Pak. J. Bot., 49(6): 2071-2078, 2017.

PRIMING SEEDS WITH PHYTOHORMONES ALLEVIATES CADMIUM TOXICITY IN MUNG BEAN (*VIGNA RADIATA* L. WILCZEK) SEEDLINGS

MEHER HASSAN[1] AND SIMEEN MANSOOR[2*]

Department of Genetics, University of Karachi, Pakistan
Corresponding author's email: simeenm@uok.edu.pk

Abstract

Cadmium toxicity has deleterious effects on human, animals and plants. Mung bean seeds were sown in petri dishes after imbibing them in 50 µM Salicylic acid (SA), 100 µM Gibberellic acid (GA_3) and distilled water (D/W) for 12 h and allowed to grow for 24h at 30°C. Seedlings were then treated with cadmium (0.3 mM and 0.5 mM) and harvested at 96h. Results revealed a significant decrease in all growth parameters and low level of protein in Cd treated seedlings as compared to control. In contrast, elevated levels of MDA, H_2O_2, antioxidant enzymes, tannin and proline were observed in cadmium treated seedlings. However, pretreatment with SA and GA_3 prior to cadmium stress showed improvement in all growth parameters and protein content with the significant decline in MDA, H_2O_2, tannin and proline under cadmium stress that confers tolerance. Antioxidant enzymes seemed to help in declining H_2O_2 and MDA contents that confer membrane stability. Conclusively these results confirm the ameliorating effects of SA and GA_3 under cadmium toxicity. As few articles are available on effect of mung bean under cadmium stress, therefore our research will have some contribution in understanding the damaging effect of cadmium and its amelioration by phytohormones in mung bean. We found that pretreatment of GA_3 and SA is an effective way to enhance tolerance against cadmium in mung bean. Furthermore, the response of GA_3 was more effective than SA under cadmium stress.

Key words: Antioxidant enzymes, Cadmium, Phytohormones, Proline, Tannin, *Vigna radiata*.

Introduction

Being sessile organisms, plants are constantly exposed during their life cycle to adverse environmental conditions that negatively affect their growth, development and productivity. In Pakistan, the main contributors to surface and ground water pollution are various industrial waste products from textile, fertilizers, petrochemicals, leather, sugar processing, steel engineering, fuel processing, mining and other industries (Tariq *et al*., 2006). In Pakistan, some urban soils and crops are contaminated with heavy metals including cadmium with the average values ranging from 5.8 to 6.1mg/kg. Studies showed that cadmium levels found in our soils and in crops grown in polluted areas exceed the safe limits of cadmium as prescribed by WHO and European standards (Saleem *et al*., 2005; Akbar *et al.*, 2009; Mahmood & Malik, 2014). Most of our farmers are using industrial waste to irrigate the crops and vegetables as it is considered as a good and inexpensive source of nutrient for plants (Nawaz *et al*., 2006; Faiz *et al*., 2009; Farid *et al*., 2015).

Mung bean is a highly consumable pulse crop having extensive cultivation area in Asia, Africa, South America and Australia. Mung bean has high nutritional value, needs low nitrogenous fertilizers, maintains soil fertility and fits in different cropping systems (Graham & Vance, 2003; Yaqub *et al*., 2010). It is cultivated over an area of 250 thousand hectares in Pakistan with an annual production of 180 thousand tons in 2009 (Hidayat *et al*., 2011). Most of Pakistan's area falls in the arid and semi-arid region (Ahmad *et al.*, 2017). Hence, the scarcity of clean water has forced poor farmers to use untreated industrial and municipal waste for irrigation of almost 32,500 hectares' land (Ensink *et al*., 2004; Hussain *et al*., 2010). This led to significant buildup of these toxic metals in the fields thereby reducing the yield of all crops, that are grown in such fields (Mahmood & Malik, 2014).

Cadmium (Cd) is a non-essential metal that is one of the most hazardous environmental pollutants for all living beings even at very low concentrations (Benavides *et al.*, 2005). Cadmium is present in abundant amounts in the soil; hence, there is a risk that it can get transferred into the beings that consume the crops cultivated in such soil. (Xiong *et al.*, 2003; Farid *et al.*, 2015). However, the level of cadmium toxicity varies among different plants. Cd is a redox in active heavy metal and can only generate ROS indirectly by enzyme inactivation (Sun *et al.*, 2007) that suppresses the growth of plants. Cadmium is also responsible to damage membranes and alter protein and enzymes needed for various metabolic processes (Sanita di Toppi & Gabbrielli, 1999; Tran & Popova, 2013; Irfan *et al*., 2013; Hassan & Mansoor, 2014). Cd is often involved in the alteration of phenolic compounds that are responsible for performing several functions in plant cells. These phenolic compounds include tannins and H_2O_2 which are often involved in responding to different kinds of abiotic and biotic stresses and may work as Reactive oxygen species (ROS) scavenging compounds (Rivero *et al.*, 2001; Olga *et al.*, 2003; Michalak, 2006). Free proline and Tannin act as osmoprotectant, metal chelators and antioxidants that protect cells from free radical damage. Free proline and Tannin also maintain a favourable cellular environment for phytochelators synthesis and Cd sequestration (Sun *et al*., 2007; Gill & Tuteja, 2010; Okuda & Ito, 2011).

Plant growth hormones like Gibberellic acid (GA_3) and Salicylic acid (SA) are endogenous signal molecules in plants and have an important role in certain physico-chemical processes. During stress their level decreases and their exogenous application will play an important role in alleviating different abiotic stresses in plants by activating certain antioxidant responses (He *et al.*, 2010; Liu *et al.*, 2012). It has been reported earlier (Ghani, 2010; Hassan & Mansoor, 2014) that mung bean is sensitive to cadmium toxicity. As insufficient research has been done on cadmium stress on mung bean and its alleviation by phytohormones, therefore the objective of the present study is to study the effect of cadmium on mung bean seedlings and the extent to which Salicylic acid and Gibberellic acid was helpful in alleviating oxidative stress caused by cadmium.

Materials and Methods

Mung bean seeds of NM 13-1 were obtained from the National Agriculture Research Council (NARC), Islamabad. Healthy seeds of uniform size were sterilized in 1% sodium hypochlorite solution for 10 min and thoroughly rinsed with distilled water several times. Seeds were imbibed in 50 µM Salicylic acid (SA), 100 µM Gibberellic acid (GA_3) and distilled water for 12 h. Experiment was set in petri dishes with three replications in CRD and allowed to germinate in distilled water for 24 h at 30°C. Then the experiment was treated with of 0.3 mM and 0.5 mM Cd for 72 h. Seedlings were harvested, saved in plastic bags and stored at 4°C for biochemical analysis.

Lengths of 96 h old seedlings were recorded in cm. Fresh weight (FW) and dry weight (DW) were measured in gm. Relative water content (RWC) was calculated in percentage (Sumithra *et al*., 2006) (Table 2).

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Protein and Antioxidant enzymes were extracted by the method of Jiang & Huang (2001). Seedlings (0.2g) were extracted in 50 mM Na_3PO_4 (pH 7.0) homogenizing buffer containing 1% w/v polyvinylpyrrolidone (PVP) and 0.2 mM ascorbic acid in a chilled mortar and pestle followed by centrifugation at 10000 rpm for 30 min. Total protein was estimated by the method of Lowry *et al*. (1951) at 750 nm against a reagent blank, using bovine serum albumin as a standard.

Guaiacol peroxidase (GPX 1.11.1.7) enzyme activity was measured by the method of Everse *et al.* (1994). Absorbance was noted at 470 nm against the reagent blank. Specific activity was calculated with an extinction coefficient of 26.6 mM^{-1} cm^{-1}. Ascorbate peroxidase (APX 1.11.1.11) enzyme activity was measured by the method of Nakano & Asada (1981). Reaction was initiated by adding 0.1 mM H_2O_2 and the increase in absorbance was measured at 290 nm for 2 min. Specific activity was calculated with an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Catalase (CAT 1.11.1.6) enzyme activity was estimated by the method of Aebi (1984). Enzyme activity was determined by following the degradation of H_2O_2 at 240 nm for 2 min. Specific activity was calculated with an extinction coefficient of 40 mM^{-1} cm^{-1}.

Lipid peroxidation was determined by measuring malondialdehyde (MDA) contents by the method of Heath & Packer (1968) at low temperature conditions. Absorbance was recorded at 532 nm and corrected for specific turbidity by subtracting the absorbance at 600 nm. MDA content was determined by using the extinction coefficient of 155 mM^{-1} cm^{-1}. H_2O_2 was determined spectrophotometrically by Sergiev *et al.* (1997). Seedlings (0.5 g) were homogenized in 0.1% TCA then centrifuged at 12000 rpm for 15 min. Supernatant (500 µl) was added to 500 µl of 10 mM potassium phosphate and 1000 µl of 1.0 M KI. Absorbance was taken at 390 nm against 0.1% TCA as blank. Reaction was performed in dark. Specific activity was calculated by extinction coefficient of 280 mM^{-1} cm^{-1}.

Tannin content was determined by Folin-Denis reagent as according to the method of Schanderl (1970) using tannic acid as standard. A calibration curve was prepared using tannic acid concentrations ranging from 0 - 100 µg. The values were expressed in µg of tannic acid equivalents per gram fresh weight.

Free Proline contents were calculated using the method described by Bates *et al.* (1973). Seedlings (0.5 g) were extracted in 5 mL of 3% sulphosalicylic acid followed by centrifugation at 10000 rpm for 10 min. Equal volume of supernatant, ninhydrin reagent and glacial acetic acid were mixed and incubated at 100°C for 1 h. The samples were immediately placed in an ice bath to stop reaction. Toluene was added to the solution twice and mixed thoroughly. The chromophore containing toluene was aspirated from the aqueous phase and warmed at room temperature. Finally, the absorbance was read at 520 nm using a spectrophotometer. Toluene was used as a blank. The concentration of proline was determined using the standard curve and expressed as µmol/g FW.

Analysis of variance (ANOVA) was performed through SPSS version 20. Mean comparison was made by Duncan's multiple range test at $p \leq 0.05$ level of significance. Similar letters in figures represent non-significant differences among the treatments. Standard errors were represented as vertical bars, which were the mean of 3 values (n = 3). Pearson correlation coefficient has been performed by SPSS 20.

Results and Discussions

Seedling length: The symptoms appearing on various plant parts are direct measures of the intensity of prevailing stress. These effects can be helpful in diagnosing stress and adopting suitable strategies to increase stress tolerance and selection of promising varieties. To characterize the impact of phytohormones on cadmium stress, seeds were pre-treated with GA_3 and SA. Table of ANOVA (Table 1) showed that all treatments were significantly different from each other for mean seedling length. Our results depicted the decrease in mean seedling length in concentration dependent manner. This finding was also supported by Muneer *et al.* (2011) and Namjooyan *et al.* (2012). Growth inhibition of plant might be due to the transmission of assimilate resulted from structural and functional disturbances in metabolic processes as supported by Kumari *et al.* (2010). It was noticed that cadmium at 0.3 mM concentration exhibited 33% inhibition in seedling length which was further inhibited to 46% in 0.5 mM Cd treated seedlings. This may be due to the decrease in nutrient uptake (Sandalio *et al.*, 2001). However, growth performance was improved when seeds were imbibed in SA and GA_3 prior to 0.3 mM and 0.5 mM cadmium stress as compared to seeds exposed to cadmium stress without pretreatment with SA and GA_3 (Fig. 1). Furthermore, our results demonstrated that pretreatment with GA_3 was more efficient in mitigating cadmium toxicity by increasing seedling growth as compared to pretreatment of SA.

Table 1. Mean sum of squares for seedling length, FW, DW, RWC of mung bean genotype NM 13-1 under pretreatment of phytohormones and cadmium stress.

SV	Df	Seedling length	MS		
			FWT	DWT	RWC
Treatment	6	6.003**	0.001**	8.29E-006**	4.910**
Error	14	0.196	0.00014	6.310E-007	0.401

**: Significant at p≤0.01

Table 2. Mean sum of squares for protein, MDA, H_2O_2, GPX, APX, CAT, tannin and proline of mung bean genotype NM 13-1 under pretreatment of phytohormones and cadmium stress.

SV	Df	Protein	MS						
			MDA	H_2O_2	GPX	APX	CAT	Tannin	Proline
Treatment	6	4613.095**	147.170**	7.127**	3516.500**	3.810**	0.006**	2810.134**	913.787**
Error	14	4.762	1.560	0.049	3.533	0.060	0.0005	18.768	3.828

**: Significant at p≤0.01

Fresh weight: Fig. 2 exhibited 23% decrease in fresh weight at 0.3mM cadmium and became 27% at 0.5mM cadmium stress. It is reported by many researchers that cadmium may alter many physiological and biochemical processes such as seed germination, photosynthesis, inactivation and denaturation of enzymes, hormonal balance, nutrient assimilation, protein synthesis, and DNA replication (Nagajyoti *et al.,* 2010;Wani *et al*., 2012; Singh *et al*., 2013).

Pretreatment with phytohormones lessens the toxic effect of cadmium by increasing fresh weight as compared to cadmium alone. Lesser inhibition in fresh weight was observed in GA_3 pretreatment samples as compared to control. The exogenous application of phytohormones seems to stabilize and protect enzymes or DNA under cadmium stress.

Dry weight: Table 1 demonstrated that there was significant difference in dry weight between treatments. The difference was non significant for dry weight between control and 0.3 mM Cd but significant between control and 0.5 mM Cd (Fig. 3). It was also noticed that the pretreatment effect of Salicylic acid at 0.3 mM Cd was non significant, however GA_3 exhibited significant increase in dry weight for 0.3 mM Cd as compared to cadmium alone. It was further observed that pretreatment of both phytohormones was not effective for the induction in mean dry weight at 0.5 mM Cd treatments.

Relative water content: Decrease in relative water content was evident in treated seedlings with 3% and 2% for 0.3 mM and 0.5 mM Cd respectively. However, pretreatments of SA and GA_3 prior to cadmium stress helped in increasing RWC in treated seedlings (Fig. 4). Cadmium stress seemed to cause physiological drought in plants by restricting water intake in plants which resulted in reduction in RWC (Farouk *et al*., 2011) and reduction in other growth parametes like fresh and dry weight. Reduction in RWC was also reported in lettuce and radish seedlings under cadmium stress (Costa & Morel, 1994; Farouk *et al*., 2011)

It is suggested that due to various abiotic stresses including metals like cadmium, there is reduction in endogenous phytohormones in growing plants. It is also reported that since under stress condition reduced plant growth could result from an altered hormonal balance, an exogenous application of plant hormone like SA has been an attractive approach to attenuate heavy metal stress (Singh *et al*., 2016). It is reported that the application of salicylic acid exogenously confered metal tolerance to the plants of *Cassia tora* (Yang *et al*., 2003). Similarly exogenous application of salicylic acid protects barley plants from cadmium stress by increasing the fresh weight of roots and shoots. This effect of SA was mediated by supressing the cadmium induced up reguation of H_2O_2 metabolizing enzymes such as CAT and APX (Metwally *et al*., 2003).

Total protein contents: Cd results in phytotoxicity by inducing complex changes at the genetic, biochemical and physiological levels. It has the affinity to phosphate cysteinyl and histidyl side chains of proteins, purines, pteridines and porphyrins that change enymes and nucleic acids and disrupt oxidative phosphorylation (Hasanuzzaman *et al*., 2012).

The cadmium treatment exhibited reduction in total protein in concentration dependent manner and Fig. 5 illustated significant decline to 54% and 67% at 0.3 mM and 0.5 mM Cd treated seedlings respectively as compared to control. It was noticed that when seeds were imbibed in SA and GA_3 prior to Cd stress, seedlings were able to synthesize more proteins as compared to cadmium stressed seedlings. Our results showed the enhancement in morphological parameters after pretreatment of salicylic acid and gibberellic acid and these results were in agreement with the findings of He *et al*. (2010), who reported that pretreated SA rice seedlings showed increase in seedling length by alleviating negative effect of Cd on growth. We found reduction in protein content under cadmium stress which suggested that cadmium was responsible for degradation and hydrolysis of various proteins and this could be one of the reasons of retarded growth. The enhancement in protein and growth parameters after the application of hormones reflects that these hormones help in the stabilization of proteins hence improve growth parameters under stress.

Antioxidant enzymes activity: We observed alterations in activities of antioxidant enzymes which indicate their protective role under oxidative stress which could be due to increased level of ROS. Plants have their enzymatic and non enzymatic system to fight against reactive oxygen species produced under oxidative stress (Michalak, 2006). Fig 6 showed the increase in guaiacol peroxidase activity by the increase in concentration of cadmium, but GPX activity was reduced when seeds were imbibed in phytohormoes prior to Cd stress as compared to cadmium stress alone. We observed increase in APX activty when seedlings were under cadmium stress (Fig. 7). There was 47% promotion in APX activity at 0.3mM Cd which further enhanced to 54% at 0.5mM cadmium while this activity was lesser when seedlings were supplemented with phytohormones before Cd stress. Similar pattern was detected for CAT (Fig. 8), where cadmium was able to cause increase in CAT activity in concentration dependent manner and pretreatment with phytohormones reduced enzyme activity as compared to Cd stress alone. Alteration in these enzymes indicated that they were helpful in neutralization of H_2O_2 and maintainung the optimal levels of redox buffer for protecting the cellular functioning (Mittler *et al*., 2004). It is suggested that the increased activity of these antioxidant enzymes under cadmium stress could be in response to high ROS production, and decreased activity of enzymes in the phytohormone pretreated samples may be due to the maintainance of production of ROS under stress. It has been earlier reported that catalase is present in large quantities in peroxisomes and plays a vital role in maintaining cellular redox balance (Corpas *et al.*, 2001). Similar results were depicted in mung bean and Soy bean under heavy metal (Namjooyan *et al.*, 2012; Hassan & Mansoor 2014). It has been described earlier that Catalases have one of the highest turn over rates amoung all enzymes. Mechanism involved in cadmium toxicity is disturbance in AsA-GSH pools with ultimately cause disturbance in Glutathione and APX enzyme activities (Anjum *et al*., 2011).

Lipid peroxidation and H_2O_2 content: H_2O_2 plays a dual role in plants at low concentartion, it act as a signal molecule that induces the expression of numerous defence genes and activate multiple defence responses to abiotic stress while excess accumulation leads to cellular oxidation damage and even programmed cell death. (Hossain *et al*., 2012; Prasad *et al*., 1994). Our results exhibited increase in MDA and H_2O_2 contents under cadmium stress, which were strong indicators of membrane damage and lipid peroxidation. Relative to control, treatment of cadmium resulted in increase in MDA and H_2O_2 contents in dose dependent manner (Figs. 9 and 10). MDA and H_2O_2 contents were reduced by the pretreatment of phytohormones, may be because cadmium caused excessive production of ROS which in turn was responsible of damage to cell membranes and on the other hand phytohormones mimic the production of ROS. One of the main reason of retarded growth under stress could be the over production of ROS and MDA. Our results are in accordance with Khavari *et al.* (2013) in which growth parametres and protein levels decreas with increase in MDA contents in tomato plants, maize and Brassica (Hussain *et al*., 2012; Kapoor *et al*., 2014) under cadmium stress but pretreatment with GA_3 alleviated adverse effects on growth against cadmium toxicity.

Tannins: Higher levels of tannin is an indicator of stress in plants. Phenolic compounds like tannins are secondary metabolites that are involved in response to different kinds of abiotic stresses. They are helpful in detoxification of H_2O_2 produced under oxidative stress, also be involved in metal chelation (Lavid *et al.*, 2001, Kamachi *et al*., 2005). Similarly, results showed increased amount of tannin under cadmium stress at 0.3 mM (138%) and 0.5 mM (92%) and it seemed that high levels of tannins were associated with heavy metals accumulation in plants (Chin *et al.*, 2009). But by the exogenous application of phytohormones prior to cadmium treatment, there was significant reduction in tannin (Fig. 11). Our results were in agreement with the findings of Guangqiu *et al.,* 2007 ; Kapoor *et al.,* 2014, who demonstrated an increase in Phenolic compounds in mangrove and Brassica under cadmium stress. High levels of tannin indicate that they act as metal chelators or directly scavenge ROS to reduce oxidative damage caused by cadmium (Michalak, 2006).

Free proline content: The mechanism of action of increased levels of proline is not sequestration, but it reduces the formation of free radicals and maintains reducing environment (Siripornadulsi *et al*., 2002). Proline is also a metal chelator due to the presence of hydroxyl and carboxyl groups that bind to metal ions particularly iron and copper (Jun *et al*., 2003). Similarly, we also noticed significant increase in level of proline in cadmium treated seedlings in concentration dependent manner (Fig. 12). There was 104% promotion in proline at 0.3mM and 138% promotion at 0.5mM cadmium stress. It was further observed that decrease in proline content after the pretreatment of hormones before cadmium may be due to the decrease in the generation of ROS . Our results were supported by (Muneer *et al.*, 2011; Michalak, 2006) who described that accumulation of proline can be an indicator of environmental stress or it may have some protective roles against stress.

Correlation Coefficients presented in Table 3 revealed significant positive correlation between morphological parameters such as seedling length, fresh weight, relative water content and protein which were negatively correlate with enzymes activity, tannin, H_2O_2 and proline. It was further observed that MDA, antioxidant enzymes, tannins, H_2O_2 showed negative correlation with morphological parameters while positive correlation among themselves in mung bean seedlings under cadmium stress alone and with phytohormones.

Table 3. Correlation coefficient (r) between morphological and biochemical parameters.

	Length	FWT	DWT	RWC	Protein	GPX	APX	CAT	MDA	Tannin	H_2O_2	Proline
Length		0.776^{**}	0.254	0.526^{*}	0.741^{**}	-0.742^{**}	-0.366	-0.102	-0.172	-0.381	-0.795^{**}	$-.850^{**}$
FWT			0.270	0.732^{**}	0.691^{**}	-0.770^{**}	-0.634^{**}	0.095	-0.106	-0.468^{*}	-0.847^{**}	-0.820^{**}
DWT				-0.452^{*}	-0.060	-0.278	-0.052	0.359	0.146	0.177	-0.208	-0.027
RWC					0.689^{**}	-0.540^{*}	-0.582^{**}	-0.136	-0.246	-0.595^{**}	-0.657^{**}	-0.741^{**}
Protein						-0.843^{**}	-0.647^{**}	-0.290	-0.340	-0.455^{*}	-0.676^{**}	-0.944^{**}
GPX							0.783^{**}	-0.150	0.487^{*}	0.585^{**}	0.855^{**}	0.890^{**}
APX								-0.383	0.302	0.520^{*}	0.658^{**}	0.671^{**}
CAT									-0.041	-0.288	-0.257	0.135
MDA										0.742^{**}	0.382	0.275
Tannin											0.724^{**}	0.488^{*}
H_2O_2												0.822^{**}
Proline												

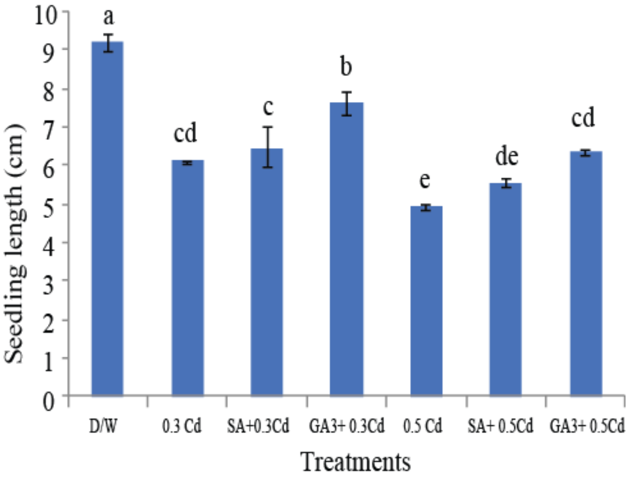

Fig. 1. Mean seedling length of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

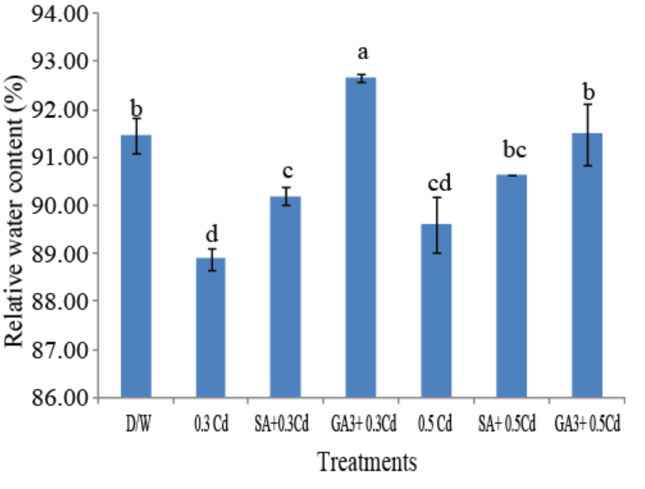

Fig. 4. Relative water content of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

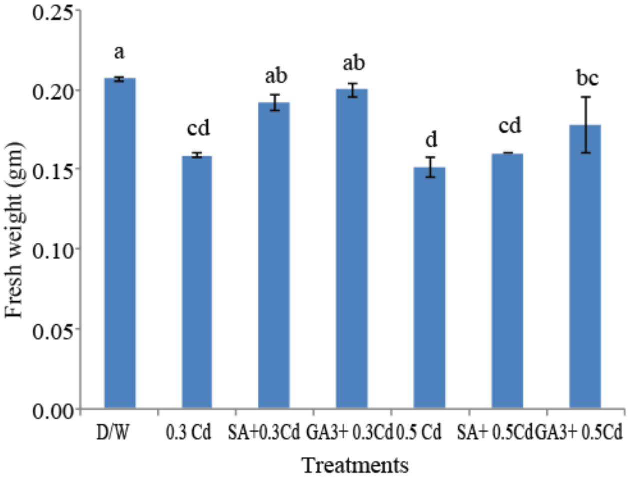

Fig. 2. Mean fresh weight of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

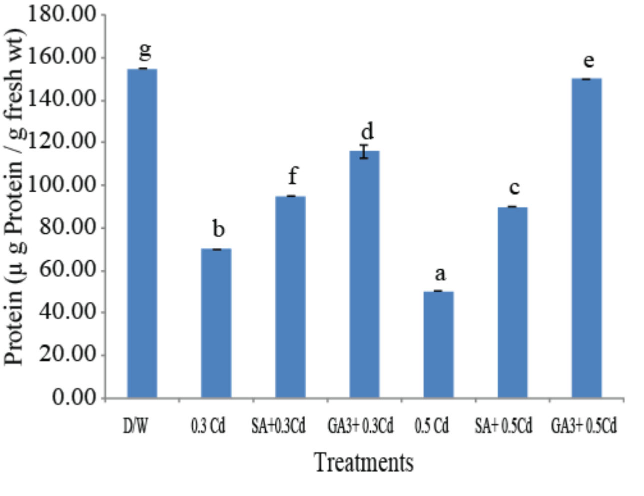

Fig. 5. Total protein contents of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

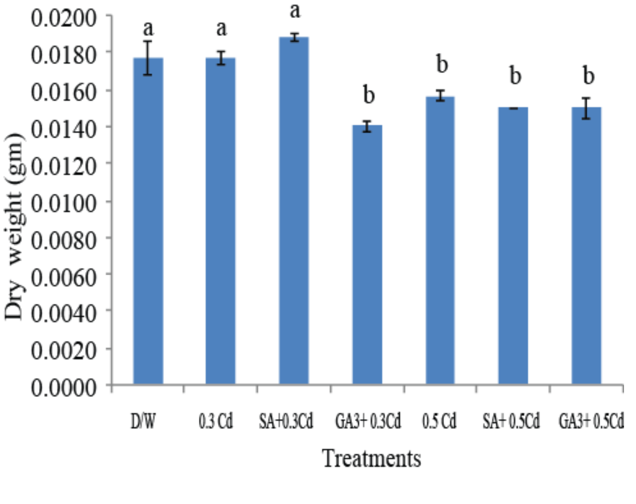

Fig. 3. Dry weight of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

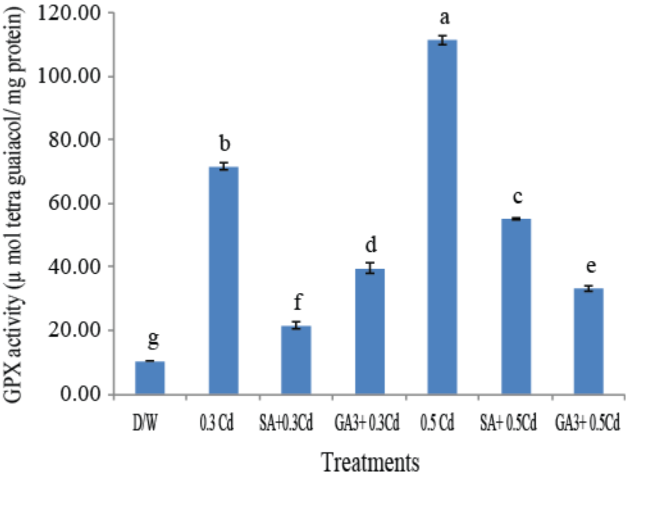

Fig. 6. GPX activity of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

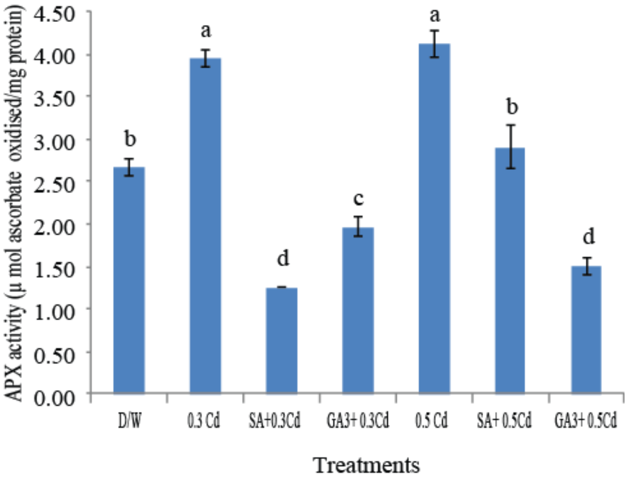

Fig. 7. APX activity of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

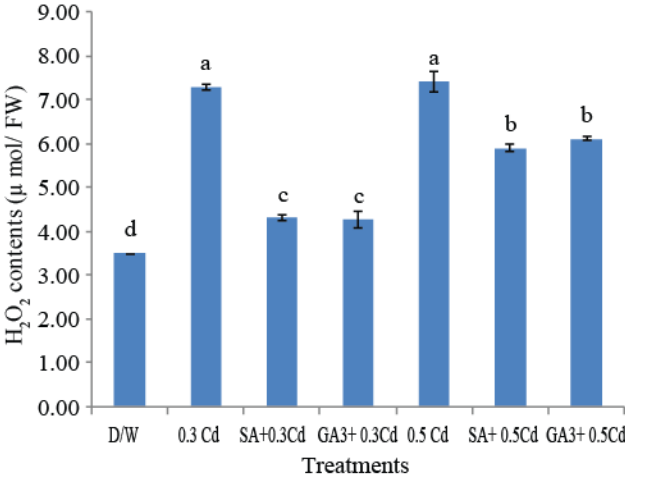

Fig. 10. H_2O_2 contents of of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

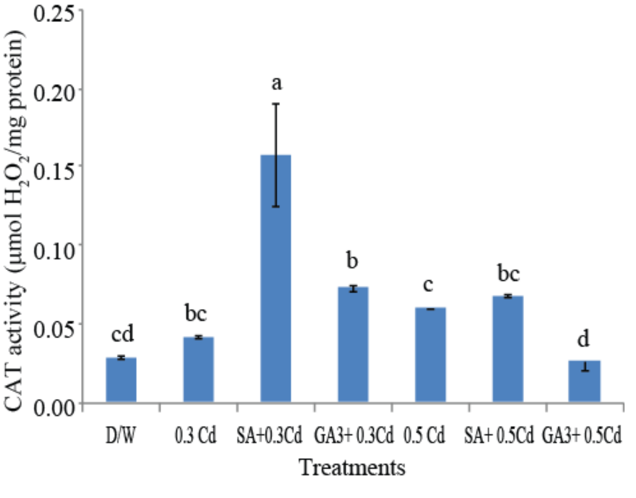

Fig. 8. CAT activity of of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

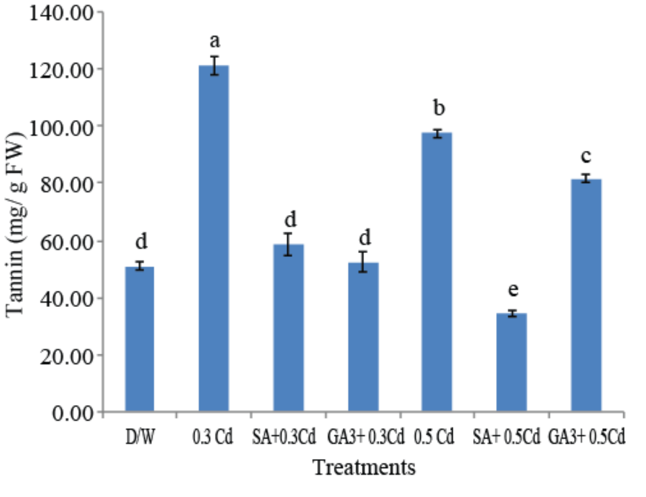

Fig. 11. Tannin contents of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

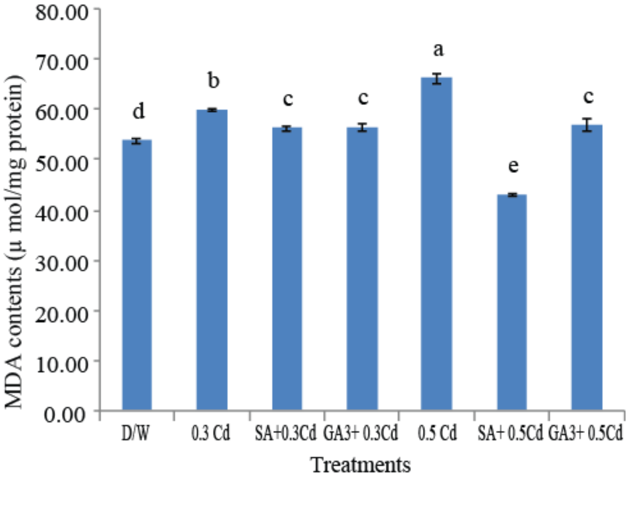

Fig. 9. MDA contents of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

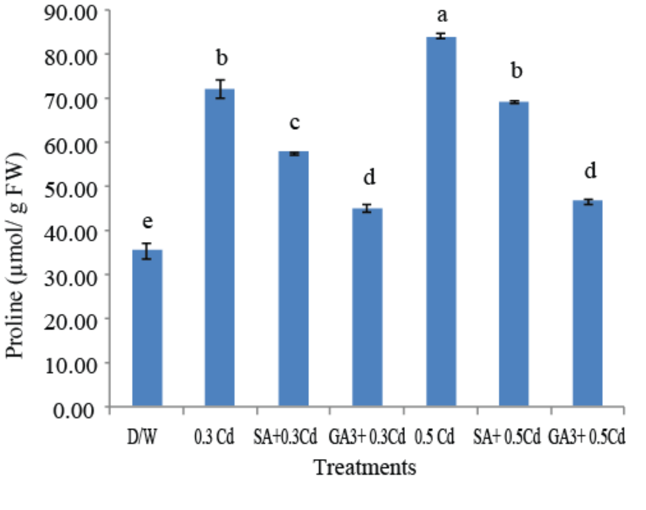

Fig. 12. Proline contents of mung bean under pretreatment of phytohormones and cadmium stress. Bars indicate standard error (n=3). Values with same letter are not significantly different at p≤0.05.

Conclusion

Thus, it is concluded that cadmium stress reduced the growth and altered other metabolic processes of mung bean seedlings by increasing MDA. The pretreatment of phyohomone ameliorate the cadmium induced deleterious effects by decreasing the oxidative stress. A negative corelation in MDA and morphological parameters were noticed.The purpose of using SA and GA_3 as pretreatment is both inexpensive and effective to reduce cadmium stress in mung bean as compared to other phytohormones like Brasinosteriods and Jasmonate. This finding may help farmers to grow mung bean in cadmium contaminated soils after pretreating the seeds with SA or GA_3, to attain more yield.

Acknowedgement

Authors are thankful to National Agriculture Research Centre (NARC), Islamabad, Pakistan, for providing mung bean seeds. This work has been presented in "The 4th International Conference on Environmental Horizon; Valuing and Conserving Nature (ICEH-2016)", during January 08-10, 2016 jointly organized by Department of Chemistry, University of Karachi and International Center for Chemical and Biological Sciences (ICCBS), University of Karachi.

Competing interest: Authors declared that they have no competing of interest.

References

Aebi, H. 1984. Catalase *In vitro. Methods in enzymology.,* 105: 121-126.

Ahmad, W., M.T. Jan, M. Ilyas, T. Shah, Moinullah, K. Azeem, A. Ahmad and S. Khan. 2017. Phenology and yield components of maize as influenced by different forms of dairy manure with supplemental nitrogen management. *Int. J. Agri and Env. Res*., 3(1): 137-146.

Akbar, F., M. Ishaq, I. IhsanUllah and S.M. Asim. 2009. Multivariate statistical analysis of heavy metals pollution in industrial area and its comparison with relatively less polluted areas, a case study from the city of Peshawar and district Dir lower. *J. Hazard Mater*., 176: 609-616.

Anjum, N.A., S. Umar, M. Iqbal and N.A. Khan. 2011. Cadmium causes oxidative stress in mongbean [*Vigna radiata* (L.) Wilczek] by affecting the antioxidant enzymes and ascorbate glutathione cycle metabolism. *Russian J. of Plant Physiol.,* 58: 92-99.

Bates, L.S., R.P. Waldran and I.D.Tear.1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-208.

Benavides, M.P., S.M. Gallego and M.L. Tomaro. 2005. Cadmium toxicity in plants. *Braz. J. Plant Physiol*., 17: 21-34.

Chin, L., D.M.W. Leung and H.H. Taylor. 2009. Correlation between endogenous tannins and lead accumulation in roots of *Symphytum officinale* L. *Aust. J. Ecotoxicol*., 15: 5-10.

Corpas, F.J., J.B. Barroso and L.A. Del Rio. 2001. Peroxisomes as a source of reaction oxygen species and nitric oxide signal molecules in plant cells. *Trends Plant Sci*., 6: 145-150.

Costa, G. and J.L. Morel J.L.1994. Water relations gas exchange and amino acid content in Cd treated lettuce. *Plant Physiol. Biochem.*, 32: 561-570.

Ensink, J.H.J., R.W. Simmons and W. Van Der Hoek. 2004. Wastewater use in Pakistan: The cases of Haroonabad and Faisalabad. In: (Eds.): Scott, C.A., N.I. Faruqui and L.R. Sally. Wastewater Use in Irrigated Agriculture, 91-99. CAB International, Wallingford, UK.

Everse, J., M.C. Jhonson and M.A. Marini. 1994. Peroxidative activities of haemoglobin derivatives. In: (Eds.): Everse J, K.D. Vandegriff and R.M. Winslow. *Methods in Enzymology*. Academic press London, 547-561.

Faiz, Y, M. Tufail, M.T. Javed, M. Chauhadry and N. Siddique. 2009. Road dust pollution of Cd, Cu, Ni, Pb, and Zn long Islamabad expressway. *Pakistan. Micro-chem J*., 92: 186-192.

Farid, G., N. Sarwar, Saif Ullah, A. Ahmad and A. Ghafoor. 2015. Heavy metals (Cd, Ni, and Pb) contamination of soils, Plants and waters in Madina town of Faisalabad metropolitan and preparation of Gis based Maps. *Adv Crop Sci Tech.*, 4: 199.

Farouk, S., A.A. Mosa, A.A. Taha, H.M. Ibrahim and A.M. El Ghamery. 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. *Var sativa)* plants subjected to cadmium stress. *J. Stress Plant Physiol. Biochem.*, 7: 99-116.

Ghani, A. 2010. Effect of cadmium toxicity on the growth and yield components of mung bean [*Vigna radiata* (L.) Wilczek]. *World App. Sci. J.,* 8: 26-29.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. and Biochem*., 48: 909-930.

Graham, P.H. and C.P. Vance. 2003. Legumes: importance and constraints to greater use. *Plant Physiol*., 131: 872-877.

Guangqiu, Q., Y. Chongling and L. Haoliang. 2007. Influence of heavy metals on the carbohydrate and phenolics in mangrove, *Aegiceras corniculatum* L., seedlings. *Bull. Environ. Contam. Toxicol.*, 78: 440-444.

Hasanuzzaman, M., M.A. Hossain and M. Fujita. 2012. Exogenous selenium pretreatment protects rapeseed seedlings from cadmium-induced oxidative stress by upregulating antioxidant defense and methylglyoxal detoxification systems. *Biol. Trace Elem. Res.*, 149: 248-261.

Hassan, M. and S. Mansoor. 2014. Oxidative stress and antioxidant defense mechanism in mung bean seedlings after lead and cadmium treatments. *Turk. J. of Agri. for*., 38: 55-61.

He, J., Y. Ren, X. Pan, Y. Yan, C. Zhu and D. Jiang. 2010. Salicylic alleviates the toxicity effect of cadmium on germination, seedling growth and amylase activity of rice. *J. Plant Nutr. Soil Sci*.., 173: 300-305.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. I kinetics and stoichiometry of fatty acid and peroxidation. *Archv. Biochem. Biophys*., 125: 189-198.

Hidayat, U., I.H. Khalil, Hidayat-ur-Rahman, F. Muhammad and I.A. Khalil. 2011. Environmental influence on heritability and selection response of morpho-physiological traits in mungbean. *Pak. J. Bot*., 43: 301-310.

Hossain, M.A., P. Piyatida, J.A.T. da Silva and M. Fujita. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J. Bot*., 872-875.

Hussain, A., G. Murtaza, A. Ghafoor, S.M.A. Basra, M. Qadir and M. Sabir. 2010. Cadmium contamination of soils and crops by long term use of raw effluent, ground and canal waters in agricultural lands. *Int. J. Agric. Biol*., 12: 851-856.

Hussain, I., M. Iqbal, S. Qurat-ul-Ain, R. Rasheed, S. Mahmood, A. Perveen and A. Wahid. 2012. Cadmium dose and exposure time dependent alterations in growth and physiology of maize (*Zea mays*). *Intl. J. Agric. Biol.*, 14: 959-964.

Irfan, M., S. Hayat, A. Ahmad and M.N. Alyemeni. 2013. Soil cadmium enrichment: Allocation and plant physiological manifestations. *Saudi J. Biol. Sci*., 20: 1-10.

Jiang, Y. and B. Huang.2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in cool season grasses. *J. Exp. Bot*., 52: 341-349.

Jun, M., H.Y. Fu, J. Hong, X. Wan, C.S. Yang and C.T. Ho. 2003. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobate* Ohwi). *J. Food Sci.,* 68: 2117-2122.

Kamachi, H., I. Komori, H. Tamura, Y. Sawa, I. Karahara, Y. Honma, N. Wada, T. Kawabata, K. Matsuda, S. Ikeno, M. Noguchi and H. Inoue. 2005. Lead tolerance and accumulation in the gametophytes of the fern *Athyrium yokoscense*. *J. Plant Res.,* 118: 137-145.

Kapoor, D., S. Kaur and R. Bhardwaj. 2014. Physiological and biochemical changes in *Brassica juncea* plants under Cd-induced stress. *Bio Med Res Intl*., Article ID 726070, http://dx.doi.org/10.1155/2014/726070.

Khavari-Nejad, R.A., F. Najafi and M. Ranjbari. 2013. The effects of GA_3 application on growth, lipid peroxidation, antioxidant enzymes activities, and sugars levels of cadmium stressed tomato (*Lycopersicon esculentum* mill cv.CH) plants. *Rom J. Biol-Plant Biol*., 58: 51-60.

Kumari, A., S. Sheokand and K. Swaraj. 2010. Nitric oxide induced alleviation of toxic effects of short term and long term Cd stress on growth, oxidative metabolism and Cd accumulation in chickpea. *Braz. J. Plant Physiol*., 22: 271-284.

Lavid, N., A. Schwartz, O. Yarden and E. Tel-Or. 2001. The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). *Planta.,* 212: 323-331.

Liu, N., J. You, W. Shi, W. Liu and Z. Yang. 2012. Salicylic acid involved in the process of aluminum induced citrate exudation in *Glycine max* L. *Plant Soil*, 352: 85-97.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Estimations of protein with the folin phenol reagent. *J. Biol. Chem*., 193: 262-275.

Mahmood, A. and R.N. Malik. 2014. Human health risk assessment of heavy metals via consumptions of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian J Chem*., 7: 91-99.

Metwally, A., I. Finkemeier, M. Georgi and K.-J. Dietz. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol*., 132: 272-281.

Michalak, A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J. Environ. Stu*., 5: 523-530.

Mittler, R., S. Vanderauwera, M. Gollery and F. Van Breusegem. 2004. Reactive oxygen gene network of plants. *Trends in Plant Science,* 10: 490-498.

Muneer, S., T.N. Qadri, Mahmooduzaffar and T.O. Siddiqui. 2011. Cytogenetic and biochemical investigations to study the response of *vigna radiata* to cadmium stress. *African J. Plant Sci*., 3: 183-192.

Nagajyoti, P.C., K.D. Lee and T.V.M. Sreekanth. 2010. Heavy metal, occurrence and toxicity for plants: a review. *Environ. Chem. Lett*., 8: 199-216.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*., 22: 867-880.

Namjooyan, S., R. Khavari-Nejad, F. Bernard, S. Namdjoyan and H. Pir. 2012. The effect of cadmium on growth and antioxidant responses in the safflower (*Carthamus tinctorius* L.) callus. *Turk. J. Agric for.,* 36: 145-152.

Nawaz, A., K. Khurshid, M.S. Arif and A.M. Ranjha. 2006. Accumulation of heavy metals in soil and rice plants (*Oryza sativa* L.) irrigated with industrial effluents. *Int. J. Agri. & Biol.,* 391-393.

Okuda, T. and H. Ito. 2011. Tannins of constant structure in Medicinal and food plants. Hydrolyzable tannins and polyphenols related to tannins. *Molecules,* 16: 2191-2217.

Olga, B., V. Eija and V.F. Kurt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Bot.*, 91: 179-194.

Prasad, T.K., M.D. Anderson, B.A. Martin and C.R. Stewart. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell.,* 6: 65-74.

Rivero, R.M., J.M. Ruiz, P.C. Garcıa, L.R. Lopez-Lefebre, E. Sanchez and L. Romero. 2001. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci*., 160: 315-321.

Saleem, M.S., M.U. Haq and K.S. Memon. 2005. Heavy metals contamination through industrial effluent to irrigation water and soil in Korangi area of Karachi (Pakistan). *Intl. J. of Agric. Biol*., 7: 646-648.

Sandalio, L.M., H.C. Dalurzo, M. Gómez, M.C. Romero-Puertas and L.A. del Rio. 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, 52: 2115-2126.

Sanita di Toppi, L. and R. Gabbrielli.1999. Response to cadmium in higher plants. *Environ. Exp. Bot.,* 41: 105-130.

Schanderl, S.H. 1970. *In: Method in Food Analysis*. Academic Press New York. 709.

Sergiev, I., V. Alexieva and E. Karanov. 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *Comp. Rend. Acad. Bulg. Sci.,* 5: 121-124.

Singh, S., P. Parihar, R. Singh, V.P. Singh and S.M. Prasad. 2016. Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. Front. *Plant Sci.*, 6: 11-43.

Singh, V.P., P.K. Srivatava and S.M. Prasad. 2013. Nitric oxide alleviates arsenic-induced toxic effects in ridged Luffa seedlings. *Plant. Physiol. Biochem.,* 71: 155-163.

Siripornadulsil, S., S. Traina, D.P.S. Verma and R.T. Sayre. 2002. Molecular mechanisms of proline-mediated tolerance to toxic heavy metal in transgenic microalgae. *Plant Cell.,* 14: 2837-2847.

Sumithra, K., P.P. Jutur, B.D. Carmel and A.R. Reddy. 2006. Salinity induced changes in two cultivars of *Vigna radiata* responses of antioxidative and proline metabolism. *Plant Growth Regul.,* 50: 11-22.

Sun, R.L., Q.X. Zhou, F.H. Sun and C.X. Jin. 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. *Environmental and Experimental Botany*, 60: 468-476.

Tariq, M., M. Ali and Z. Shah. 2006. Characteristics of industrial effluents and their possible impacts on quality of underground water. *Soil & Environment*, 25: 64-69.

Tran, T. A., & Popova, P. P. 2013. Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turk. J. Bot.,* 37*:* 1-13.

Wani, P.A., M.S. Khan and A. Zaidi. 2012. Toxic effects of heavy metal on germination and physiological processes of plants," in Toxicity of Heavy Metal to Legumes and Bioremediation, (Eds.): Zaidi, A., P.A. Wani and M.S. Khan (Springer-Verlag Wien), 45-66.

Xiong, X., G. Allinson, F. Stagnitti and J. Peterson.2003. Metal contamination of soils in the Shenyang Zhangshi irrigation area. *Bull. Environ. Contam. Toxicol*., 70: 935-941.

Yang, Z.M., J. Wang, S.H. Wang and L.L. Xu. 2003. Salicylic acid induced aluminium tolerance by modulation of citrate efflux from roots of *Cassia tora* L. *Planta*., 217: 168-174.

Yaqub, M.,T. Mahmood, M. Akhtar, M.M. Iqbal and S. Ali. 2010. Induction of mungbean [*Vigna radiata* (L.) Wilczek] as a grain legume in the annual rice-wheat double cropping system. *Pak. J. Bot.,* 42: 3125-3135.

(Received for publication 11 December 2016)