

IMPACT OF DIFFERENT CADMIUM CONCENTRATIONS ON TWO *PISUM SATIVUM* L. GENOTYPES

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Abstract

This study was conducted to evaluate the effect of different concentrations of cadmium (Cd) (0, 100, 200, and 400 μ M Cd) on two genotypes (AG-10 and AP-3) of *Pisum sativum*. Cd stress reduced the length, fresh weight, and dry weight of roots and shoots in both genotypes. Chlorophyll 'a' and 'b' and carotenoid content was also decreased and greater decreases were observed for higher concentrations of Cd. As Cd stress increased, levels of proline, glycine betaine, and soluble proteins were increased and sugar content was decreased. The application of Cd also caused an increase in hydrogen peroxide, lipid peroxidation, and electrolyte leakage in both genotypes. The activities of defensive antioxidant enzymes like superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and glutathione S-transferase were also increased with increasing Cd concentrations; however, the increase in AG-10 was greater than in AP-3. The accumulation of phenols was decreased with Cd stress in both pea genotypes, with the greatest decreases were observed in AP-3. In conclusion, Cd caused a marked reduction in growth, biomass yield, and pigment content in both pea genotypes. However, enhanced accumulation of osmolytes and antioxidant enzyme activities alleviated the adverse effects of Cd. AG-10 was more tolerant genotype than AP-3, as AG-10 showed less Cd induced damage and higher antioxidant enzyme activities.

Key words: Cadmium; *Pisum sativum*; Growth; Lipid peroxidation; Osmolytes; Antioxidants.

Introduction

Soil is polluted with different kinds of heavy metal elements, one of which is cadmium (Cd). Non-ferrous metal industries, fossil fuel combustion, and waste incineration primarily contribute to soil pollution (Ahmad *et al.*, 2016). Cd is highly toxic and has detrimental effects on plant and animal life (Ahmad *et al.*, 2011; Ahmad, 2012). After entering the human body through the food chain, Cd causes various health issues such as kidney problems, pulmonary dysfunction, neurotoxicity, and endocrine disruption (Godt *et al.*, 2006). Plant roots easily absorb and transport Cd to other parts of the plant, causing toxicity there (Talukdar, 2012). Cd primarily affects plants by inhibiting seed germination, reducing plant growth (Siddiqui *et al.*, 2012), reducing chlorophyll synthesis (Mobin & Khan, 2007), changing Calvin cycle enzyme activities (Mobin & Khan, 2007; Shamsi *et al.*, 2008), and closing stomata (Perfus-Barbeoch *et al.*, 2002). Cd also affects the synthesis of carbohydrates, proline, and protein (Siddiqui *et al.*, 2012; El-Beltagi & Mohamed, 2013; Mondal *et al.*, 2013) and the uptake of mineral elements (Sandalio *et al.*, 2001; Siddiqui *et al.*, 2012). In addition, excessive Cd exposure leads to the generation of reactive oxygen species (ROS), causing oxidative stress in plants (Ahmad *et al.*, 2010; Ahmad *et al.*, 2015). ROS are very reactive and have the capacity to oxidize organic molecules such as lipids, proteins, and nucleic acids (Ahmad *et al.*, 2010; Ahmad *et al.*, 2015). However, plants are equipped with antioxidants, which act as a defense mechanism against ROS (Ahmad *et al.*, 2010; Ahmad *et al.*, 2015). Plants contain both enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants comprise superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR),

glutathione S-transferase (GST), and peroxidase (POD), whereas non-enzymatic antioxidants include ascorbic acid (AsA) and glutathione (GSH) (Ahmad *et al.*, 2010; Ahmad *et al.*, 2015).

Legumes are susceptible to Cd stress, which leads to global reductions in plant biomass and crop production (Shamsi *et al.*, 2008). Pea (*Pisum sativum*) is an important leguminous crop, serving as the main protein source for the growing population of humans worldwide. Thus, this study was conducted to determine which variety of pea is most tolerant to Cd pollution in soil.

Materials and Methods

Pisum sativum seeds were sterilized (using NaOCl 5%), placed in Petri dishes lined with blotting paper for germination, and provided with distilled water. Healthy germinated seedlings were transferred to pots filled with reconstituted sand that was supplemented with vermicompost. Seedlings were thinned to 10 per pot and grown for another 10 days. Seedlings were irrigated with full-strength (200 mL) Hoagland's nutrient solution every other day. After 10 days, a modified Hoagland solution supplemented with different concentrations of CdCl₂ (0, 100, 200 and 400 μ M) was provided for another 20 days. Pots were maintained in a greenhouse with the following conditions: day/night temperatures of 26°C/16°C, relative humidity of 70%-75%, and photoperiod of 18 h light/6 h night. Plants were harvested at 30 days of age for further analysis.

Plant length and dry weight: A manual scale was used to measure length and plant tissue was oven-dried overnight at 70°C to determine dry weight.

Photosynthetic pigments: To extract photosynthetic pigments, fresh leaf tissues were macerated in 80% acetone. Homogenates were centrifuged at 3000xg for 20 min and absorbance was measured at 480, 645, and 663 nm using a spectrophotometer (Beckman 640 D, USA) (Arnon, 1949).

Proline, glycine betaine, protein, and sugar content: Proline was measured using the method described by Bates *et al.*, (1973). To extract proline, leaf tissue was first homogenized in sulfosalicylic acid. The resulting supernatant was mixed with ninhydrin acid and incubated in boiling water for one hour. After cooling on ice, proline was separated using toluene and optical density was recorded at 520 nm using a spectrophotometer (Beckman 640 D, USA).

Glycine betaine was quantified using the method described by Grieve & Grattan (1983). Dry samples were extracted in distilled water. Extracted samples were mixed with a KI-I₂ (potassium iodide and iodine solution) reagent and the periodide crystals that formed were measured at 365 nm using a spectrophotometer (Beckman 640 D, USA). A graded concentration of glycine betaine was used for the standard curve.

Protein was estimated using the method described by Bradford (1976) and absorbance was recorded at 595 nm. Bovine serum albumin was used as the standard. Sugar was measured using the method described by Dey (1990) and absorbance was recorded at 485 nm using a spectrophotometer (Beckman 640 D, USA).

Determination of hydrogen peroxide (H₂O₂), lipid peroxidation (MDA), and electrolyte leakage:

Electrolyte leakage was determined by incubating fresh leaf tissue at different temperatures and measuring electrical conductivities (Dionisio-Sese & Tobita, 1998). Leakage was calculated using the following formula:

$$\text{Electrolyte leakage (\%)} = (\text{EC1} - \text{EC0}) / (\text{EC2} - \text{EC0}) \times 100$$

For lipid peroxidation, fresh tissue was macerated in trichloroacetic acid. Malondialdehyde content was measured after a known volume of supernatant was reacted with thiobarbituric acid (Madhava Rao & Sresty, 2000).

To determine hydrogen peroxide (H₂O₂) content, fresh tissue was extracted in trichloroacetic acid and optical density was measured at 390 nm (Velikova *et al.*, 2000).

Assay of antioxidant enzyme activities: Antioxidant enzymes were extracted by homogenizing 1 g fresh tissue in ice-cold 100 mm potassium phosphate buffer (pH 7.0) supplemented with Polyvinylpyrrolidone (PVP, 1%). Samples were centrifuged at 12000xg for 15 min at 4°C and the resulting supernatants were used for enzyme assays.

To determine superoxide dismutase activity, inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) was measured at 560 nm. The assay mixture was exposed to fluorescent light for 15 min and read against unexposed samples (Dhindsa & Matowe, 1981).

The catalase assay was conducted by monitoring the decrease in optical density at 240 nm. The assay mixture contained a phosphate buffer (pH 6.0), Ethylenediamine tetraacetic acid (EDTA), and enzyme. H₂O₂ was used to initiate the reaction (Aebi, 1984).

Ascorbate peroxidase activity was determined in a reaction mixture containing a phosphate buffer (pH 7.5), EDTA, ascorbate, enzyme, and H₂O₂, and changes in absorbance were recorded at 290 nm (Nakano & Asada, 1981).

To determine glutathione reductase activity, the assay mixture consisting of phosphate buffer (pH 7.0), EDTA, oxidized glutathione, nicotinamide adenine dinucleotide phosphate (NADPH), and enzyme was observed for 3 min to measure changes in optical densities at 340 nm (Foster & Hess, 1980).

The activity of glutathione S-transferase was measured using the method described by Hasanuzzaman & Fujita (2013). Changes in optical densities were recorded at 340 nm using a spectrophotometer (Beckman 640 D, USA).

Determination of total phenolics: Phenols were extracted with ethanol and measured after reacting with a Folin-Ciocalteu reagent (Chun *et al.*, 2003). The Gallic acid standard was used for calculations.

Statistical analysis

Data were analyzed using analysis of variance. Duncan's multiple range test was used to determine the least significant difference (LSD) for a completely randomized design at 0.05% significance. Values represent the mean (±SE) of three replicates.

Results and Discussion

Growth and Biomass Yield: In this study, Cd treatment resulted in reduced growth of *Pisum sativum* cultivars. Lengths, fresh weights, and dry weights of roots and shoots were lower when Cd treatment was applied (Fig. 1A-F). "At 400 μM, the length, fresh weight, and dry weight of cultivar AG-10 was 13.48, 37.11, 30.94% (shoot) and 12.28, 35.79, 9.7% (root) lower than the control, respectively. The length, fresh weight, and dry weight of cultivar AP-3 was 31.43, 37.11, 43.33% (shoot) and 20.53, 62.03, 43.37% (root) lower than the control, respectively."

Cd alters growth, activates leaf necrosis, and disrupts cell division and elongation (Liu *et al.*, 2004). Cd stress has been shown to reduce growth in *Lycopersicon esculentum* (Jing *et al.*, 2005; Ahmad *et al.*, 2018), *Brassica juncea* (Ahmad *et al.*, 2011; Per *et al.*, 2016a, 2016b), *Hordeum vulgare* (Juknys *et al.*, 2012) and *Festuca arundinacea* (Huang *et al.*, 2017). Differences were observed between cultivars, as AG-10 exhibited a greater tolerance to increasing Cd concentrations. Cd can potentially disrupt the activities of key metabolic enzymes that control growth (Amaya-Carpio *et al.*, 2009). In addition, growth reductions are a result of the irreparable effect of Cd on membrane leakage and proton pump activity (Karcz & Kurtyka, 2007). Cd stress hinders the hydraulic conductivity and extensibility of the cell wall, which damages the morphology of the plant (Ehlert *et al.*, 2009).

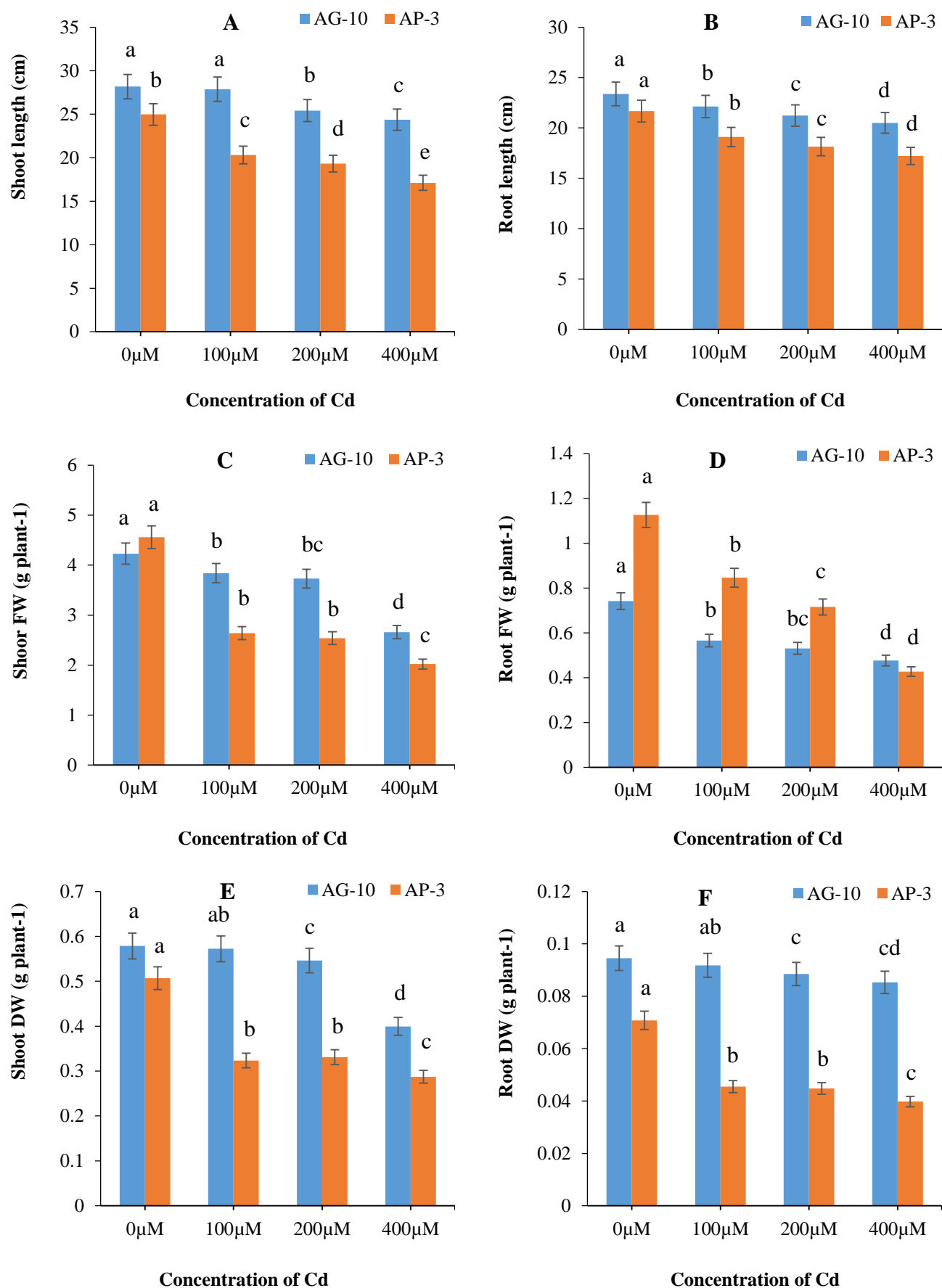


Fig. 1. Effect of different concentrations of Cd on (A) shoot length, (B) root length, (C) shoot FW, (D) root FW, (E) shoot DW, and (F) root DW in two genotypes of *Pisum sativum* L. Data presented are the means \pm SE ($n = 5$). Different letters indicate significant difference at $p \leq 0.05$.

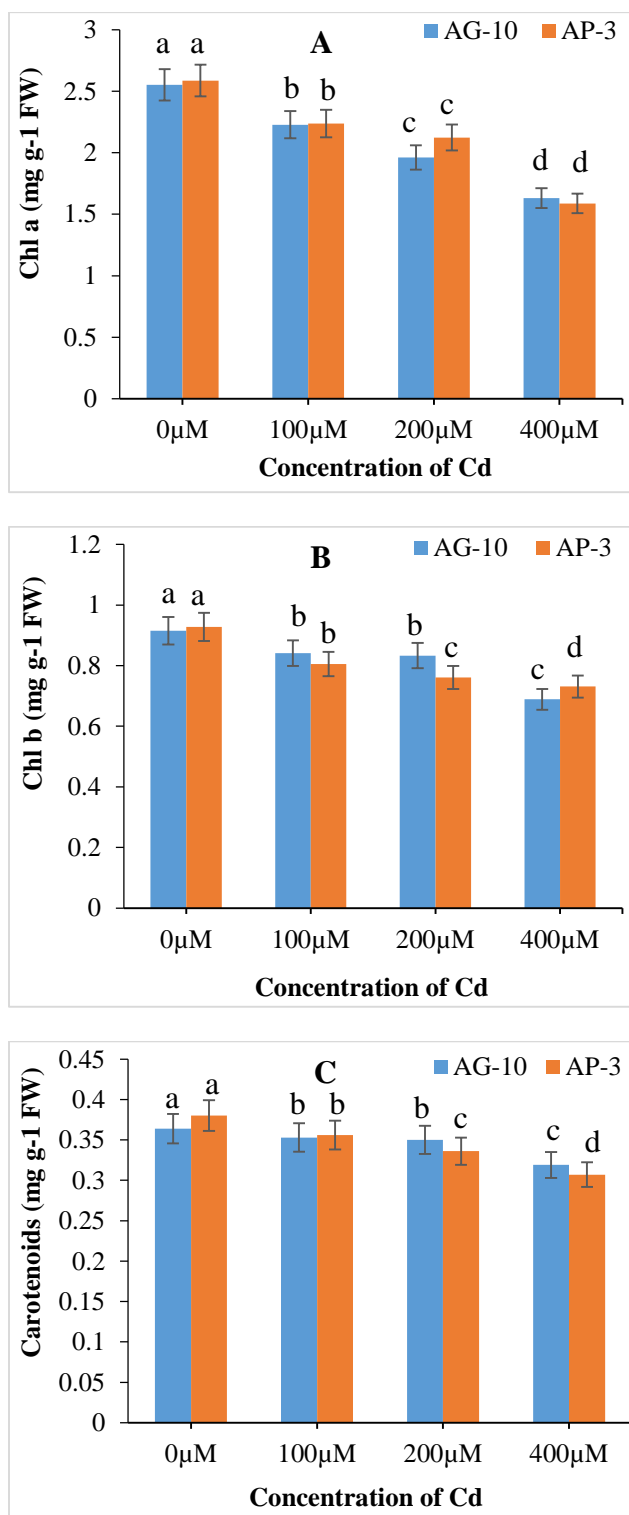


Fig. 2. Effect of Cd toxicity on (A) chlorophyll 'a', (B) chlorophyll 'b', and (C) carotenoid content in two genotypes of *Pisum sativum* L. Data presented are the means \pm SE ($n = 5$). Different letters indicate significant difference at $p \leq 0.05$.

Pigment content: Treatment of *Pisum sativum* cultivars with Cd led to a considerable reduction in chlorophyll synthesis, with greater reductions was observed when a high level of Cd (400 μ M) was applied. The observed reductions in Chl a, Chl b, and carotenoids were 36.08, 24.69, 12.29% (AG-10) and 38.61, 21.18, 19.15% (AP-3), respectively, at 400 M μ Cd (Fig. 2A-C).

Similarly, declines in pigment synthesis due to Cd application have been previously reported for *Helianthus annuus* (Rivelli *et al.*, 2012), *Abelmoschus esculentus* (Mangal *et al.*, 2013) and *Gossypium hirsutum* (Liu *et al.*, 2014). Heavy metals (including Cd) have the potential to impede the biosynthesis of chlorophyll pigments (Hsu & Kao, 2003), potentially due to the enhanced activity of chlorophyll-degrading enzymes like chlorophyllase (Singh & Jain, 1981). Ahmad *et al.*, (2011) and Khan *et al.*, (2015) also observed chlorophyll degradation in mustard and wheat respectively due to Cd stress.

Osmolytes

The effect of Cd on the accumulation of osmolytes, including proline, glycine betaine (GB), total protein, and soluble sugar, is shown in Fig. 3A-D. Significant increases in proline, GB, and protein accumulation were observed for both cultivars when higher Cd concentrations were applied. Relative to the controls, proline and GB increased up to 75.47% and 85.98%, respectively, in AG-10 and 68.09% and 77.18%, in AP-3 at 400 μ M Cd stress (Fig. 3A-D). However, soluble protein content increased at 200 μ M Cd stress by 47.11% and 2.48% in AG-10 and AP-3, respectively, but decreased at 400 μ M Cd stress. Sugar content declined at all Cd concentrations, with the greatest declines observed at 400 μ M Cd (AG-10: 33.84%, AP-3: 46.53%).

Accumulation of proline, GB, and sugars have been implicated in the maintenance of water balance and thus, cell osmolarity (Abdul Jaleel *et al.*, 2007; Ahanger *et al.*, 2014). Environmental stress conditions enhance the activity of proline-synthesizing enzymes and down-regulate its catabolism (Ahmad *et al.*, 2010; Ahanger *et al.*, 2014), resulting in proline accumulation. Similar to the results of this study, increased accumulation of proline in Cd-fed seedlings has been shown in *Helianthus annuus* (Zengin & Munzuroglu, 2006), *Lycopersicon esculentum* (Ahmad *et al.*, 2018), and *Brassica juncea* (Irfan *et al.*, 2014). Although the increased accumulation of osmolytes, including proline, GB, and sugars, does not interfere with metabolic pathways of cells, these osmolytes do replace water in key metabolic processes (Zhifang & Loescher, 2003). Accumulation of proline, GB, and sugars helps prevent stress-induced oxidative damage to plants by maintaining enzyme activity, membrane functionality, and by eliminating ROS (Ahanger & Agarwal, 2017). GB, which is exclusively found in chloroplasts, protects the thylakoid membranes, Rubisco activity, oxygen evolving complex, and, thus, the photosynthetic efficiency (Yokoi *et al.*, 2002). GB accumulation stabilizes extrinsic PSII complex proteins at non-physiological conditions (Papageorgiou & Murata, 1995; Chen & Murata, 2011). Soluble sugars (such as disaccharides, oligosaccharides in the raffinose family, and fructans) and the enzymes involved in their regulation are believed to have a strong correlation with stress-induced ROS accumulation in plants (Keunen *et al.*, 2013). Ahanger & Agarwal (2017) observed higher stress tolerance in *Triticum aestivum* L. as a result of sugar accumulation. Accumulated sugars regulate the interplay between ROS and other tolerance strategies and enhance growth and crop production (Sami *et al.*, 2016).

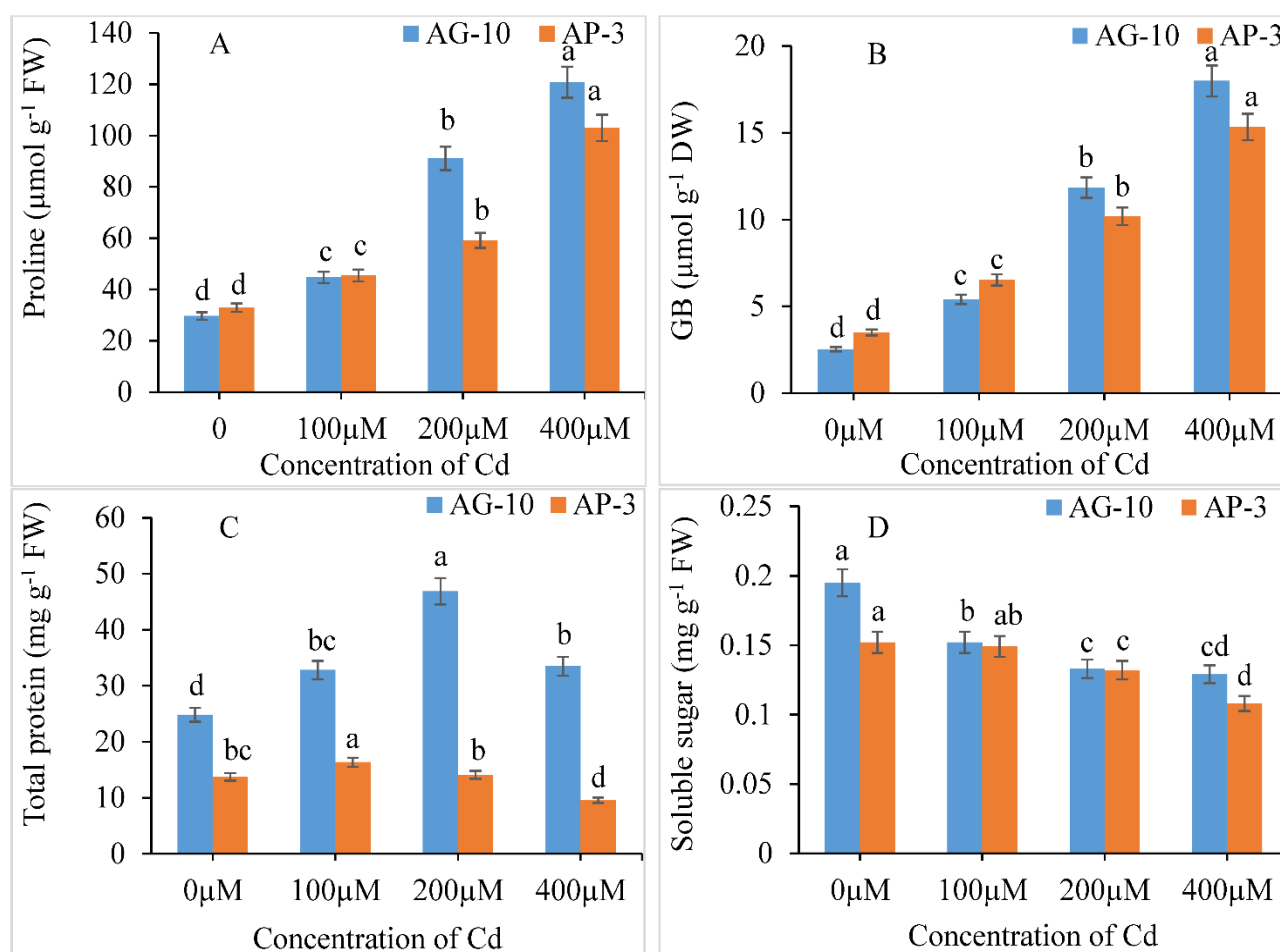


Fig. 3. Effect of different concentrations of Cd on (A) proline, (B) glycine betaine, (C) total protein, and (D) soluble sugar content in two genotypes of *Pisum sativum* L. Data presented are the means \pm SE ($n = 5$). Different letters indicate significant difference at $p \leq 0.05$.

H₂O₂, MDA, and EL: H₂O₂, MDA, and EL increased by 40.63%, 43.70%, and 27.92% (AG-10) and 39.30%, 42.14%, and 33.46% (AP-3), respectively, relative to control (Fig. 4A-C). AG-10 had lower reductions in H₂O₂, MDA, and EL compared to AP-3, indicating that AG-10 has a higher tolerance to Cd. Greater H₂O₂, MDA, and electrolyte leakage has been observed for Cd-stressed tomato (Ouariti *et al.*, 1997), *Chrysanthemum morifolium* (Hossain *et al.*, 2006), and mustard (Ahmad *et al.*, 2011). Lipid membranes are sensitive to excess ROS and often trigger oxidation, causing the generation of peroxide radicals through a chain of reactions (Ahanger & Agarwal, 2017; Ahmad *et al.*, 2018). This results in the loss of structural and functional integrity and leakage of essential elements and other cellular constituents (Djebali *et al.*, 2005). Mahmood *et al.*, (2007) reported reduced membrane stability leading to the leakage of essential ions in wheat, barley, and rice due to heavy metal stress. Heavy metal stress enhances lipoxygenase activity and production of peroxides and hydroxyl radicals, thereby deteriorating the structural and functional integrity of membranes and other cellular components (Macri *et al.*, 1994; Djebali *et al.*, 2005). Increased generation of ROS are responsible for oxidative stress and growth reduction (Kuo & Kao, 2004).

Antioxidants: The activity of antioxidant enzymes SOD, CAT, APX, GR and GST was increased significantly at all levels of Cd stress. Relative to the control, SOD, CAT,

APX, GR and GST activities increased by 12.9%, 15.52%, 16.70%, 20.29% and 8.82% (AG-10) and by 13.83%, 14.95%, 15.94%, 16.95% and 6.25% (AP-3), respectively, at 100 μM Cd (Fig. 5A-E). At a higher concentration of Cd (400 μM), antioxidant activity was increased in both genotypes; however, the greatest increase was observed in AG-10.

Within the antioxidative protective system, SOD is a key component that actively eliminates toxic superoxide radicals, leading to greater protection of cellular structures and processes such as the electron transport chain. Our results support the findings of Milone *et al.*, (2003), Shan *et al.*, (2012), Irfan *et al.*, (2014), Per *et al.*, (2016b), and Ahmad *et al.*, (2018). Greater SOD activity brings modulation in Haber-Weiss reaction substrates, superoxides, and H₂O₂, leading to reductions in the generation of toxic hydroxyl (OH[•]) radicals. SOD generated H₂O₂ is scavenged either by CAT or APX and GR via the ascorbate-glutathione cycle, where AsA and GSH are the key catalytic components (Ahmad *et al.*, 2010; Ahanger *et al.*, 2017). Greater activity of CAT, GR, and GST mediates the efficient removal of toxic H₂O₂ to maintain the functional and structural integrity of cells, ultimately leading to improved stress adaptation (Ahanger & Agarwal, 2017). John *et al.*, (2009) also showed a gradual increase in the activities of antioxidant enzymes in mustard with increasing Cd concentrations in soil.

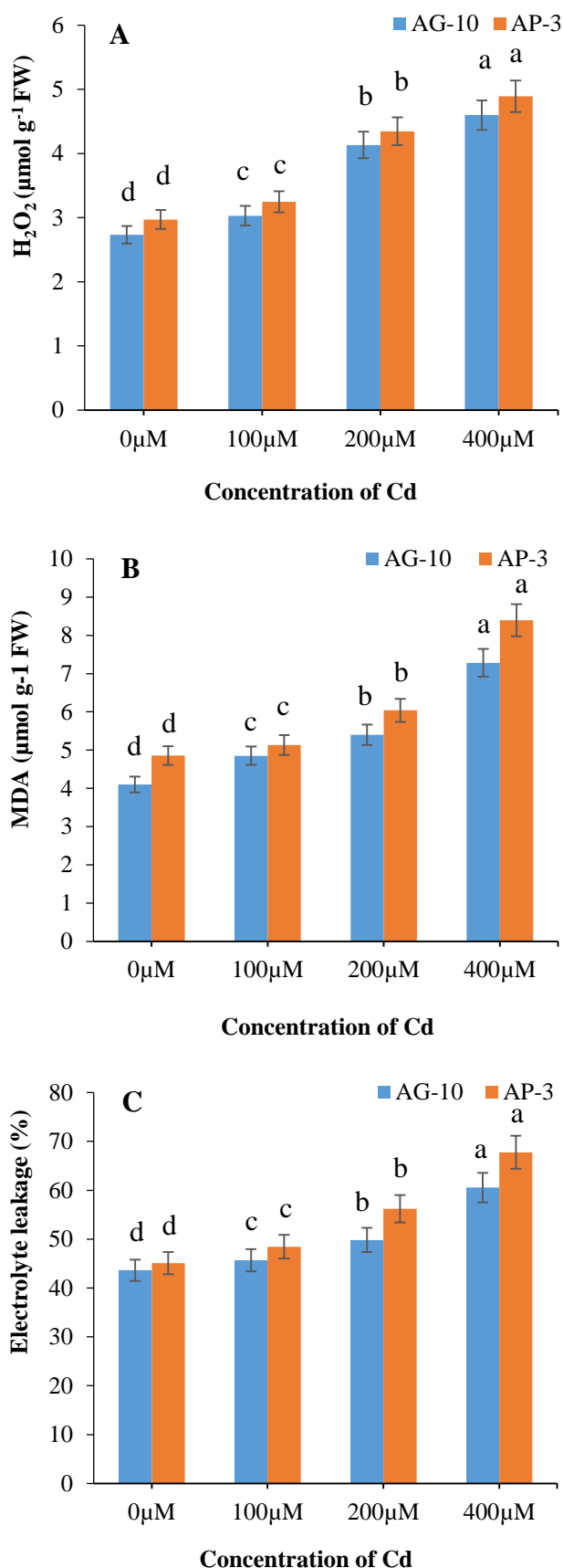


Fig. 4. Enhanced levels of (A) hydrogen peroxide, (B) malondialdehyde, and (C) electrolyte leakage in two genotypes of *Pisum sativum* L. subjected to different concentrations of Cd. Data presented are the means \pm SE ($n = 5$). Different letters indicate significant difference at $p \leq 0.05$.

Phenols: Cultivar AP-3 showed greater declines in the accumulation of phenols with increasing Cd concentrations (Fig. 5F). Phenol content was decreased by 22.91% (AG-10) and 39.14% (AP-3) at 400 μ M Cd relative to the controls. This also indicates that AG-10 is more tolerant to Cd than AG-3.

Higher synthesis of phenols and phenol derivatives has been shown to confer stress tolerance in crop plants (Tomar & Agarwal, 2013; Ahanger & Agarwal, 2017). Plant species maintaining higher phenol content are known to have higher peroxidase enzyme activities, leading to better growth (Zupan *et al.*, 2014). Polyphenols have a strong capacity to donate hydrogen ions, and can, therefore, be among the best antioxidant molecules to eliminate ROS (Hernández *et al.*, 2009). Multiple studies have shown that increased induction of several genes under stress conditions (e.g., drought, metal toxicity, nutrient deprivation, and wounding) causes increase in phenol levels (Dixon & Paiva, 1995; Winkel-Shirley, 2002; Ahanger & Agarwal, 2017). Griesser *et al.*, (2015) demonstrated that the high photosynthetic efficiency of *Vitis vinifera* was due to the enhanced accumulation of wide variety of polyphenolic compounds, which led to the maintenance of a higher water potential.

Conclusions

The results from this study showed that Cd toxicity decreased plant growth and photosynthetic activity through decreased chlorophyll content in two pea genotypes. Cd leads to increase in ROS, which are dangerous for biomolecules. However, osmolytes (such as proline, GB, and protein) help pea plants to withstand Cd stress. Antioxidant enzyme activities also increased in order to maintain the redox balance under Cd stress. The genotype AG-10 showed smaller reductions in growth, biomass yield, and pigment content under Cd stress than AP-3. AG-10 also showed higher accumulations of proline and GB and enhanced antioxidant enzyme activities than AP-3, indicating that the AG-10 genotype is more tolerant to Cd than AP-3.

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References

- Abdul Jaleel, C., R. Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and R. Panneerselvam. 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *S. Afr. J. Bot.*, 73(2): 190-195.
- Aebi, H. 1984. Catalase in vitro. In: *Methods Enzymol.* (Eds.): Colowick, S. and N. Kaplan. Elsevier, Florida, pp. 121-126.
- Ahanger, M.A. and R. Agarwal. 2017. Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. *Plant Physiol. Biochem.*, 115: 449-460.

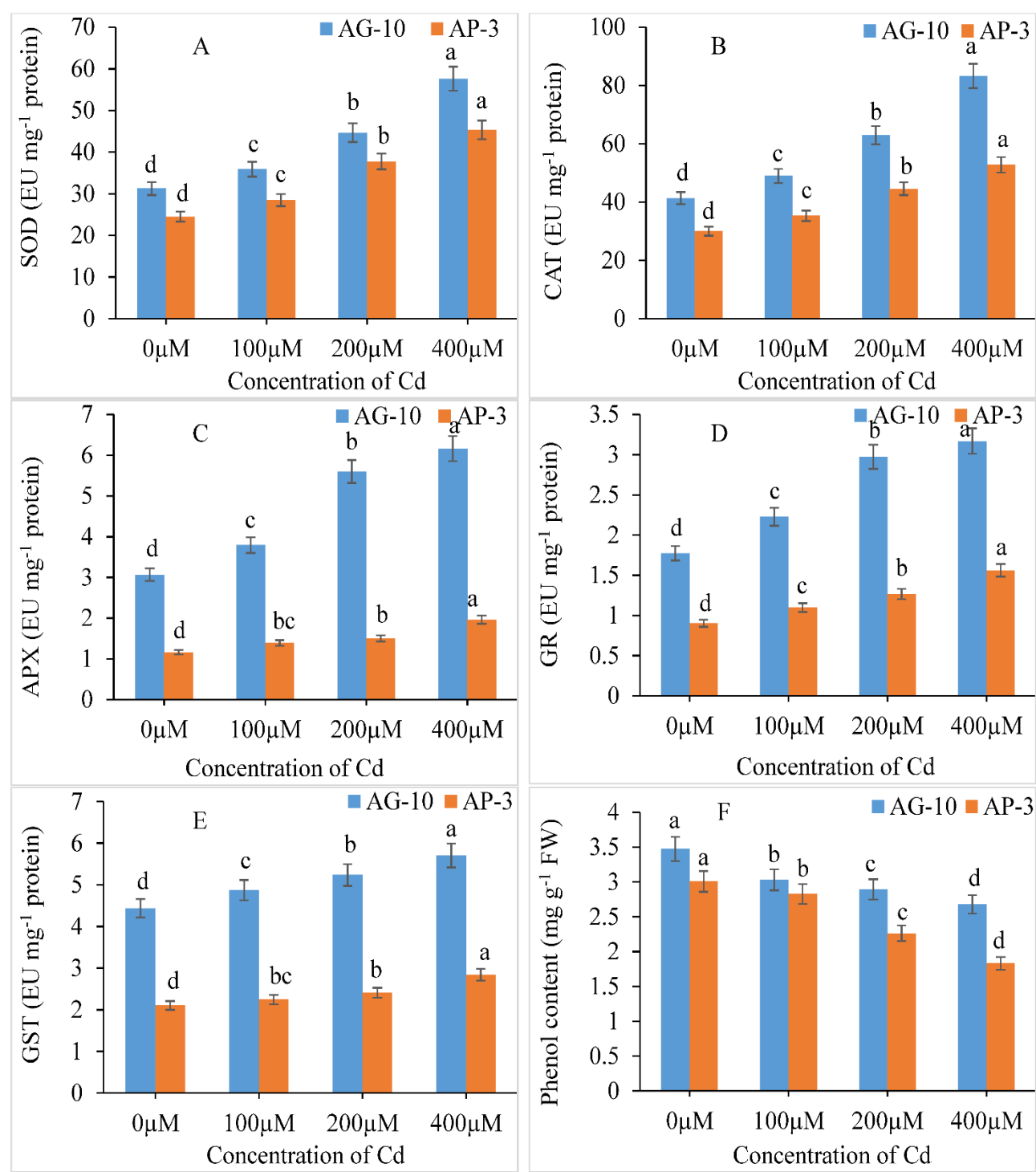


Fig. 5. Effect of different concentrations of Cd on (A) superoxide dismutase, (B) catalase, (C) ascorbate peroxidase, (D) glutathione reductase, (E) glutathione S-transferase, and (F) phenol content in two genotypes of *Pisum sativum* L. Data presented are the means \pm SE ($n = 5$). Different letters indicate significant difference at $p \leq 0.05$.

Ahanger, M.A., N.S. Tomar, M. Tittal, S. Argal and R.M. Agarwal. 2017. Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiol. Mol. Biol. Plants*, 23(4): 731-744.

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.K.R. Hakeem, A. Kumar, M. Ashraf and N.A. Akram. 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr. J. Biotechnol.*, 11(11): 2694-2703.

Ahmad, P., E.F. Abd Allah, A. Hashem, M. Sarwat and S. Gucl. 2016. Exogenous application of selenium mitigates cadmium toxicity in *Brassica juncea* L. (Czern & Cross) by up-regulating antioxidative system and secondary metabolites. *J. Plant Growth Regul.*, 35(4): 936-950.

- Ahmad, P., E.F. Abd-Allah, M.N. Alyemeni, L. Wijaya, P. Alam, R. Bhardwaj and K.H.M. Siddique. 2018. Exogenous application of calcium to 24-epibrassinosteroid pre-treated tomato seedlings mitigates NaCl toxicity by modifying ascorbate–glutathione cycle and secondary metabolites. *Sci. Rep.*, 8: 13515.
- Ahmad, P., C.A. Jaleel and S. Sharma. 2010. Antioxidant defense system, lipid peroxidation, proline-metabolizing enzymes, and biochemical activities in two *Morus alba* genotypes subjected to NaCl stress. *Russ. J. Plant Physiol.*, 57(4): 509-517.
- 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.
- Ahmad, P., M. Sarwat, N.A. Bhat, M.R. Wani, A.G. Kazi and L.S.P. Tran. 2015. Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. *PLOS One*, 10(1): e0114571.
- Amaya-Carpio, L., F.T. Davies, T. Fox and C. He. 2009. Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* ssp. *fistulosa*. *Photosynthetica*, 47(1): 1-10.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant Physiol.*, 24(1): 1-15.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- Chen, T.H. and N. Murata. 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.*, 34(1): 1-20.
- Chun, O.K., D.O. Kim and C.Y. Lee. 2003. Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *J. Agric. Food Chem.*, 51(27): 8067-8072.
- Dey, P.M. 1990. Oligosaccharides. In: *Methods in Plant Biochemistry*. Elsevier: pp: 189-218.
- 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.
- Dixon, R.A. and N.L. Paiva. 1995. Stress-Induced Phenylpropanoid Metabolism. *Plant Cell*, 7(7): 1085.
- Djebali, W., M. Zarrouk, R. Brouquisse, S. El Kahoui, F. Limam, M.H. Ghorbel and W. Chaïbi. 2005. Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biol.*, 7(4): 358-368.
- 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.
- El-Beltagi, H.S. and H.I. Mohamed. 2013. Alleviation of cadmium toxicity in *Pisum sativum* L. seedlings by calcium chloride. *Not. Bot. Horti Agrobot. Cluj. Na.*, 41(1): 157.
- Foster, J.G. and J.L. Hess. 1980. Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. *Plant Physiol.*, 66(3): 482-487.
- Godt, J., F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich and D.A. Groneberg. 2006. The toxicity of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol.*, 1(1): 22.
- Griesser, M., G. Weingart, K. Schoedl-Hummel, N. Neumann, M. Becker, K. Varmuza, F. Liebner, R. Schuhmacher and A. Forneck. 2015. Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). *Plant Physiol. Biochem.*, 88: 17-26.
- Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. *Plant Soil*, 70(2): 303-307.
- Hasanuzzaman, M. and M. Fujita. 2013. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. *Ecotoxicol.*, 22(3): 584-596.
- Hernández, I., L. Alegre, F. Van Breusegem and S. Munné-Bosch. 2009. How relevant are flavonoids as antioxidants in plants? *Trends Plant Sci.*, 14(3): 125-132.
- Hossain, Z., A.K.A. Mandal, S.K. Datta and A.K. Biswas. 2006. Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: in vitro mutagenesis and selection by salt stress. *Funct. Plant Biol.*, 33(1): 91.
- Hsu, Y.T. and C.H. Kao. 2003. Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. *Plant Cell Environ.*, 26(6): 867-874.
- Huang, M., H. Zhu, J. Zhang, D. Tang, X. Han, L. Chen, D. Du, J. Yao, K. Chen and J. Sun. 2017. Toxic effects of cadmium on tall fescue and different responses of the photosynthetic activities in the photosystem electron donor and acceptor sides. *Sci. Rep.*, 7: 14387.
- 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.
- Jing, D., W. Fei-bo and Z. Guo-ping. 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang Univ-Sci B.*, 6(10): 974.
- John, R., P. Ahmad, K. Gadgil and S. Sharma. 2009. Cadmium and lead-induced changes in lipid peroxidation, antioxidative enzymes and metal accumulation in *Brassica juncea* L. at three different growth stages. *Arch. Agron. Soil Sci.*, 55(4): 395-405.
- Juknys, R., G. Vitkauskaitė, M. Račaitė and J. Vencloviėnė. 2012. The impacts of heavy metals on oxidative stress and growth of spring barley. *Cent. Eur. J. Biol.*, 7(2): 299-306.
- Karcz, W. and R. Kurtyka. 2007. Effect of cadmium on growth, proton extrusion and membrane potential in maize coleoptile segments. *Biol. Plant.*, 51(4): 713-719.
- Keunen, E.L.S., D. Peshev, J. Vangronsveld, W.I.M. Van Den Ende and A.N.N. Cuypers. 2013. Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant Cell Environ.*, 36(7): 1242-1255.
- Khan, M.I.R., 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.
- Kuo, M.C. and C.H. Kao. 2004. Antioxidant enzyme activities are upregulated in response to cadmium in sensitive, but not in tolerant, rice (*Oryza sativa* L.) seedlings. *Bot. Bull. Acad. Sin.*, 45: 291-299.
- Liu, D., W. Jiang and X. Gao. 2004. Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biol. Plant.*, 47(1): 79-83.
- Liu, L., H. Sun, J. Chen, Y. Zhang, D. Li and C. Li. 2014. Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton (*Gossypium hirsutum* L.). *Plant Omics*, 7(4): 284.
- Macri, F., E. Braidot, E. Petrusa and A. Vianello. 1994. Lipoxygenase activity associated to isolated soybean plasma membranes. *Biochim. Biophys. Acta*, 1215(1-2): 109-114.

- Madhava Rao, K.V. and T.V.S. Sresty. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.*, 157(1): 113-128.
- Mahmood, T., K. Islam and S. Muhammad. 2007. Toxic effects of heavy metals on early growth and tolerance of cereal crops. *Pak. J. Bot.*, 39(2): 451.
- Mangal, M., M. Agarwal and D. Bhargava. 2013. Effect of cadmium and zinc on growth and biochemical parameters of selected vegetables. *J. Pharmacog. Phytochem.*, 2(1): 106-114.
- Milone, M.T., C. Sgherri, H. Clijsters and F. Navari-Izzo. 2003. Antioxidative responses of wheat treated with realistic concentration of cadmium. *Environ. Exp. Bot.*, 50(3): 265-276.
- Mobin, M. and N.A. Khan. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.*, 164(5): 601-610.
- Mondal, N.K., C. Das, S. Roy, J.K. Datta and A. Banerjee. 2013. Effect of varying cadmium stress on chickpea (*Cicer arietinum* L.) seedlings: An Ultrastructural study. *Ann. Environ. Sci.*, 7: 59-70.
- Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22(5): 867-880.
- Ouariti, O., N. Boussama, M. Zarrouk, A. Cherif and M. Habib Ghorbal. 1997. Cadmium- and copper-induced changes in tomato membrane lipids. *Phytochem.*, 45(7): 1343-1350.
- Papageorgiou, G.C. and N. Murata. 1995. The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex. *Photosyn. Res.*, 44(3): 243-252.
- Per, T.S., N.A. Khan, A. Masood and M. Fatma. 2016a. Methyl jasmonate alleviates cadmium-induced photosynthetic damages through increased S-assimilation and glutathione production in mustard. *Front. Plant Sci.*, 7: 1933.
- Per, T.S., S. Khan, M. Asgher, B. Bano and N.A. Khan. 2016b. Photosynthetic and growth responses of two mustard cultivars differing in phytoalexin activity under cadmium stress. *Photosynthetica*, 54(4): 491-501.
- Perfus-Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier. 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32(4): 539-548.
- Rivelli, A.R., S. De Maria, M. Puschenreiter and P. Gherbin. 2012. Accumulation of Cadmium, Zinc, and Copper by *Helianthus annuus* L.: impact on plant growth and uptake of nutritional elements. *Int. J. Phytoremed.*, 14(4): 320-334.
- Sami, F., M. Yusuf, M. Faizan, A. Faraz and S. Hayat. 2016. Role of sugars under abiotic stress. *Plant Physiol. Biochem.*, 109:54-61.
- Sandalio, L.M., H.C. Dalurzo, M. Gómez, M.C. Romero-Puertas and L.A. del Río. 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, 52(364): 2115-2126.
- Shamsi, I.H., G. Jilani, Z. Guo-Ping and W. Kang. 2008. Cadmium stress tolerance through potassium nutrition in soybean. *Asian J. Chem.*, 20(2): 1099.
- 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. Agri. Sci.*, 4(6): 142-151.
- Siddiqui, M.H., M.H. Al-Wahaibi, A.M. Sakran, M.O. Basalah and H.M. Ali. 2012. Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. *Int. J. Mol. Sci.*, 13(6): 6604-6619.
- Singh, G. and S. Jain 1981. Effect of some growth regulators on certain biochemical parameters during seed development in chickpea under salinity. *Ind. J. Plant Physiol.*, 25: 167-179.
- Talukdar, D. 2012. Exogenous calcium alleviates the impact of cadmium-induced oxidative stress in *Lens culinaris medic.* seedlings through modulation of antioxidant enzyme activities. *J. Crop Sci. Biotechnol.*, 15(4): 325-334.
- Tomar, N.S. and R.M. Agarwal. 2013. Influence of treatment of *Jatropha curcas* L. leachates and potassium on growth and phytochemical constituents of wheat (*Triticum aestivum* L.). *Amer. J. Plant Sci.*, 04(05): 1134-1150.
- Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. *Plant Sci.*, 151(1): 59-66.
- Winkel-Shirley, B. 2002. Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol.*, 5(3): 218-223.
- Yokoi, S., F.J. Quintero, B. Cubero, M.T. Ruiz, R.A. Bressan, P.M. Hasegawa and J.M. Pardo. 2002. Differential expression and function of *Arabidopsis thaliana* NHX Na⁺/H⁺ antiporters in the salt stress response. *Plant J.*, 30(5): 529-539.
- Zengin, F.K. and O. Munzuroglu. 2006. Toxic effects of cadmium (Cd⁺⁺) on metabolism of sunflower (*Helianthus annuus* L.) seedlings. *Acta Agric ScandB Soil Plant Sci.*, 56(3): 224-229.
- Zhifang, G. and W.H. Loescher. 2003. Expression of a celery mannose 6-phosphate reductase in *Arabidopsis thaliana* enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimer. *Plant Cell Environ.*, 26(2): 275-283.
- Zupan, A., M. Mikulic-Petkovsek, A. Slatnar, F. Stampar and R. Veberic. 2014. Individual phenolic response and peroxidase activity in peel of differently sun-exposed apples in the period favorable for sunburn occurrence. *J. Plant Physiol.*, 171(18): 1706-1712.

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