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EFFECT OF CADMIUM STRESS ON ANTIOXIDANT ENZYMES ACTIVITY IN DIFFERENT BEAN GENOTYPES

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ABSTRACT

Oxidative stress is induced by a wide range of environmental factors including heavy metals stress such as Cadmium. Therefore, antioxidant resistance mechanisms may provide a strategy to enhance metal tolerance, and processes of antioxidant responses to metal stress must be clearly understood. And also identification of tolerant cultivars and study of tolerance mechanisms to heavy metal stress is necessary in order to evaluate Cadmium stress effect on super oxide dismutase, catalase and glutathione peroxydase activity, a pot experiment was done in the form of factorial in randomized complete block design (RCBD) in research greenhouse of Islamic Azad University, college of agriculture and natural resources branch of karaj. In this experiment 6 bean seeds genotypes grew in greenhouse conditions in the soil consist of $CdCl_2$ with concentration of 0, 45, 90 mg/kg. The results of analysis of variance indicated that effect of Cd stress on above traits in different bean genotypes were significant. Thus, amount of SOD, CAT and GPX enzymes activities, in the highest level of Cd toxicity, were increased 146.2%, 96.2% and 92%, respectively in compared to control that these results can be the effects of tolerance mechanisms of plants against Cd stress. Mean comparison showed that Emerson and G-01437 genotypes have the most activity in the highest level of Cd toxicity by comparison with control. According to received results, increase in antioxidant activity in special genotypes is referred to the index of tolerance to Cadmium stress.

Keywords: cadmium, oxidative stress, bean genotypes, antioxidant enzymes.

INTRODUCTION

Heavy metal toxicity is one of the main current environmental health problems, and potentially harmful because of bioaccumulation through the food chain and plant products for human consumption. Therefore, heavy metal contaminations of soils and plants have become an increasing problem especially by industrial effluents and agricultural improvement. Heavy metal contents of food plants can be affected by the anthropogenic factors such as the application of fertilizers, sewage sludge or irrigation with wastewaters (Frost, H.L., L.H. Ketchum., 2000). Amongst the heavy metals, Cadmium (Cd) is one of the most toxic metals in the present environment with a long biological half-life, which is caused increasing international concern much higher than other heavy metals due to its toxicity. This element is readily and easily taken up by plants (Sarkar, B., 2002), by means of their roots and translocated to different plant parts (Baker *et al*., 1994). In stress conditions by metal toxicity, higher activities of antioxidant enzymes and higher contents of non-enzymatic constituents are recognizable for plants to tolerate the stress. One of the biochemical changes occurring in plants subjected to heavy metals stress conditions such as Cd stress, is the production of reactive oxygen species (ROS) like super oxide radical (O_2^-), hydrogen peroxide, singlet oxygen and hydroxyl radicals (OH^-) (Cho and Park, 2000). The ROS are strong oxidizing agents that cause oxidative damage to biomolecules such as lipids and proteins and eventually lead to cell death (Molassiotis *et al*., 2006). Mechanisms for the generation of ROS in biological systems are represented by both non-enzymatic and enzymatic reactions. There are many reports show that the mechanism of Cd toxicity is related to oxidative stress in plant cells. Cd can promote the generation of AOS (Olmos *et al*., 2003), inhibit or stimulate the activities of antioxidant enzymes (Iannelli *et al*., 2002) and also treatment with Cd results in cellular oxidative damage or lipid peroxidation (Chien *et al*., 2002).Overall, when plants are subjected to environmental stresses oxidative damage may result because the balance between the production of ROS and heir detoxification by the antioxidative system is altered (Gómez *et al*., 1999). Tolerance of damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify activated oxygen species (Foyer *et al*., 1994).

Furthermore, there is evidence that the tolerance of plants is related with increasing amounts of antioxidants and activity of radical scavenging enzymes. The antioxidant defense system in the plant cell includes both enzymatic, such as super oxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and non-enzymatic antioxidants such as ascorbate, glutathione and α-tocopherol. As a major scavenger SOD is a metalloprotein that catalyzes the dismutation of super oxide to H_2O_2 and molecular oxygen (Allen, 1995). However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and/or peroxidase (POD) to water and oxygen (Zhu *et al*., 2004).

In Iran development of industrial manufactories and use of industrial waste leads to contamination of soil with heavy metals in important zones under cultivation of bean. Therefore the dominant goal of agriculture is selection of suitable crop plants and production of new varieties which grow in such conditions and have proper economical efficiency. And also identification of tolerant cultivars and study of tolerance mechanisms to heavy metal stress is necessary. This study was carried out to evaluate the effect of Cadmium Chloride on growth parameters of different bean genotypes. Our primarily observation seems that these genotypes from genetically difference, regarding to Cd exposed most be various. Hence, studying on these genotypes can provide a good system for next investigations in future.

MATERIALS AND METHODS

The present study was carried out in the research greenhouse of Agricultural and Natural Resources Faculty, Karaj in 2011. The relative humidity of the greenhouse was 60% and the minimum and maximum temperatures were 16 and 32°C, respectively. The experimental treatments were laid out in factorial form based on randomized complete block design with four replications. In this experiment the first factor was three levels of cadmium ($CdCl_2$) include 0-45-90 mg/kg soil and the second factor was genotypes in six levels. Treatments consisted of 18 pots in each replication that six genotypes of the plant species bean (*phaseolus vulgaris* L) grew under different concentrations of cadmium.

The experiment was also carried out to study the physiological tolerance of bean species in soils contaminated with cadmium and evaluated by measuring of antioxidant enzymes super oxide dismutase (SOD), catalase (CAT) and Glutathione per oxidase (GPX). The field soil was sampled from 0-30 cm and then examined to determine soil physical and chemical properties particularly heavy metal of cadmium. The studied soil was loam sandy and EC (electrical conduction) equaled to 5.61 ds mG; pH was 7.8 and total nitrogen percentage equaled to 0.64% and organic matter content was 0.063%. The level of cadmium was 0.06 mg/kg. This content of cadmium is not toxic for plants. So, the soil was contaminated with concentrations more than the permitted levels. The soil colloids were fragmented and passed through 4 mm sieve. In order to more chelating of the elements with soil colloids and preparing contamination with elements, the treated pots were remained in such a situation for 45 days and after that the cultivation was done. To analyze the effects of the experimental treatments on the plants, sampling was done in early flowering stage and was transported to laboratory.

Greenhouse experiments

The selected pots were all of the same sizes and 24 cm in upper diameter and 20 cm in lower diameter; the height of the pots were also 25 cm. and several trays were used beneath each pots to prevent pots were filled with given mass of soil up to a certain height; subsequently, the soil and pots were weighed using a digital scale with a high accuracy (0.01). Each pot was filled with 6800 g soil. After solution spreading the soil to help chelating the elements with soil colloids and preparing contamination with the elements the treated pots remained in such a condition for 45 days and afterwards the cultivation was done.

Preparation of enzyme extracts

Leaves from each plant were washed with distilled water and homogenized in 0.16M Tris buffer (pH = 7.5) at 4°C. Then, 0.5mL of total homogenized solution was used for protein determination by the Lowery *et al*. (1951) method. Based on the amount of protein per volume of homogenized solution; the following enzymes were assayed in the volume containing a known protein concentration in order to calculate the specific activities of the enzymes. The activity of following enzymes was expressed as specific activity ($Umg.protein^{-1}$).

Determination of superoxide dismutase (SOD)

The activity was measured based on Misra and Fridovich (1972), in which it was measured on the basis of its ability to inhibit free radical chain oxidation in which O_2^- was a chain propagating radical and the auto oxidation of epinephrine (0.25 mM) was induced. A SOD standard was used for calibration of activity.

Catalase (CAT) activity

Catalase activity was measured at 25°C as previously described by Paglia and Valentine (1987), that used hydrogen peroxide as substrate and 1 k of catalase activity was defined as the rate constant of the first order reaction.

Glutathione peroxidase (GPX) activity

The activity was measured by the Paglia and valentine (1987) method in which 0.56M (pH = 7) phosphate buffer, 0.5M EDTA, 1 mM $NaNO_3$, 0.2 mM NADPH were added to the extracted solution. GPX catalyses the oxidation of glutathione (GSH) by cumene hydro peroxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Statistical analysis

All data were analyzed using SAS Institute Inc., Version 9.1 software. And first analyzed by ANOVA to determine significant ($P \leq 0.01$) cadmium treatment and genotype effects Mean comparison was conducted using the Duncan's Multiple Range Test (DMRT) at 1%.

RESULT AND DISCUSSIONS

Effect of Cd on superoxide dismutase activity (SOD)

The results of analysis of variance (Table-1) indicated that the main effect and interactions of studied traits were significant (P<0.01). According to results, it showed that Cd treatment in the highest level of toxicity (90 mg/kg) causes increase of 146.2% in SOD activity compared to control (Figure-1). Among the genotypes, Emerson and G-01437 had maximum amount for super oxide dismutase activity in concentration of 90mg/kg (Figure-2). SOD is considered a key enzyme in the regulation of intracellular concentrations of ROS. Thus, increased SOD activity in plant cells showed that it plays a positive role in controlling the cellular level of these ROS and/or repairing oxidative damage (Miller *et al*., 2008). In this experiment increase in activity of super oxide dismutase against free radicals by cadmium stress was observed. Similar increase in SOD activity has also been reported by other researches (Scebba *et al*., 2006; Mobin and Khan, 2007).

Table-1. Analysis of variance for experimental traits under normal and cadmium stress conditions.

Sov	Df	Ms		
		SOD	CAT	GPX
Rep	3	$10.15^{n.s}$	14.36^{**}	$3.12^{n.s}$
A (Cd levels)	2	6575.58^{**}	4307.15^{**}	1569.62^{**}
B (genotypes)	5	119.02^{**}	157.05^{**}	37.64^{**}
A*B	2	136.4^{**}	221.31^{**}	157.36^{**}
Error	51	6.632	2.651	1.742
% C.V		9.62	5.68	7.63

ns and **: Non significant and significant at 1% levels of probability, respectively.

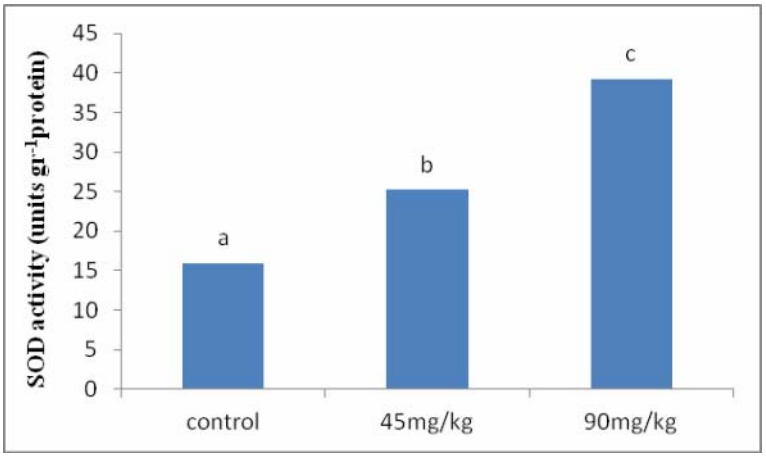

Figure-1. Comparison of SOD activity different cadmium concentrations.

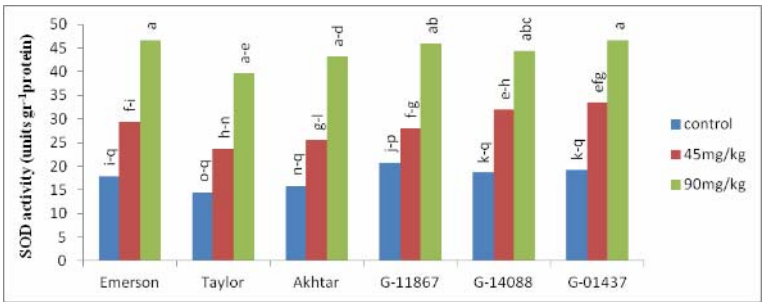

Figure-2. SOD enzyme activity and comparing between bean genotypes in different Cd concentrations.

Effect of Cd on catalase activity (CAT)

The effects of Cadmium on Catalase activity were assessed and the obtained results were given in Table-1. The results of analysis of variance indicated that the main effect and interactions of studied traits were significant (P<0.01). As shown in Figure-3, Cd treatment causes increase of 96.2% in catalase activity compared to control. In Cd highest concentration (90mg/kg), the most amount of CAT activity was obtained from G-01437 and Cos-16 genotypes (Figure-4). CAT is one of the major antioxidant enzymes that eliminate hydrogen peroxide by converting it into oxygen and water (Miller *et al*., 2008). Many reports indicated that CAT activity was significantly influenced by cadmium stress. This recommended that CAT activity, plays a very important role in the protection against oxidative damage caused by cadmium (Scebba *et al*., 2006; Mobin and Khan, 2007; Zhang *et al*., 2009).

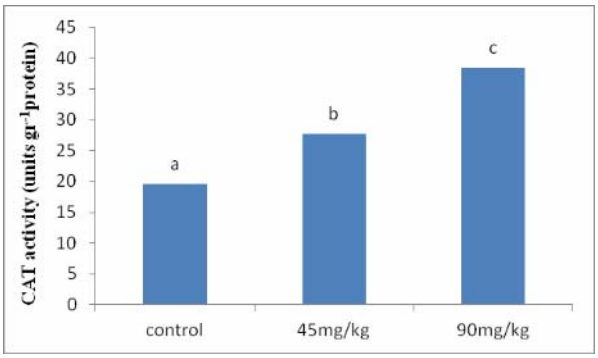

Figure-3. Comparison of CAT activity different cadmium concentrations.

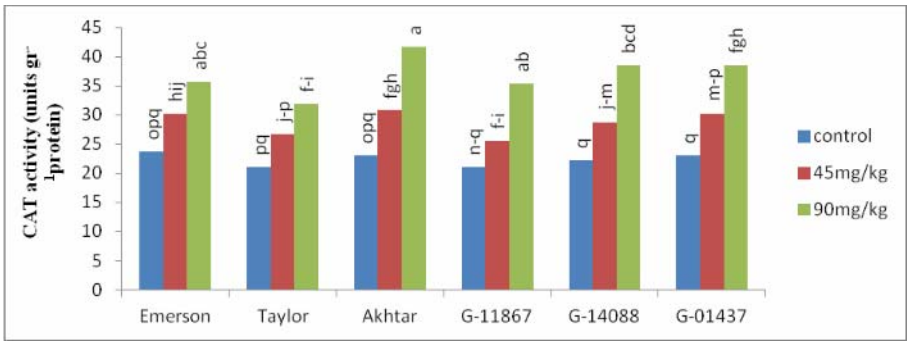

Figure-4. CAT enzyme activity and comparing between bean genotypes in different Cd concentrations.

Effect of Cd on glutathione per oxidase activity (GPX)

The results of analysis of variance (Table-1) indicated that the main effect and interactions of studied traits were significant (P<0.01). Increasing cadmium concentrations in the soil led to increase in Gpx activity in which 92% increase in the highest level of Cd concentration observed, compared to control (Figure-5). Among the genotypes, Emerson and G-01437 had maximum amount for Glutathione Peroxidase activity at the maximum level of cadmium in the soil (Figure-6). GPX is a well known enzyme which plays a vital role in protection of lipid membrane against oxidative damages. Mashhadi Akbar Boojar and Goodarzi (2007) in a research on three plant species including Malva sylvestris, Chenopodium ambrosiodes and Datura stramonium in a cupper mine, observed that GPX increased its antioxidant enzymatic activity in reaction to toxicity.

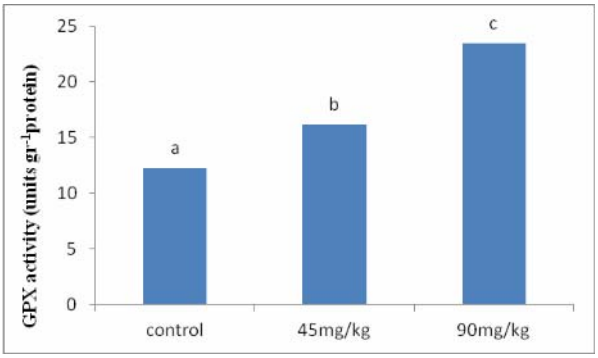

Figure-5. Comparison of GPX activity different cadmium concentrations.

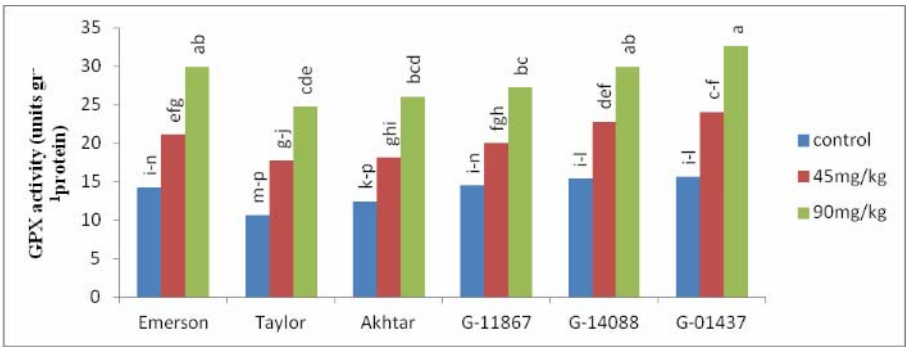

Figure-6. GPX enzyme activity and comparing between bean genotypes in different Cd concentrations.

CONCLUSIONS

In conclusion, it can be said that increase the activity of antioxidant enzymes could be attributed to the increased tolerance to Cd. Results of our findings can be a useful indicator of metal tolerance to plantation of these genotypes in metal contaminated regions. The data evaluations of the antioxidant responses in different cadmium concentrations allow the suggestion that Emerson and G-01437genotypes are more tolerant than others. Additional research is necessary to provide further insight concerning the specific relationship between heavy metal stress and the antioxidant response.

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