EFFECT OF CADMIUM CHLORIDE ON GROWTH PARAMETERS OF DIFFERENT BEAN GENOTYPES (*Phaseolus vulgaris* L.)

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ABSTRACT

Cadmium (Cd) is a toxic heavy metal which causes oxidative stress in plants and has a high level of toxicity for plants, animals and human. Present study carried out in order to evaluate the Cd stress effect on growth parameters in bean seedlings. This experiment was conducted in the form of factorial in randomized complete block design (RCBD) by three replications per treatment. In addition, 25 bean seeds genotypes grew in laboratory conditions consist of solution $CdCl_2$ with concentration of 4 mg/lit and distilled water (as a control). The seedlings were harvested after 8 days and the germination percentage, root and shoot length and its stability, R/S ratio and fresh weight were recorded. The results revealed that Cd adversely influenced on these traits. As a result, Cd treatment in all genotypes reduced the germination percentage 9.9%, root and shoots length 83.9% and 66.3%, respectively, R/S ratio 52.2%, and fresh weight 42.3%, in compared to control. Based on the results we concluded that, these traits of bean plant are seriously affected by Cd treatment and also these are symptoms of toxicity of Cd element. Therefore less amount of reduction in a special genotype is referred to the index of tolerance to Cd.

Keywords: bean, cadmium chloride, genotypes, growth parameters, oxidative stress.

INTRODUCTION

Nowadays environmental pollution by heavy metals has increased because of manifold industrial activities, solid waste management and agricultural improvements. Hereupon, soil pollution through the food chain threats health of humankind (McLaughlin *et al*., 2009). Scientists and engineers have started to generate efficacious technologies that contain the utility of microorganisms, biomass and some plants for the process of removing polluted zones (Ebbs, Kochian, 1997; Wasay *et al*., 1998). Among heavy metals Cadmium is one of the toxic elements that don’t play a vital role for live organs. It is unnecessary for plants, animals and human and has long biological persistence causes leaf rolls chlorosis and reduction of root and stem growth (Smeets *et al*., 2005; Mishra *et al*., 2006). Several investigations on the effect of Cadmium on plants have been carried out that most of them conducted using seedling or adult plants (Chatterjee, and Chatterjee, 2000; Gratton *et al*., 2000; Oncel *et al*., 2000; Pitchel *et al*., 2000). Studies show that Cadmium limits the process of germination and growth and development of seedling (Rascio *et al*., 1993). Cd treatment with 1μM in 24 hours reduced the root-growth up to 30% and this inhibit had positive correlation with the reduction of root cells viability (Siroka *et al*., 2004). One of the biochemical changes occurring in plants subjected to various environmental stress conditions such as Cd stress is the production of reactive oxygen species (ROS) that leads to oxidative stress (Cho, and Park, 2000). The ROS has a definite role in lipid peroxidation, membrane damage and consequently in plant senescence (Zhang *et al*., 2003). Furthermore photosynthesis is sensitive to Cd because it straightly affects chlorophyll biosynthesis (Gadallah, 1995) and the reasonable development of chloroplast ultrastructure (Stoyanova and Tchakalova, 1997).

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 must be various. Hence, studying on these genotypes can provide a good system for next investigations in future.

MATERIAL AND METHODS

Preparation of samples

Present experiments were conducted in laboratory of Biotechnology of Karaj Islamic Azad University, Faculty of Agriculture and Natural Resources, Iran. Twenty five (25) bean seeds were prepared from Agricultural Research Institute Khomeyn, Iran, and seeds as the same size and form were selected and then sterilized by 2.5% (v/v) sodium hypochloride for 15min to avoid fungi contamination. All seeds were washed carefully with distilled water. 12 seeds of every genotype were germinated in 2 rolled Whatman filter papers and placed in special cultivation vessels including solution $CdCl_2$ with concentration of 4mg/lit and distilled water (as a control). Level of the liquid in vessels was daily examined in order to reduce the inaccuracy of water absorption condition. For providing the optimum temperature of bean development, daily mean temperature during the study was adjusted to 24ºC (75.2ºF).

The process of trait measurement

The seedlings were harvested after 8 days and the germination percentage, root and shoot length and its stability, R/S ratio and fresh weight were recorded. The reason of choosing 8 days is referred to the trait measurements which should be done before the process of plant leaving. Root and shoot lengths were obtained with a ruler with the precision of 1mm and the average of 12 seedlings of each genotype noted for every replication Then the ratio of R/S were calculated. Also the fresh weight of the seedlings was accounted by using a scale with the precision of 0.01gr. For determination of germination percentage, the number of seed emergences in the end of treatment period was counted.

Statistical analysis

This experiment was carried out in the form of factorial in randomized complete block design (RCBD) by three replications per treatment. All data were analyzed with SAS Institute Inc., Version 9.1 software and first analyzed by ANOVA to determine the significant ($P \leq 0.01$) Cd treatment and genotype effect. Significant difference between individual means was determined using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSIONS

Effect of cadmium chloride on root and shoot length and R/S ratio of bean seedling

The effects of $CdCl_2$ on growth parameters were assessed and the obtained results were given in Table-1. The results of analysis of variance indicated that Cd significantly affect on said traits. According to Figures 1 and 2, it showed that Cd treatment causes reduction of 83.9%, 66.3% and 52.3% in root and shoot length and R/S ratio of bean seedling, respectively by comparison with control. Among the 25 available genotypes Emerson and Wa4502-1, jules and Ks-41126, respectively have the most account of root and shoot length while Shokofa and Pak, Shokofa and G-11867 have the least one in Cd treatment (Figures 5 and 6). And also the results related to R/S ratio showed that Daneshkadeh and Pak have the highest and lowest amount (Figure-7). The result of present study revealed that Cd was an inhibitor factor on root and shoot length growth and the effect of Cd on root length growth was more obvious than shoot length growth. These gained resulted in relation with the influence of Cd on limitation and reduction of growth of plant species is in conformity with the findings of other researchers. For example, application of Cd reduced the shoot length in bean, wheat and alfalfa (Chaoui *et al*., 1997; Bhardwaj *et al*., 2009; Veselov *et al*., 2003; Aydinalp and Marinova, 2009) and also Mihalescu *et al*. (2010) were of the opinion that the reduction in root length and height of the plant was due to Cd accumulation in Zea mays. Furthermore they induced that such a decrease was directly proportional with the increase of metal concentration. The reduction in root length affected by Cd treatment in bean (Bhardwaj *et al*., 2009), Solanum melongena (Siddhu *et al*., 2008) and alfalfa (Aydinalp and Marinova, 2009) was recorded that these results are similar to our outcomes.

The reason for the reduced seedling growth in metal treatments could be as a result of the reduction in meristematic cells present in this region and some enzymes contained in the cotyledons and endosperm. Cells become active and begin to digest and store food which is converted into soluble form and transported to the primary root and shoot tips for enzyme amylase which converts starch into sugar and proteases act on proteins. So, when activities of hydrolytic enzymes are affected, the food does not reach to the primary root and shoot, thereby affecting the seedling length (Kabir *et al*., 2008). Several authors reported that, the inhibition of root elongation caused by heavy metals may be due to metal interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis (Jiang *et al*., 2001; Huillier *et al*., 1996; Radha *et al*., 2010; Liu *et al*., 2003), which can be effected on seedling growth and explain the inhabitation of seedling growth in this investigation.

Seed germination percentage and fresh weight

The percentage of germination and fresh weight reflected in Table-1, indicated that Cd significantly affect on said traits. Cd treatment 9.9% decreased seed germination percentage by comparison with the control (Figure-3) and Zodras, Pak and Taylor had the most amounts while Jules had the least one under Cd stress (Figure-8). Findings of this investigation were in conformity with the results of other researchers (Hoshmandfar and Moraghebi, 2011). For instance, Rahman *et al*., (2010) reported that treatment with nickel and cobalt caused a reduction in seed germination in Chickpea plant. According to Shafiq *et al*., (2008) decrease in seed germination of plant can be characterized to the accelerated breakdown of stored food materials in seed by the application of Cd. Furthermore, Reduction in seed germination can be attributed to changes of selection permeability properties of cell membrane.

Growth is the best indices plant reaction to environmental stress. Mean fresh weight in genotypes under Cd treatment reduced 42.3% by comparison with control (Figure-4). The G-14088 and G-11867 genotypes had maximum and minimum amount for fresh weight correspondingly (Figure-9). Bhardwaj *et al*., (2009) in their investigation on the bean plant observed a reduction in total biomass and fresh weight of seedling and also similar reports have been found by other researchers in T. sativum and Lens esculanta. Reduction in fresh weight may be referred to toxicity of $CdCl_2$, thereby this toxic material can breakdown normal physiological mechanisms and finally have negative influences on biomass.

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

Sov	Df	MS				
		Root	Shoot	R/S	Vigour	Weight
Rep	2	11.83$^{n.s}$	1.855$^{n.s}$	0.002$^{n.s}$	59.71$^{n.s}$	0.39$^{n.s}$
A (Cd level)	1	2376.2**	2013.4**	8.05**	2334.1**	2625.7**
B (Genotype)	24	8.51**	6.98**	0.068**	748.48**	60.93**
A*B	24	10.31**	7.23**	0.060**	174.27$^{n.s}$	23.02**
Error	98	0.755	1.008	0.015	139.09	2.49
C.V%		15.79	13.58	19.39	14.12	10.13

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

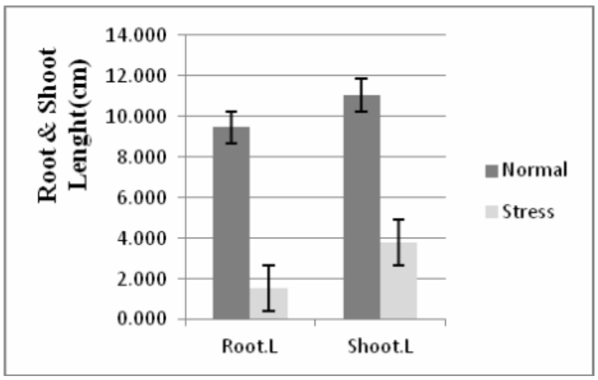

Figure-1. Comparison of root and shoot length in normal and stress conditions. Each bar represents mean (±SE) of three different samples.

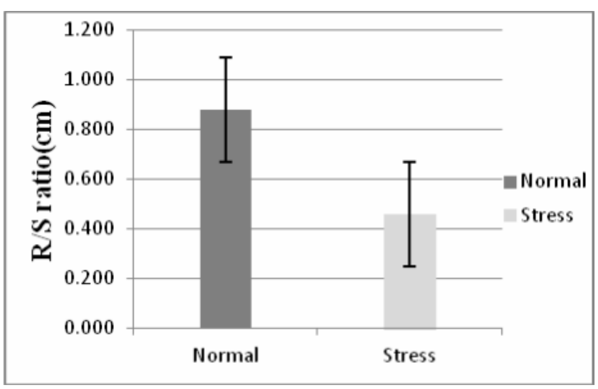

Figure-2. Comparison of R/S ratio in normal and stress conditions. Each bar represents mean (±SE) of three different samples.

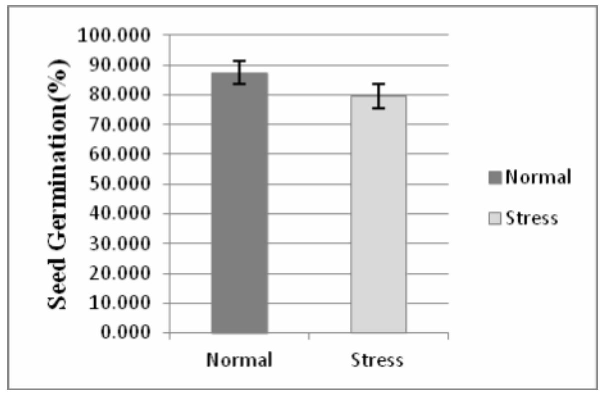

Figure-3. Comparison of seed germination in normal and stress conditions. Each bar represents mean (±SE) of three different samples.

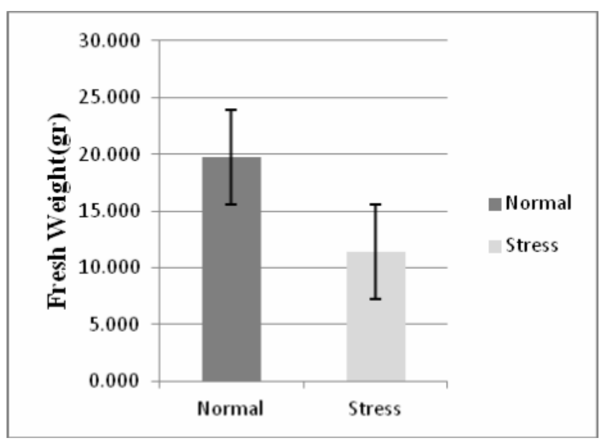

Figure-4. Comparison of fresh weight in normal and stress conditions. Each bar represents mean (±SE) of three different samples.

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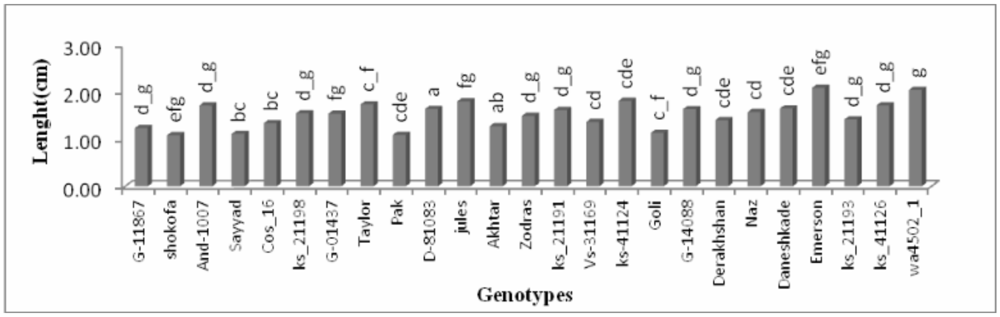

Figure-5. Mean comparison of root length in different genotypes under stress condition for a given means within each column of each genotype followed by the same letter are not significantly different at p = 1% according to DMRT.

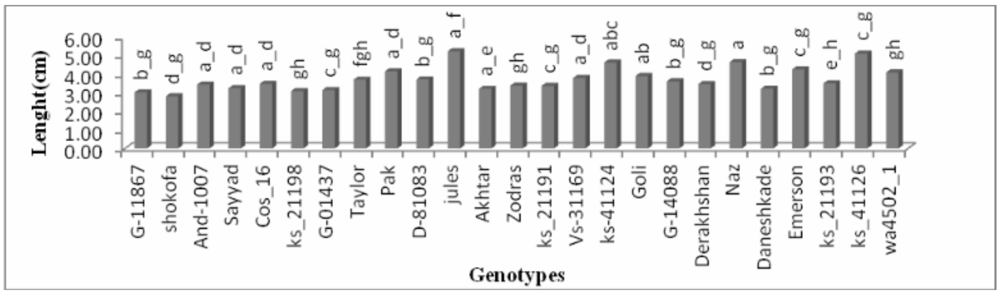

Figure-6. Mean comparison of shoot length in different genotypes under stress condition for a given means within each column of each genotype followed by the same letter are not significantly different at p = 1% according to DMRT.

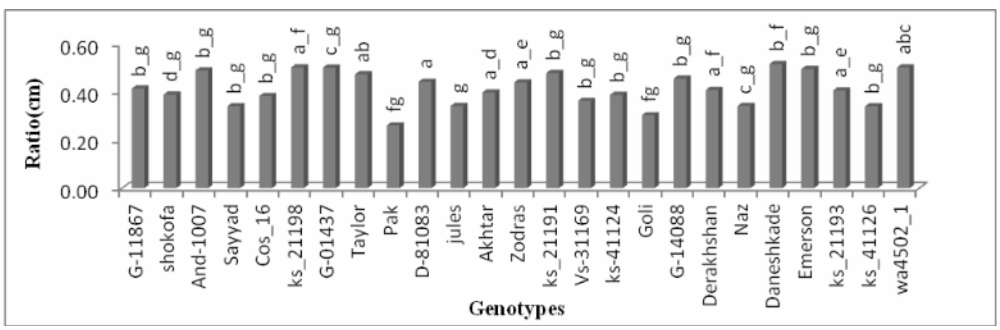

Figure-7. Mean comparison of R/S ratio in different genotypes under stress condition for a given means within each column of each genotype followed by the same letter are not significantly different at p = 1% according to DMRT.

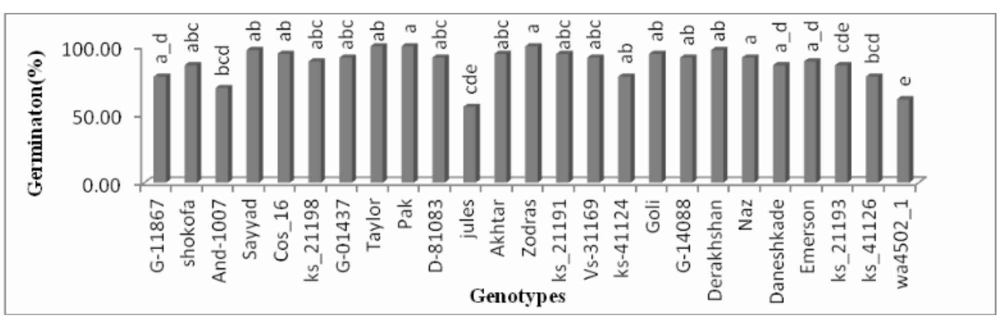

Figure-8. Mean comparison of seed germination in different genotypes under stress condition for a given means within each column of each genotype followed by the same letter are not significantly different at p = 1% according to DMRT.

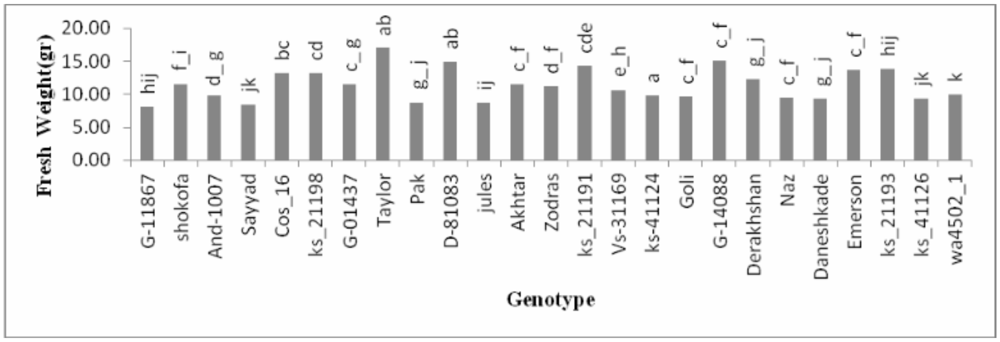

Figure-9. Mean comparison of fresh weight in different genotypes under stress condition for a given means within each column of each genotype followed by the same letter are not significantly different at p = 1% according to DMRT.

CONCLUSIONS

Overall, the results of our investigation showed that the toxicity of cadmium in bean seedlings impressed. This information can be considered a contributing step in exploring and finding of the tolerance limit of bean genotypes at 4mg/lit concentration of treated cadmium And the fact that the growth response of bean seedlings strongly depends on to genetic variation among the genotypes. The data evaluating the growth responses in both normal and cadmium stress allow the suggestion that Cos-16, D-81083, Akhtar, Taylor and Naz genotypes are more tolerant than other genotypes. Results of the findings can be useful indicator of metal tolerance to some extent for plantation of these genotypes in metal contaminated area. Finally, in the metal contaminated areas, further research is needed to determine different levels of metals in the environment and various parts of the plants.

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