owing to its effects on electron transport systems, which leak electrons to molecular oxygen (Gill & Tuteja, 2010), and its interference with the enzymes involved in redox homeostasis (Cuypers *et al.*, 2011), and the overproduction of ROS alters almost every physiological and biochemical attribute (Ahmad *et al.*, 2011).

To avert the deleterious effects of induced oxidative stress, plants use a variety of defense mechanisms, including the partitioning and compartmentalization of toxic metal ions into vacuoles (Wu & Wang, 2011), overproduction of phytochelatins and subsequent compartmentalization of phytochelatin-metal complexes into vacuoles (Salt *et al.*, 1995), accumulation of compatible organic osmolytes, and increased antioxidant activity (Ahmad *et al.*, 2011). Partitioning and compartmentalization help plants to maintain toxic ions and substances within physiological limits. For quickly neutralizing and scavenging ROS, plants have developed antioxidant defense systems that include both enzymatic and non-enzymatic components (Ahmad *et al.*, 2010, 2011; Abd_Allah *et al.*, 2016), the former of which include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR).

Soybean (*Glycine max*) is a major source of protein, oil, and livestock feed across the globe. The crop has the potential to accumulate high levels of cadmium (Wolnik *et al.*, 1983), and different genotypes have been reported to accumulate different levels (Jamali *et al.*, 2009). Soybean is considered sensitive to cadmium (Finger-Teixeira *et al.*, 2010). The aim of the present study was to study the impact of cadmium stress on the growth, lipid peroxidation, and antioxidant responses of five soybean genotypes.

Material and Methods

Plant materials and experimental design: Certified and viable seeds of five soybean genotypes (Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24) were sterilized using sodium hypochlorite ($NaOCl_2$), and after washing, the seeds were sown in pots containing 5 kg of sand and vermicompost (3:1) and were kept under glasshouse conditions (day/night temperature of 27°C/16°C and photoperiod of 8-11 h). The experiment was performed using a completely randomized design, and every treatment was represented by five replicates. After germination (4 d), the seedlings were thinned to two per pot and supplied with Hoagland's solution for 1 wk. Nutrient solutions with different cadmium ($CdSO_4 \cdot 8H_2O$) concentrations (0, 50, 100, and 150 mg L^{-1}) were prepared and supplied to the pots on alternate days, however, the control plants were only supplied with nutrient solution. After 35 d of treatment (46-d-old plants), the plants were carefully uprooted for analysis. The composition of the Hoagland's solution was: (mg L^{-1}): 270 N (KNO_3), 31 P (KH_2PO_4), 234 K (KNO_3), 200 Ca ($Ca(NO_3)_2.4H_2O$), 64 S ($MgSO_4.7H_2O$), 48 Mg ($MgSO_4.7H_2O$), 2.8 Fe (Fe-EDTA), 0.5 Mn ($MnCl_2.4H_2O$), 0.5 B (H_3BO_3), 0.02 Cu ($CuSO_4$), 0.05 Zn ($ZnSO_4.7H_2O$) and 0.01 Mo ($H_3MoO_4.H_2O$).

Growth and biomass: The lengths of the shoots and roots were measured manually, and dry weight (DW) was determined after drying for 72 h in an oven at 65°C.

Relative leaf water content, electrolyte leakage and Proline content: To measure relative leaf water content (RLWC), leaf discs were punched using a sharp cork borer and then weighed both before (fresh weight, FW) and after (turgid weight, TW) floating on water for 1 h, which was performed to gain turgidity, as well as after drying in an oven at 85°C to constant weight (dry weight, DW), according to Smart & Bingham (1974). Afterward, LWC was calculated using following formula:

$$RLWC\ (\%) = (FW - DW)/(TW - DW) \times 100\%$$

The method of Dionisio-Sese & Tobita (1998) was used to estimate electrolyte leakage. Briefly, 20 leaf discs were placed in tubes that contained 10 ml deionized water, and the initial electrical conductivity (EC_0) was measured. Thereafter, the same samples were heated in a water bath at 50°C (EC_1) and 100°C (EC_2) for 25min and 10 min respectively and electrolyte leakage was calculated using the following formula:

$$Electrolyte\ leakage\ (\%) = (EC_1 - EC_0)/(EC_2 - EC_0) \times 100\%$$

Proline was extracted in sulphosalicylic acid, and its concentration was estimated by reacting the extract with a known quantity of supernatant with ninhydrin reagent and measuring its absorbance at 520 nm (Bates *et al.,* 1973).

Hydrogen peroxide (H_2O_2) content and membrane lipid peroxidation: To measure H_2O_2 content, fresh leaf samples (500 mg) were macerated in 5 ml trichloroacetic acid (TCA, 0.1%) and centrifuged at 12,000 rpm for 15 min. The resulting supernatants (0.5 ml) were then mixed with equal volumes of 10 mM potassium phosphate buffer (pH 7.0) and 1.0 ml potassium iodide (1.0 M), and the absorbance of the solutions was measured at 390 nm. Afterward, the H_2O_2 content of the extracts was calculated from the absorbance measures using a standard curve (Velikova *et al.*, 2000).

To measure lipid peroxidation, in terms of malondialdehyde (MDA) content), fresh tissue (500 mg) was macerated in 2.5 ml TCA (1%) and centrifuged at 10,000 rpm for 5 min. The resulting supernatants (1.0 ml) were then mixed with 4.0 ml thiobarbituric acid (TBA, 0.5%) and incubated for 30 min at 95°C. After cooling in an ice bath, the samples were centrifuged again at 5000 rpm for 5 min, and the absorbance of the supernatants at 532 and 600 nm was determined. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to determine the MDA concentration (Heath & Packer, 1968).

Antioxidant enzymes: To extract antioxidant enzymes, fresh leaves (10 g) were homogenized in a mixture of Tris-HCl (100 mM, pH 7.5), dithiothreitol (DTT, 5.0 mM), $MgCl_2$ (10 mM), ethylenediaminetetraacetic acid (EDTA, 1.0 mM), magnesium acetate (5.0 mM), phenylmethanesulfonyl fluoride (PMSF, 1.0 mM), and PVP (1.5%) and then centrifuged at 10,000 rpm for 15 min. Except for APX assay, the resulting supernatant was used as the enzyme source. For measuring APX, ascorbate (2.0 mM) was also included in the extraction buffer. The method of Bradford (1976) was used to estimate enzyme levels, and bovine serum albumin was used as a standard.

The SOD (EC 1.15.1.1) activity (Unit mg^{-1} protein) was assayed using the method of Van Rossun *et al.* (1997), i.e., by observing the photoreduction of nitroblue tetrazolium (NBT) at 560 nm. Briefly, reaction mixtures that contained phosphate buffer (50 mM, pH 7.8), EDTA (0.1 mM), methionine (13 mM), NBT (75 µM), riboflavin (2.0 µM), and 100 µl enzyme solution were incubated under light for 15 min.

The CAT (EC 1.11.1.6) activity (Unit mg^{-1} protein) was measured by observing the disappearance of H_2O_2 at 290 nm for 3 min (Luck, 1974). The assay mixture contained 50 µl enzyme extract and 50 mM phosphate buffer (pH 7.0) and was initiated using 20 mM hydrogen peroxide. An extinction coefficient of 0.036 mM^{-1} cm^{-1} was used for the calculation of enzyme activity.

The APX (EC1.11.1.11) activity (Unit mg^{-1} protein) was measured using the method of Nakano & Asada (1981), i.e., by observing the oxidation of ascorbate at 290 nm after adding H_2O_2. An extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for the calculation of enzyme activity.

The GR (EC 1.6.4.2) activity (Unit mg^{-1} protein) was measured by observing the oxidation of NADPH at 340 nm for 2 min in an assay mixture of 0.75 µl potassium phosphate buffer (pH 7), 2.0 mM EDTA, 2.0 mM NADPH, and 20 mM GSSG, in a final volume of 1.0 ml (Carlberg & Mannervik, 1985). An extinction coefficient of 6.2 mM^{-1} cm^{-1} was used for the calculation of enzyme activity.

Cadmium accumulation: The cadmium content (µM g^{-1} DW) of the shoots and roots was estimated using a Perkin-Elmer (Analyst Model 300) atomic absorption spectrophotometer.

Statistical analysis: Data is presented as the mean of five replicates, and least significant differences were calculated using ANOVA in SPSS version 17. Different letters denote differences that are statistically significant at the $P < 0.05$ level.

Results

Growth and biomass: The exposure of the soybean genotypes to cadmium stress resulted in considerably reduced growth. Shoot and root length, as well as the fresh and dry weights of shoots, decreased with increasing cadmium concentration. However, the effect was more obvious in PK-416 and Pusa-24 than in Pusa-37 or Pusa-16, which exhibited slight reductions in growth (Fig. 1A-D). More specifically, cadmium stress (150 mg L^{-1}) reduced the shoot length of Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 by 26.45, 29.39, 32.50, 34.93, and 33.50%, respectively, and reduced root length by 19.24, 21.59, 25.17, 27.91, and 37.82%, respectively. Cadmium stress also induced the reduction of fresh and dry weight, and these parameters decreased substantially with increasing cadmium concentration. At 150 mg L^{-1}, cadmium stress reduced the shoot FW of Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 by 40.45, 42.51, 45.06, 49.03, and 54.12% over that of the control plants, respectively, and reduced shoot DW by 36.64, 41.99, 42.49, 43.70, and 60.40%, respectively.

Relative leaf water content, electrolyte leakage, and proline content: The effect of different cadmium concentrations on the RLWC of the different soybean genotypes is depicted in Fig. 2A. At the cadmium level of 150 mg L^{-1}, higher reductions were observed in the PK-416 and Pusa-24 genotypes (48.70 and 56.60%, respectively) than in the others.

Cadmium treatment dramatically increased the electrolyte leakage of the Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 genotypes by 4.65-, 4.85-, 4.95-, 5.37-, and 6.07-fold, respectively (Fig. 2B).

Soybean genotypes treated with different concentrations of cadmium accumulated increased levels of proline (Fig. 2C). However, tolerant genotypes accumulated higher levels of proline than the sensitive genotypes. At higher doses of cadmium (150 mg L^{-1}), the Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 genotypes accumulated proline levels of 472.71, 457.81, 451.44, 391.24, and 346.28% respectively over that of the control plants.

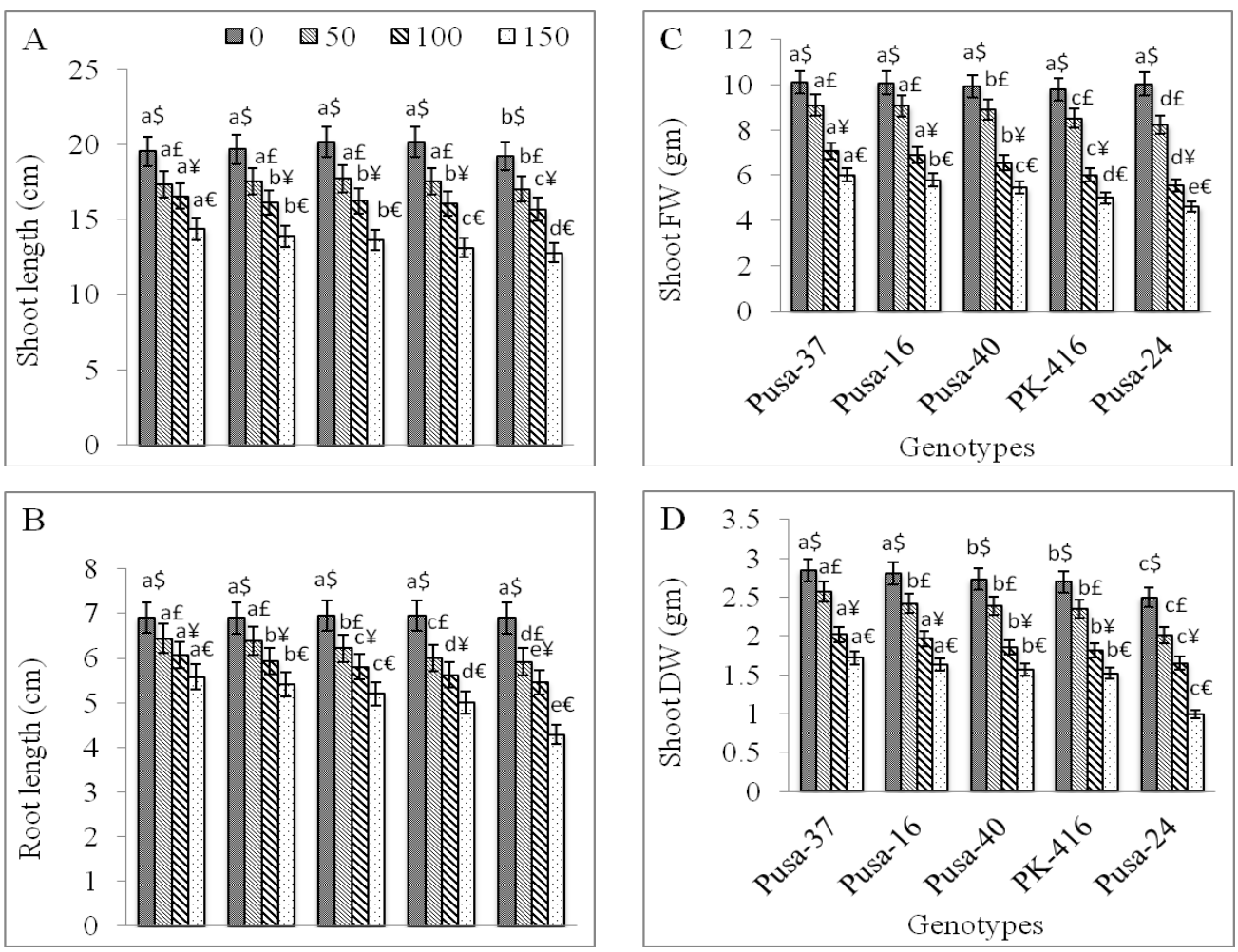

Fig. 1. Effect of Cd concentration on the (A) shoot length, (B) root length, (C) shoot fresh weight (FW), and (D) shoot dry weight (DW) of various soybean genotypes. Values indicate means ± SE (n = 5). Different letters indicate significant differences among treatments at the $p \leq 0.05$ level.

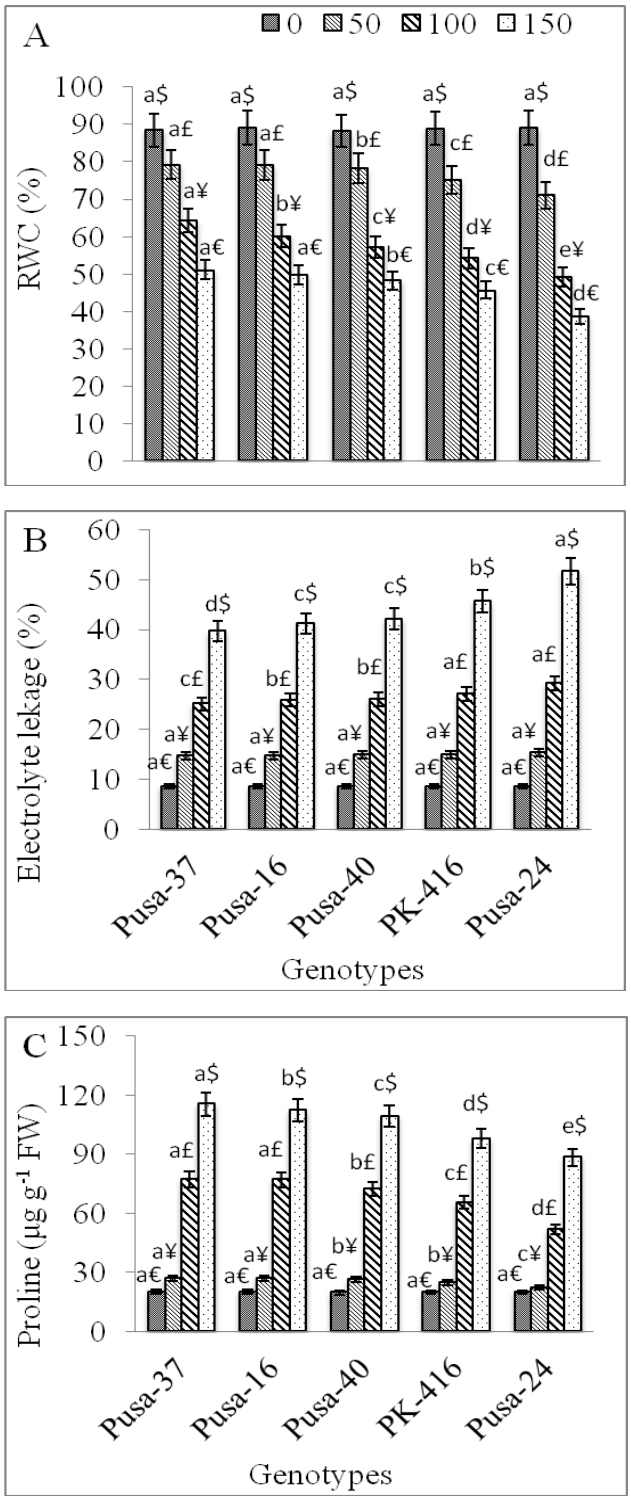

Fig. 2. Effect of Cd concentration on the (A) leaf relative water content, (B) electrolyte leakage, and (C) proline content of various soybean genotypes. Values indicate means ± SE (n = 5). Different letters indicate significant differences among treatments at the $p \leq 0.05$ level.

Hydrogen peroxide content and membrane lipid peroxidation: The H_2O_2 levels of the soybean plants increased with increasing concentrations of cadmium, and the elevation of H_2O_2 was more obvious at 150 mgL^{-1} cadmium in all five genotypes. The sensitive genotypes (PK-416 and Pusa-24) accumulated more H_2O_2 (214.90 and 248.79%, respectively) than the Pusa-37 and Pusa-16 genotypes (189.37 and 194.28%, respectively; Fig. 3A). Cadmium stress also induced the peroxidation of membrane lipids, which was measured in terms of MDA content. In the present study, MDA content increased with cadmium concentration in all the genotypes, reaching maximum values at 150 mg L^{-1}. However, genotype differences were also evident, with the Pusa-37 and Pusa-16 genotypes showing lower levels of lipid peroxidation than the sensitive genotypes (PK-416 and Pusa-24), which yielded higher MDA levels (Fig. 3B).

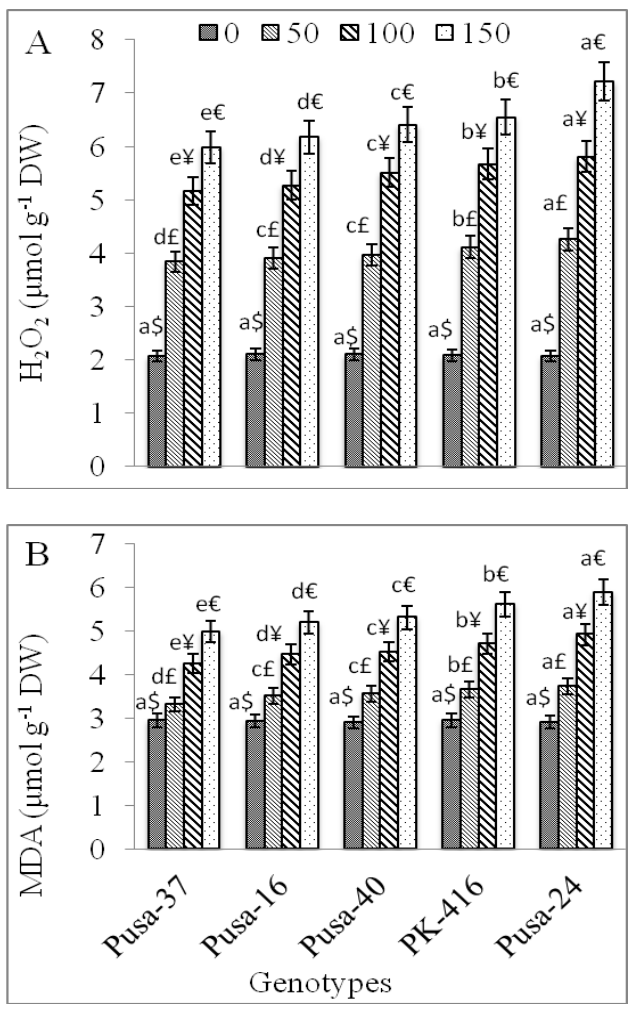

Fig. 3. Effect of Cd concentration on the (A) hydrogen peroxide (H_2O_2) and (B) malondialdehyde (MDA) content of various soybean genotypes. Values indicate means ± SE (n = 5). Different letters indicate significant differences among treatments at the $p \leq 0.05$ level.

Antioxidants: The influence of cadmium concentration on the SOD activity of soybean genotypes is shown in Fig. 4A. The activity of SOD varied greatly among genotypes, and at 150 mg L^{-1} cadmium, the SOD activity of genotypes Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 were 193.17, 177.31, 173.31, 154.01, and 88.64% greater than that of the control, respectively. The CAT activity also increased with increasing cadmium application (Fig. 4B), and at 150 mg L^{-1}, the CAT activity of the genotypes varied considerably, higher activity in the Pusa-37 (271%), Pusa-16 (259.51%), and Pusa-40 (226.48%) genotypes than in the PK-416 (196.18%) and

Pusa-24 (150.38%) genotypes. Meanwhile, APX activity was higher in the Pusa-17 and Pusa-16 genotypes at all cadmium concentrations (Fig. 4C), and GR activity was increased by cadmium treatment in all the genotypes, with a greater increase in genotype Pusa-37 at all concentrations of cadmium (Fig. 4D). More specifically, at 150 mg L^{-1} cadmium, the GR activity of the Pusa-37, Pusa-16, Pusa-40, PK-416, and Pusa-24 genotypes was 369.89, 349.85, 344.59, 331.54, and 278.27% greater than that of the control, respectively.

Cadmium accumulation: All five soybean genotypes accumulated higher levels of cadmium in their roots than in their shoots. However, there were obvious differences between the genotypes, and the concentration of accumulated cadmium was very low in the control plants. In addition, both the roots and shoots of genotypes PK-416 and Pusa-24 accumulated higher contents of cadmium than the Pusa-37, Pusa-16, and Pusa-40 genotypes (Fig. 5A-B).

Discussion

In the present study, the exposure of soybean genotypes to cadmium drastically reduced plant growth, an observation that is usually attributed to altered nutrient uptake and reduced water content (Gomes *et al.*, 2013). Indeed, heavy metals hamper vital growth processes, like cell division and elongation, through their irreversible effect on the functioning of membranes and proton pumps (Karcz & Kurtyka, 2007). Therefore, the cadmium-induced growth reduction observed in the present study corroborates several previous reports (e.g., Ahmad *et al.*, 2011; Irfan *et al.*, 2014; Roy *et al.*, 2016). The reduced RLWC observed in the present study may be due to cadmium-induced reductions in hydraulic conductivity, which significantly reduces cellular turgor (Ehlert *et al.*, 2009). Reduction in RLWC have also been observed in *Atriplex halimus* (Manousaki & Kalogerakis, 2009) and *Brassica juncea* (Ahmad *et al.*, 2011) under Cd stress. Cadmium-induced water content reduction hampers cell wall extension and, thus, cell division, thereby reducing morphological attributes such as leaf area, length, and weight (Marshner, 2012).

In the present study, cadmium stress also caused marked increases in the electrolyte leakage of all genotypes and had greater effects on the electrolyte leakage of the sensitive genotypes (Pusa-24 and PK-416) than tolerant genotypes (Pusa-37 and Pusa-16), which exhibited superior growth. These results are similar to those observed in mustard (John *et al.*, 2009; Ahmad *et al.*, 2011). Hossain *et al*. (2006) also reported higher electrolyte leakage in sensitive genotypes of *Chrysanthemum morifolium* that were subject to salt stress. Stress-induced loss of membrane integrity is attributed to enhanced peroxidation of membrane lipids.

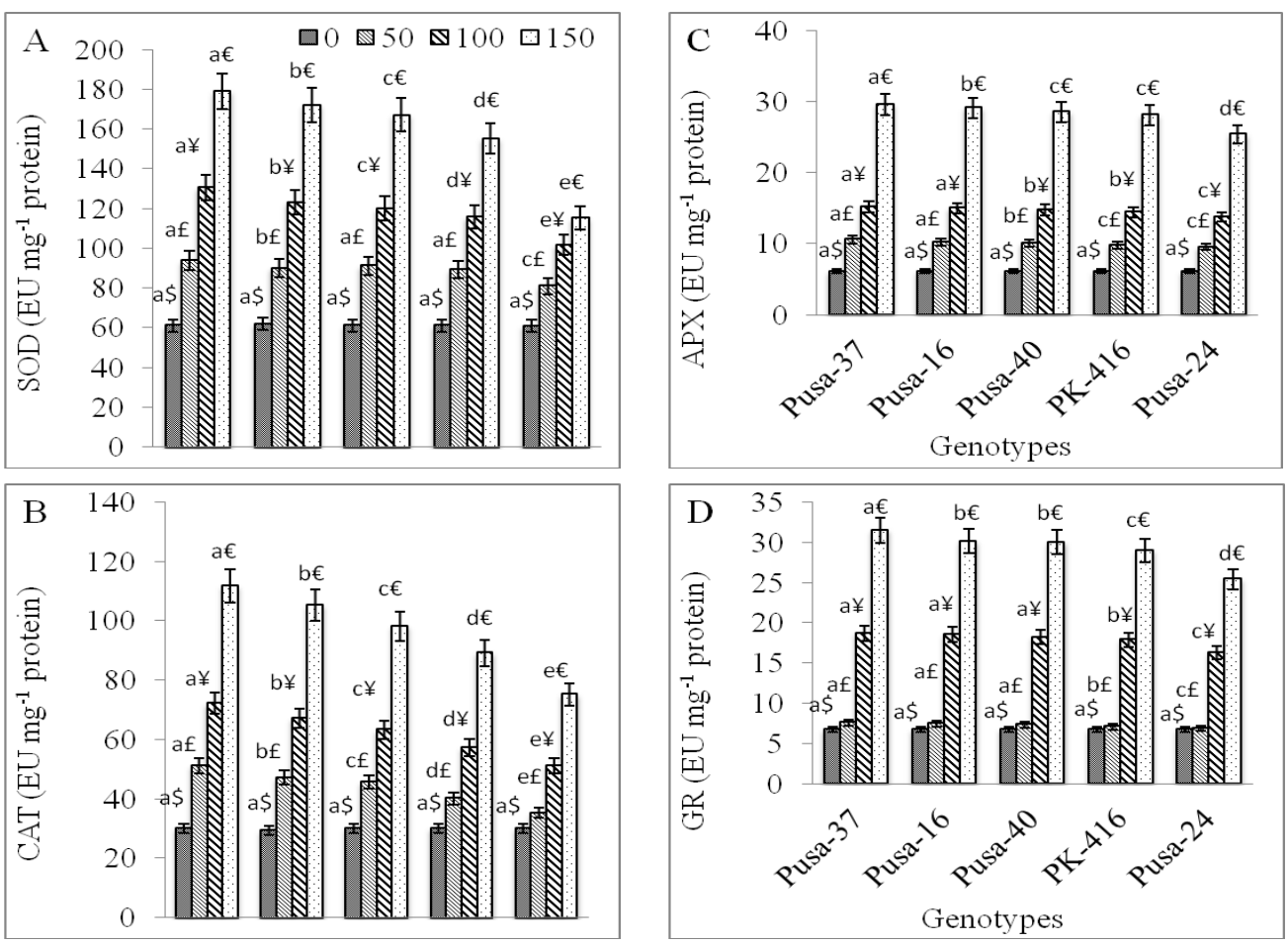

Fig. 4. Effect of Cd concentration on the activities of (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) ascorbate peroxidase (APX), and (D) glutathione reductase (GR) in various soybean genotypes. Values indicate means ± SE (n = 5). Different letters indicate significant differences among treatments at the $p \leq 0.05$ level.

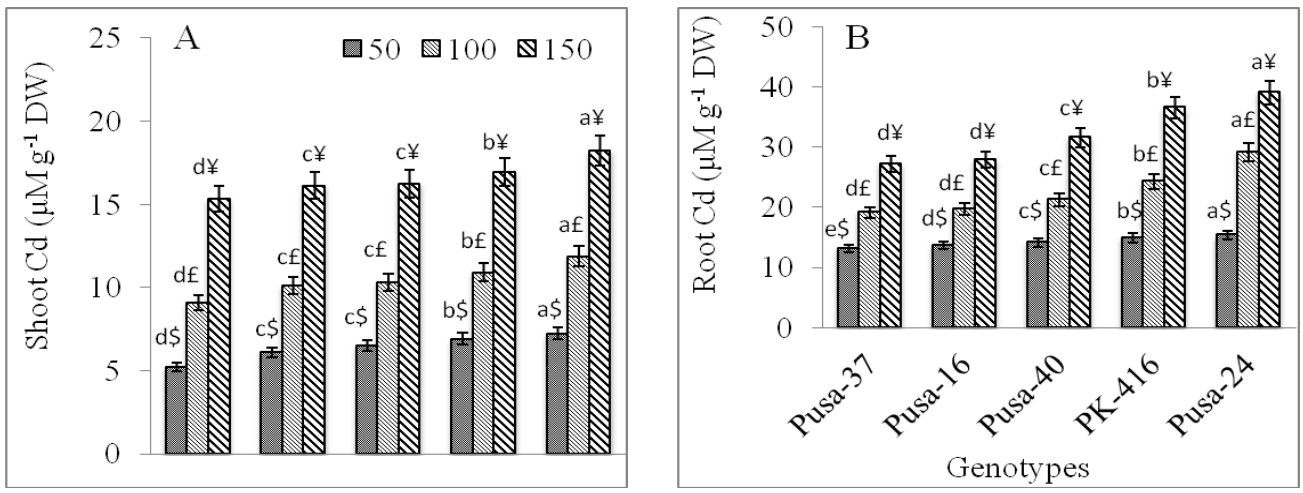

Fig. 5. Effect of Cd concentration on the accumulation of Cd by soybean (A) shoots and (B) roots. Values indicate means ± SE (n = 5). Different letters indicate significant differences among treatments at the $p \leq 0.05$ level.

Hydrogen peroxide, which is one of the potentially toxic ROS, also increased with cadmium exposure in all the examined genotypes. Previous studies have established that H_2O_2 production is greatly increased upon exposure to environmental stresses (Ahmad *et al.,* 2016 a,b; Hashem *et al.,* 2016), and H_2O_2 is also produced as a byproduct of various physiological and biochemical processes, such as photorespiration, the dismutation of superoxide radical, and β-oxidation (Ahmad *et al.,* 2010). Furthermore, H_2O_2 and other toxic ROS usually target molecules like membrane lipids and DNA, which results in the formation of lipid peroxides (Ahmad *et al.,* 2010), and cadmium-induced increases in H_2O_2 production have been previously reported in tobacco (Olmos *et al.,* 2003), other soybean genotypes (Yang *et al.,* 2007), and mustard (Ahmad *et al.,* 2011). In the present study, cadmium stress-induced considerable increases in lipid peroxidation (i.e., MDA content), which is widely used to measure the magnitude of oxidative stress (Ahmad *et al.,* 2011), since the exposure of plants to stress enhances the lipoxygenase activity mediateding lipid peroxidation (Macri *et al.,* 1994). In agreement with the findings of the present study, Ahmad *et al.* (2011) also observed the lipid peroxidation increased with cadmium concentration in *Brassica juncea*, and several other studies (e.g., Zhang *et al*., 2007; Monteiro *et al.,* 2009; Noriega *et al.,* 2012; Singh & Prasad, 2014) have reported the increased accumulation of MDA in cadmium-exposed plants, thereby supporting the use of lipid peroxidation as a biomarker for heavy-metal stress and tolerance.

In addition, the accumulation of organic osmolytes has been shown to enhance the stress tolerance of plants by mediating the maintenance of cell water content (Tester & Davenport, 2003), a process in which proline plays an irreplaceable role *via* osmotic adjustment, membrane stabilization, and stress mitigation. Supra-optimal levels of proline do not affect enzyme activity but, rather, hydrate enzymes and help restore their activity (Kishor *et al.,* 2005). Indeed, cadmium-tolerant plants accumulate higher levels of compatible osmolytes. For example, in cadmium-stressed *B. juncea*, Irfan *et al.* (2014) demonstrated that tolerant genotypes accumulated higher levels of proline than sensitive genotypes, as observed in the present study, and similar observations were also reported by Zengin and Munzuroglu (2006) and Ahmad *et al.* (2011) in sunflower and *B. juncea*, respectively. Furthermore, Jaleel *et al*. (2007) also reported that the activity of proline synthesizing enzymes is upregulated under stressful conditions, whereas the activity of catabolizing enzymes is downregulated.

Among the enzymes involved in the antioxidant network, both SOD and APX play important roles in the process of scavenging superoxide radicals. However, SOD activity is more important for the normal maintenance of cellular functioning of plants under oxidative stress (Slooten *et al.,* 1995). Different SODs present in various cellular organelles are upregulated to avert oxidative damage in plants subjected to stress (Ahmad *et al.,* 2010). In the present study, cadmium stress-induced increases in the SOD activity of all five soybean genotypes, although enhanced activity was observed in genotypes Pusa-37 and Pusa-16, which indicates quick scavenging of superoxide radicals. Cadmium-induced increases in SOD activity have also been reported in *Glycine max* L. (Melo *et al.,* 2011), *Arachis hypogea* L. (Shan *et al.,* 2012), *B. juncea* (Ahmad *et al.,* 2011; Irfan *et al.,* 2014), and *Solanum melongena* L. (Singh & Prasad, 2014).

The activity of CAT was also increased markedly in genotypes Pusa-37 and Pusa-16, suggesting that these genotypes exhibited superior H_2O_2-scavenging abilities, when compared to the other genotypes. This conclusion is supported by the fact that CAT, a heme-containing defense enzyme, catalyzes the conversion of H_2O_2 to oxygen and water (Srivalli *et al.,* 2003) and the observation that genotypes PK-421 and Pusa-24, which possessed lower CAT activity, also exhibited increased H_2O_2 levels. In *Solanum melongena* L., Singh & Prasad (2014) demonstrated that cadmium stress significantly enhances CAT activity and, thereby, mediates the rapid scavenging of toxic ROS. In plants, increased antioxidant activity is a general strategy used to counteract ROS-induced oxidative stress and to enhance tolerance. Therefore, enhanced CAT activity could be mediated by the upregulation of CAT-coding genes that are induced by elevated H_2O_2 levels (Dixit *et al.,* 2001). Indeed, in five

genotypes of *Arachis hypogaea* L., Shan *et al.* (2012) also demonstrated that CAT activity increased with cadmium concentration.

The increased activity of APX in the cadmium-stressed soybean genotypes coincide with the results reported by Schutzendubel *et al.* (2001) and Ahmad *et al.* (2011) for pine and *B. juncea*, respectively. APX, which uses ascorbate as an electron donor, plays important roles in scavenging H_2O_2 in both the water-water cycle and ascorbate-glutathione pathway (Kangasjarvi *et al.*, 2008). In wheat, Milone *et al*. (2003) demonstrated that cadmium stress enhanced APX activity and that the effect was more evident in roots than in shoots. The higher APX activity observed in cadmium-stressed soybean plants suggest that it plays a pivotal role in the detoxification of H_2O_2 in soybean, as well.

In addition, both GR and APX are crucial enzymes of the ascorbate-glutathione pathway and catalyze the NADPH-dependent reduction of GSSH to glutathione (GSH), in order to maintain a higher GSH/GSSH ratio, which enhances the stress tolerance of plants by mediating the generation of sufficient ascorbate levels and by activating enzymes related to carbon metabolism (Noctor & Foyer, 1998). Reduced GSH serves as an electron donor for the conversion of dehydroascorbate to ascorbate, which then serves as an electron donor for APX-mediated H_2O_2 scavenging (Noctor & Foyer, 1998). The finding of the present study, that GR activity was elevated in cadmium-stressed plants, is consistent with previous observations in *Crotolaria juncea* (Pereira *et al.*, 2002), *Ceratophyllum demersum* (Arvind & Prasad, 2005), *B. juncea* (Ahmad *et al.*, 2011). Moreover, higher GR activity also helps maintain a higher NADP/NADPH ratio, which allows NADP to quickly become available as an electron acceptor for the electrons released from the photosynthetic electron transport system and, thereby, restricts both the flow of electrons to oxygen and the subsequent formation of O_2^- (Ahmad *et al.*, 2010).

In the present study, soybean roots accumulated more cadmium than did the shoots, which indicated that the toxic cadmium ions were sequestered efficiently, and genotype differences were quite clear, with higher levels of cadmium observed in the roots of the tolerant genotypes (Pusa-37 and Pusa-16). The enhanced uptake and accumulation of toxic heavy metals in upper plant parts causes osmotic shifts and induces the overproduction of ROS, which results in oxidative stress (Gill & Tuteja, 2010). Relatively higher levels of cadmium accumulation in plant roots, compared to shoots, has also been reported in *B. juncea* (Ahmad *et al.*, 2011; Irfan *et al.*, 2014; Liu *et al.*, 2014).

Conclusion

Soybean is an important legume crop that is often exposed to abiotic stressors. In the present study, cadmium stress was shown to adversely affect various morpho-physiological attributes. Both H_2O_2 and lipid peroxidation increased with cadmium stress in all genotypes, which indicated the deleterious impact of cadmium exposure on membrane integrity. Furthermore, tolerance was clearly related to the enzymatic antioxidant system, which is important for maintaining redox homeostasis. Among the five soybean genotypes analyzed in the present study, Pusa-37 and Pusa-16 exhibited efficient proline accumulation and enhanced antioxidant enzyme activity and, thus were more tolerant to cadmium stress than the other genotypes.

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