Azarian Journal of Agriculture

Azarian J. Agric. VOL (8) ISSUE 1, 2021: 1-11

http://dx.doi.org/10.52547/azarinj.047

www.azarianjournals.ir

Azarian Journals

Research article **ISSN:2383-4420**

Effects of cadmium stress on antioxidant responses of *Vigna radiate* L.Wilcziek

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Article Info

Accepted:
29 Jan. 2021

Keywords:
Bioconcentration, Contamination, Heavy metals stress, Oxidative potential, Plant Tolerance

ABSTRACT

The current study evaluated the effects of cadmium stress on the antioxidant responses of *Vigna radiata* L. Wilcziek. The treatments consisted of 0, 25, 50, 75, and 100 ppm of $CdCl_2$ $2H_2O$. Seeds were placed in plastic trays and watered with distilled water. After 24 hours, the seeds germinated, and then they were transferred to pots. We irrigated each pot with their desired treatment solution until the end of the growth phase. A sampling of mature leaves at the flowering time was done. The concentration of cadmium in soil and other tissues of the plant, the level of leaves soluble proteins, ascorbate peroxidase (APx) and peroxidase enzymes (POD), total antioxidant capacities (TAC), and concentration of hydrogen peroxide (H_2O_2) in the samples were measured and compared to control. The lowest level of soluble protein (73.75±6.8 mg L^{-1}) was observed in the 50 mg Cd/L treatments. The maximum rate of H_2O_2 (1.82±0.06 µM g^{-1} frw) and APx (0.83±0.04 U $gfrw^{-1}$) activity was measured in treatment of 25 mg Cd/L and the lowest was observed in the treatment of 100 mg Cd/L (1.16±0.08 µM g^{-1} frw and 0.19±0.02 U $gfrw^{-1}$, respectively). The highest and lowest level of POD was observed in the treatment of 75 mg Cd/L (1.93±0.03 U $gfrw^{-1}$) and 100 mg Cd/L (0.12±0.02 U $gfrw^{-1}$), respectively. The Mung bean plant has a resistance to cadmium stress even up to 100 ppm. This resistance appears to be due to the high total antioxidant capacity of the *V. radiata*. Hereupon, mung bean can provide a safe culture for cadmium-contaminated environments.

INTRODUCTION

Heavy metal-contaminated environment is one of the major eco-environmental concerns of human societies that can affect the life and health of biota on earth (Saxena et al. 2019). Drinking water contaminated by humans or animals directly endangered public health. Instead, in contaminated soil, these effects indirectly showed by the reducing yield and quality of compounds within the plant. On the other hand, if humans/animals used this plant as food maybe suffering acute and chronic toxicity. Among the heavy metals (HMs), cadmium has received greater attention due to the high mobility, rapid absorption by plants, non-biodegradable, and toxicity (Palutoglu et al. 2017). Cadmium can be toxic to the nervous system, liver, kidneys, and many other body tissues damage (Olalekan et al. 2018).

Mung bean (Moong bean), *Vigna radiata* L.Wilcziek plants (Fabaceae) for thousands of years is one of the most important crops in the human diet (Figure 1). This plant has been originated from Indian and Asian regions (Aklilu and Abebe 2020). Now, two varieties of this plant, with names of Gohar (Omran) and Parto are grown in Iran. The ancient chines used the plant for disposal heat, detoxification of the body, and diuretic (www.pingminghealth.com), but in Iran, this plant is also used to treat constipation. In most societies, especially in low-income communities is a good source of vegetable protein. Mung bean contains significant amounts of folic acid, potassium, manganese, and selenium (USDA

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National Nutrient Database). Antioxidant and therapeutic properties of *V. radiata* were also reported (Cao et al. 2011; Shi et al. 2016). Mung bean and other legumes have a substance called protease inhibitors, which could have a protective effect against breast cancer. Protease inhibitor has reduced the division of cancer cells and thus prevents tumor formation (Xu and Chang 2012; Ganesan and Xu 2018).

Figure 1. Vigna radiata L.Wilczek plant and its seeds yield

Plants in their tissues and cells have polyphenolic compounds as source of natural antioxidant agents but their abundance depends on the species. These chemical compounds have phenyl ring attached to hydroxyl groups, including functional derivatives such as glycosides, esters, etc (Pabón-Baquero et al. 2018). To reducing the oxidative damage caused by reactive oxygen species (ROS) plant used a mechanism of non-enzymatic antioxidant that includes components such as ascorbate, glutathione, tocopherols, carotenoids, and flavonoids. Enzymes from photosynthetic systems of plants such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APx), peroxidase (POD), polyphenol oxidase (PPO), and glutathione reductase (GR) which could play a protective role against environmental stresses (Kasote et al. 2015). ROS was another symptom of stress produced in the cellular organelles such as chloroplasts, peroxisome, and mitochondria (Choudhury et al. 2017). Antioxidant enzyme activities play an important role in the clearance of ROS. The enzyme involved in this reaction can increase stress tolerance in plants and delay aging (Kasote et al. 2015). In living plant cells, the production of superoxide radicals (o^{-}) meanwhile, the electron transport chain in mitochondria and chloroplasts will be scavenged by SOD and then converted to hydrogen peroxide (H_2O_2). Hydrogen peroxide is destroyed by the APx in different parts of the cell. Also, antioxidant enzymes (CAT, POD, and APx) are the most important neutral hydrogen peroxide enzymes in plants (Arora et al. 2002). Rahimi and Ronaghi (2012) reported that by using cadmium in wheat fields, the concentration of hydrogen peroxide as an oxidizing agent was increased. Also, Rodríguez-Serrano et al. (2001) found that cadmium affected the intracellular metabolic redox reactions of pea (*Pisum sativum* L.) roots and increase their activity of catalase and superoxide dismutase. Further, cadmium toxicity can change the antioxidant activity of plants by increase superoxide radical production and lipid peroxidation (Khan et al. 2013). The high concentration of Cd treatments influenced the germination and seedling growth of corn, and increased the ascorbate peroxidase activity (Malekzadeh et al. 2007), but, the presence of the toxic heavy elements in the plant can make tension (Fathi Amirkhiz et al. 2011). A research objective addresses the effect of different levels of cadmium on the antioxidant properties of *V. radiata* under natural conditions and the Cd-accumulation potential of leaf plants.

MATERIALS AND METHODS

Chemicals

All reagents and chemicals were obtained from Merck and Sigma Aldrich (St. Louis, MO). The solutions were prepared using double-distilled water (ddH_2O).

Agricultural Practices

This study was carried out on pot cultivation in completely randomized design bases and by means of 5 treatments and 3 replications. Irrigation treatments included the five solutions, of 0, 25, 50, 75, and 100 ppm of $CdCl_2 2H_2O$. The garden soil was suitable for *V. radiata* cultivation. Mung seeds were placed in plastic trays and then watered with distilled water. After 24 hours, the seeds germinated and then they were transferred to pots. Each of the pots was irrigated with their desired treatment solution until the end of the growth phase. A sampling of mature leaves at the flowering time was done. Immediately we put samples into a plastic bag containing a label, moved them into the freezer (-20℃) until the tests were performed. In order to measure the elements in various organs, plant bushes were harvested from a centimeter above the soil surface (collar region of the stem). Also, the roots have been isolated from soil and then they were transferred to the laboratory. Leaves, stems, and roots were rinsed with ddH_2O and placed in the oven at temperature of 60 ℃ for 24 hours to dry.

Initial Plant and Soil Analysis

For measurement of microelements, air-dried plants were powdered. Then 0.5 g of each sample was weighed and transferred inside the electric furnace for 2.5 hours at 550 °C. The ash was mixed with 5 mL of HCl (2N), transferred to the water bath, and then the mixture was passed through a

Whatman filter paper (No. 41). The volume of extract was adjusted to 50 mL by using ddH_2O. The optical density (OD) of the samples was determined by Atomic Absorption Spectrometer (AAS) ContrAA 700 (Analytik Jena, Germany). The level of cadmium (mg kg^{-1}) was calculated using the following formula (Suchowilska et al. 2012):

Cadmium (mg kg^{-1}) = Cadmium concentration (ppm) * (V/M) (1)

Where: V= Extract volume (50 mL), and M= Dry weight of plant (g)

To measure the concentration of cadmium in soil, first, a solution of diethylene try amine Penta-acetic acid (DTPA) was prepared (to make one liter of solution DTPA, 1.967 g of the DTPA mixture in 13.3 mL of Triethanolamine). Then, add 1 g of calcium chloride to it. Then add 100 mL of ddH_2O and the mixture was stirred. Set the pH of the solution to 7.2 by HCl (1N). The mixture volume was adjusted to 1 liter with distilled water. 5 g of soil poured into a flask and add 10 mL of DTPA (0.005 mM). Put the suspension on a shaker for 30 min and then pass the mixture through the filter paper. Then, the absorbance of the samples was determined by AAS ContrAA 700 (Analytik Jena, Germany). Calculation methods have been described such as micro-elements. Standard solutions prepared with a concentration of 0.2, 0.3, 0.5, 1, and 1.5 mg L^{-1} from a stock solution of 1000 mg L^{-1} (Cd (NO_3). HNO_3).

Soluble Protein Assays

For soluble protein assays, Tris- HCl 1M (pH= 6.8), sodium chloride solution 0.15 M, Bradford reagent, and protein extraction buffer are needed. To prepare the Bradford solution (Bradford,1976), Coomassie brilliant blue 250 G (100 mg) was dissolved in 95% ethanol (50 mL) and stirred for one hour. Then, 85% phosphoric acid (100 mL) added dropwise to the solution, and gradually adding distilled water to a volume of one liter. This mixture was passed through the Whatman 41 filter paper. Protein extraction buffer was prepared by 5 mL of Tris**-**HCl 1M, 200 mL of sodium chloride solution 0.15 M, and 40 µL of 2 - Mercaptoethanol 0.04 % (V/V) that added to the solution and finally, was adjusted to 100 mL by adding distilled water. Put 0.5 g of fresh leaves in a porcelain mortar that was placed in the ice bath, and then crushed in a mortar with a pestle to obtained uniform extract. Add 6.25 mL of protein extraction buffer to the sample and continue to erode. Pour 15 mL of the resulting extract into a Falcon (mL 15). Keep the samples in a refrigerator for 24 hours. Then centrifuge with around 16000 g (13000 rpm) for 30-40 min at 2-4 ^{0}C. Remove 0.1 mL of the supernatant and poured it into a Falcon (15 mL). Add 5 mL of Bradford reagent to it and vortexed. After 25 min, the absorbance of samples was read by a spectrophotometer (595 nm). The standard stock solution was prepared by using bovine serum albumin (BSA) (100 µg mL^{-1}). For this purpose, 10 mg of BSA in 30 mL of sodium chloride solution 0.15 M was dissolved and added distilled water to a volume of 100 mL. In order to draw a standard curve, the solutions with a concentration of 5, 10, 15, 25, 50, and 100 µg BSA/mL are prepared. The result of total soluble protein was reported in terms of µg protein /mL.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of mung bean was measured using Benzie and Strain (1996) method. In this FRP assay, the antioxidant compound present in the samples react FeIII-TPTZ to form FeII-TPTZ, which then has a blue-colored product. For FRP assay, four solutions were prepared separately: 1-Acetate buffer, 2-Diluted hydrochloric acid solution, 3-TPTZ solution, 4-$FeCl_3.6H_2O$. In order to preparation of working solutions, 3.1 g of sodium acetate $3H_2O$ weighted and put in a beaker of 100 cc. Added 16 mL of acetic acid glacial. This solution is kept so long in the refrigerator. To prepare the solution of diluted hydrochloric acid, 1 mL of hydrochloric acid (11 mM) was poured into a balloon with a volume of 1 liter. To make the 2,4,6-tri[2- Pyridyl]-s-Triazine (TPTZ) solution, also, 0.155 g of TPTZ were weighed and poured into a beaker of 100 mL of ddH_2O. Then a bit of hydrochloric acid solution add to resolve it. The mixture volume was adjusted to 50 mL with distilled water. This solution should be prepared fresh and daily and stored in the fridge. The fresh iron chloride solution was produced using 0.27g of $FeCl_3.6H_2O$, which weighed and transferred into a beaker of 50 mL of ddH_2O under mild magnetic stirring to resolve it. The solution of FRAP was prepared by add 400 mL of acetate buffer and 40 mL of TPTZ solution in a beaker of 500 cc. Then add 48 mL of distilled water to it. FRAP solution is covered with foil and placed in a water bath at 37 ^{0}C. At this stage, the color of the solution becomes purple or blue. Then the solution was taken out of the bath and add 400 mL of ddH_2O to it, then 3.6 mL of FRAP reagent was added to the sample mixture. The samples were put into a water bath at 37 °C to allow the temperature of both solutions to rise. Standard solutions of $FeSO_4.7H_2O$ with a concentration from 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM were provided. Absorbance was measured directly at 593 nm. The result of total antioxidant capacity (TAC) was reported in terms of mM L^{-1}.

Antioxidant enzymes activity

APx and POD assay

The solution of KH_2PO_4 (1mM), $K_2H\ PO_4$ (1mM), are prepared separately. Then a solution of potassium phosphate buffer (PPB) (0.1 M, pH= 7.4) using the above solutions was prepared as follows: Add 1.98 mL of KH_2PO_4 (1mM) to 8.04 mL of $K_2H\ PO_4$ (1mM). To adjust the pH (7.4), use the solutions of KH_2PO_4 and $K_2H\ PO_4$. 0.5 g from the fresh leaves was weighed and placed in a porcelain mortar, and then crushed in a mortar with a pestle to obtained uniform extract. Porcelain mortar should be in the ice bath. The extract was transferred into a falcon (15 mL) that placed on the ice. Then pour 10 mL of phosphate buffer (0.1 M, pH= 7.4) on each sample. Samples were centrifuged at 15000 rpm and 4 °C for 25 minutes. The upper phase (supernatant) was used for ascorbate peroxidase and peroxidase enzymes assay.

The activity of APX in the leaf was determined using a spectrophotometer with a wavelength of 290 nm (Nakano and Asada 1978). The total reading time for each sample is 2 minutes. Every 10 seconds, the device will record the reading. To provide a blank, 100 µL of ascorbate (0.5 mM) pour into quartz covet. Add 2 mL of PPB (0.05 M, pH=7), and 100 µL of EDTA (0.1 mM) to it. Then, immediately add 100 mL of hydrogen peroxide (1%), close the covet door, and shake by hands. Then put in the device and Caliber. For reading sample, add 100 µL of supernatant to blank. This enzyme has a decreasing curve. Decompose of H2O2 was measured at 290 nm. By using the extinction coefficient of ascorbate (ε = 2.8 mM−1 cm−1) APX activity was calculated.

The activity of POD in the leaf was spectrophotometrically assayed according to the method of Hemeda and Klein (1990). To blank preparation, 100 µL of PPB (pH= 6.6) pour in covet. Then, 200 mL of guaiacol 1% and 20 mL of hydrogen peroxide (H_2O_2) 0.3 % were added to covet. Put into the device, with a wavelength of 470 nm to the caliber. For reading sample 10 µL of enzyme extract added to blank. The total reading time for each sample is 2 m. One unit of POD activity is defined as a change in OD per 0.001 min.

$$\text{Enzyme activity (U mL}^{-1}) = \frac{\Delta A \times H_2O_2 \text{Coefficient} \times V_t \times d_f}{\varepsilon \times l \times t \times V_s}$$

Where: ΔA_{290}= Subtracting the absorbance at 290 nm wavelength

Coefficient H_2O_2 = for Ascorbate peroxidase, 0.5 and for peroxidase, 4

V_t = The total volume of material within covet for each sample

d_f = 1=Dilution factor of enzyme extract

t = Reading time (minute) for each sample

$\boldsymbol{\varepsilon}$ $(mM^{-1}c\ m^{-1})$ = Extinction coefficient for each enzyme has a specific number, for APx, 2.8, for CAT, 39.4, and for POD, 26.6.

The specific activity of the enzyme (units mg^{-1}) = $\frac{\text{enzyme activity}}{\text{soluble proteins}}$

(Units mg^{-1} protein in 0.5g fresh weight of plant)

Hydrogen Peroxide Assay

For measurement of aqueous hydrogen peroxide (HP) in leaf use only1 g of fresh leaves that pulverized in a porcelain mortar. Then was added 5 mL of trichloroacetic acid solution (W/V) 1% to leaf extracts (Velikova et al. 2000). The contents were transferred to a tube (10 mL) and centrifuged at 12000 g for 15 minutes at 4 ℃. The tube is covered with foil to keep away from the light. In a tube add 0.5 mL of PPB (10 mM, pH=7) and 1 mL of potassium iodide solution, and 5 mL of the supernatant, and then, the lid of the tube was closed and stirred. The absorbance of samples was read by a spectrophotometer (390 nm). Blank is prepared such as the sample, without extract. Standard solutions of 2, 4, 6, 8, and 10 mM of hydrogen peroxide were produced and were interpreted by a spectrophotometer (390 nm). The result of hydrogen peroxide activity was reported in terms of mM. We put the results of the standard curve in the following formula to obtain the amount of hydrogen peroxide:

H_2O_2 (mM L^{-1} H_2O_2in1g fresh weight of leaf) = 5 × 2 × Number of the standard curve

Experimental design and statistical analysis

To analyze of the variance of the raw data, SPSS 19.0 (IBM, SPSS) software package for Windows was used. The data are reported as mean ± SD (n=3). Comparisons among multiple groups were performed by using Duncan's multiple range tests in ANOVA. P< 0.05 was presumed significant.

RESULTS AND DISCUSSION

Mung bean, *V. radiata* is one of the most popular and important edible legume crops in Asia (Thomas et al. 2003). In this regard, many efforts have been made to cultivate it under various stress conditions (Alzarqaa et al. 2014). Abiotic stresses (such as HMs) can affect plant tolerability at the cellular as well as the whole-plant level (Ashraf et al. 2004), and their molecular, physiological, and

Table 1. Effect of different concentrations of Cd on mean values of studied parameters.

Parameters	(Control)	Treatments			
	Distilled water	25(mg L^{-1})	50(mg L^{-1})	75(mg L^{-1})	100(mg L^{-1})
Soil Cd (mg Kg^{-1})	7.83 ± 0.7^{c}	139.1 ± 18^{c}	1075.2 ± 20^{a}	787.93 ± 25^{b}	706.65 ± 32^{b}
Root Cd (mg Kg^{-1})	7.65 ± 0.6^{c}	11.55 ± 0.7^{c}	32.35 ± 2.7^{b}	24.15 ± 1.4^{bc}	54.35 ± 12^{a}
Stem Cd (mg Kg^{-1})	4.10 ± 0.3^{c}	4.6 ± 0.7^{c}	4.2 ± 0.4^{c}	6.1 ± 0.5^{b}	6.95 ± 0.2^{ab}
Leaf Cd (mg Kg^{-1})	5.50 ± 0.2^{b}	6.2 ± 0.7^{b}	7.5 ± 0.3^{b}	14.35 ± 1.8^{a}	11.75 ± 1.7^{a}
Shoot Cd mg Kg^{-1})	14.43 ± 3^{b}	15.2 ± 2^{b}	17.15 ± 4^{b}	14.35 ± 1.8^{a}	11.75 ± 1.7^{a}
Total plant Cd (mg Kg^{-1})	22.08 ± 4^{d}	26.75 ± 5^{cd}	42.6 ± 9^{bc}	49.26 ± 9^{b}	77.68 ± 10^{a}

Data are expressed mean+SE of three experiments, performed in duplicate. $P < 0.05$

morphological interactions will be complex (Alzarqaa et al. 2014).

According to the results, the treatment of 100 mg Cd/L showed the highest Cd levels in the root, stem, and total plants (Table 1). However, in the treatment of 50 mg Cd/L, the high concentration of cadmium was absorbed by soil and root, and also for the leaf a similar trend was observed in the treatment of 75 mg Cd/L (Table 1). There is a reverse relationship between the soil Cd and the plant absorbency levels. These phenomena are due to the existing mechanisms in *V. radiata*, for the prevention of cadmium transfer to its shoot. Different treatments showed a significant difference ($p< 0.05$) in terms of the root, stem, leaf,

shoot, and total plant Cd content. By increasing the concentration of soil Cd, the increase of Cd in root was observed, but there is a negative and significant correlation between soil and root Cd. This trend was due to the fact that although more Cd had entered the soil, the amount absorbed by the roots and by other plant organs was not proportional to such increase. The Cd absorption by the roots was proportionally slightly higher than that of other organs (Table 1).

Data analysis showed that there is a significant difference between the treatments in terms of soluble proteins ($p\leq 0.01$). So, the dissolved cadmium affected the soluble proteins of the *V. radiata* (Table 2; Figure 2). According to the mean comparison (Table 2), the highest soluble proteins have been observed in the control (ddH_2O) and the lowest in the treatment of 50 mg Cd/L. Further, as shown in Table 3, there is a negative and significant correlation between APx and soluble protein content, because the increase in enzyme activity of ascorbate peroxidase caused to reduction in content of soluble protein. Subsequently, the reduction of soluble proteins caused a decrease in peroxidase activity and total antioxidant capacity. Excess cadmium in soil, roots, leaf, shoot, biomass, and total plant, caused to slightly reduce the soluble proteins marker. Previous studies have shown that mung bean albumin peptides showed high metal chelating and radical scavenging activities (Kusumah et al. 2020). These findings conformed to our results and Sandalio et al. (2001) reports.

In general, the imbalance in production and metabolism of ROS, oxidative stress is created, which is necessary for cell survival. Poorakbar and Ashrafi (2011) expressed that cadmium is one of the factors that caused to the generation of ROS. In the plant, ROS such as O^{-2}, OH^{-} , and H_2O_2 are produced under stress conditions and attacked various biomolecules. In fact, these oxygen species act as mediators in regenerative -O_2 to H_2O_2. In the presence of metals such as copper and iron, H_2O_2 can be converted more active OH^{-} via the Fenton reaction. Metal ions oxidized are converted into superoxide radicals by rehabilitation. Using the Haber Weiss, OH^{-} to H_2O_2 and O^{2-} is made directly. The possible formation mechanism of Fenton and Haber Weiss reactions can be written as follows (Crichton 2009):

$H_2O_2 + Fe^{2+}/ Cu^{+} \rightarrow OH + OH^{-} + Fe^{3+}/Cu^{2+}$ (Fenton)

$O^{2-} + Fe^{3}/ Cu^{2+} \rightarrow Fe^{2}/ Cu + O_2$

$H_2O_2 + O_2^{\cdot-} \rightarrow OH + OH^{-} + O_2$ (Haber Weiss)

Heavy metals (e.g, Cd), unlike redox metals, cannot directly generate ROS by participating in the Haber Weiss/Fenton reactions. However, these metals are indirectly induced ROS production through various mechanisms in the plant cells such as activation of NADPH oxidase, cation exchange capacity of enzymes, and control of enzyme activity based on their -SH groups affinity (Shahid et al. 2014). To ROS levels control and protect plant cells, a total plant antioxidant capacity is the set of all non-enzymatic substances (e.g, ascorbic acid, glutathione, vitamin C and E, flavonoids, anthocyanins, and carotenoids) and also enzymatic antioxidants (e.g, SOD, APX, and CAT) (Kasote et al. 2015). Each of these materials in various plants has different types and quantities. The results of the current study showed that TAC decreased in a dose-dependent manner under-tested. Based on the results, among the different treatments compared to control, a significant difference has not been observed (Table 2). In other words, cadmium

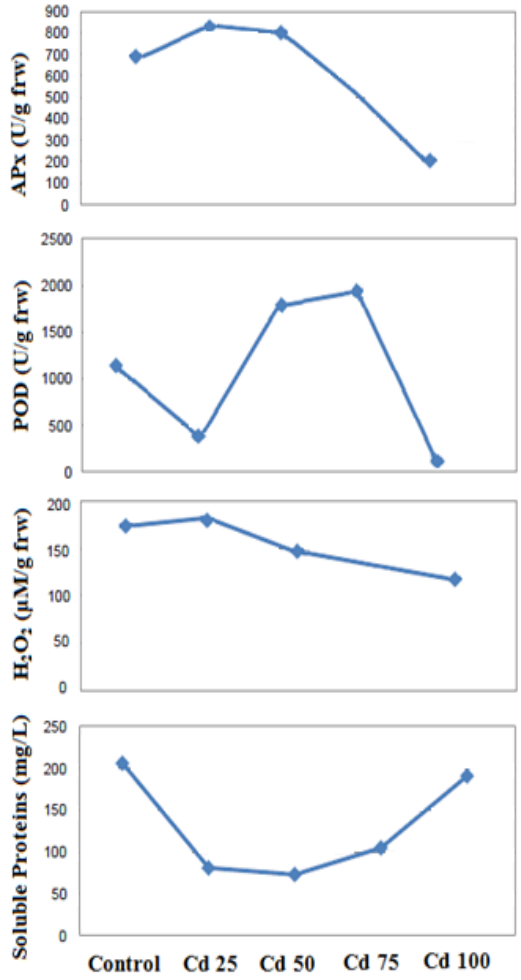

Figure 2. The impact of cadmium on H_2O_2, POD, and APx activity and soluble proteins content of the leaf extracts of *V. radiata* under different treatments.

treatments have been unable to affect the *V. radiata* TAC under extreme conditions of stress. Although in the treatment of 100, the amount of both POD and APx was declined, the plant antioxidant system has to deal with stressful situations. Non-protein thiol groups, particularly glutathione plays an important role in protecting plants from environmental stresses, especially to Cd toxicity (Zagorchev et al. 2013). Flavonoids in citrus fruit have significant amounts of hydroxy acids, frolic, and coumaric, which have high antioxidant properties and it has an important role in controlling cancer (Shojaa et al. 2011). Also, polyphenols are a variety of antioxidants that play a role in preventing many diseases, including cancer (Malakoot Tabari et al. 2013). These compounds are very diverse and have different effects. Phenolic compounds, including vitamins, pigments, and flavonoids, are anti-mutation and anti-tumor. Antioxidant activity of phenolic and related compounds are mainly due to the power of restoration and their chemical structure, that it is able to donate electrons to free radicals and neutralize free radicals, the formation of complex, metal ions and oxygen molecules triple makes off (Michalak 2006). Phenolic compounds by donating electrons to free radicals were inhibited the lipid oxidation reactions (Ferrari 2012). Therefore, at the highest concentration of cadmium, plants may reduce phenolics compounds to avoid an impaired antioxidative system responses based on phenoxyl radicals product (Márquez-García et al. 2012). In this line, Domínguez et al. (2010) reported that *Spartina densiflora* is not able to counteract high cadmium exposition, but this plant by synthesizing non-enzymatic substances such as ascorbic acid or glutathione is a successful response to moderate concentrations of cadmium. These findings agreed with our study. According to the study on antioxidant potentials protect *V. radiata* (L.) Wilczek plants from soil cobalt stress done by Abdul Jaleel et al. (2009), the increase in metal concentration decreased the CAT activity and increased the peroxidase (POX) and polyphenol oxidase activities (PPO) activities and improve growth and photosynthetic pigments composition.

Hydrogen peroxide is a substance for which a wide range of reactions dependent peroxidase acts as an electron acceptor (Zand et al. 2010). It seems that, peroxidases act as enzyme detoxification of ROS, peroxidase and catalase are known as the main enzymes H_2O_2-eliminating enzymes (Caverzan et al. 2012). Apparently, the cell wall is the main site of aaccumulation of a number of peroxidase isoenzymes. Some of the peroxidase isoenzymes have links in the cell surface, that can be easily released under stress conditions and they go to the apoplast. Some surface peroxidases have a strong bond with the plasma membrane (Minibayeva et al. 2009). Biosynthesis and exudation apoplastic peroxidases initially available, may be adjusted by changing environmental conditions. The classification of enzymes, peroxidase, and ascorbate peroxidase are in the oxidoreductase group.

Among the different treatments of cadmium, there are significant differences in terms of enzyme activity of ascorbate peroxidase ($p \leq 0.01$). Mean comparison showed that the highest levels of APx activity were observed in the treatment of 25 mg Cd/L and lowest in 100 mg Cd/L (Table 2; Figure 2). According to correlation Table 3, increasing cadmium in soil, root, stem, leaf, shoots and total plant caused to slight decrease in ascorbate peroxidase enzyme activity. Increasing to 25 mg L^{-1} of cadmium has been able to increase the ascorbate peroxidase enzyme activity. However, with the increase in the concentration of cadmium, the activity of APx in *V. radiata* was decreased. Poorakbar & Ashrafi (2011) also showed that with cadmium concentrations of 50 Mm L^{-1}, the activity

Table 2. The average concentrations of soluble proteins, ascorbate peroxidase, peroxidase and hydrogen peroxide was measured in total *V. radiata* in response to different concentrations of cadmium chloride.

Parameters	(Control)	Treatments			
	Distilled water	25(mg L^{-1})	50 mg L^{-1})	75(mg L^{-1})	100(mg L^{-1})
Soluble proteins(mg L^{-1})	206.25 ± 15.2^{a}	81.66 ± 11.7^{b}	73.75 ± 6.8^{b}	105.83 ± 17.2^{b}	191.66 ± 21.2^{a}
Total antioxidant capacities(mM g frw^{-1})	35.0 ± 4^{a}	43.3 ± 5^{a}	40.3 ± 5^{a}	38.6 ± 3^{a}	35.0 ± 7^{a}
Ascorbate peroxidase(U g frw^{-1})	0.682 ± 0.07^{a}	0.835 ± 0.04^{a}	0.799 ± 0.03^{a}	0.524 ± 0.02^{ab}	0.193 ± 0.02^{b}
Peroxidase(U g frw^{-1})	1.079 ± 0.03^{b}	0.373 ± 0.01^{c}	1.786 ± 0.02^{a}	1.935 ± 0.03^{a}	0.122 ± 0.02^{d}
Hydrogen peroxide(µM g frw^{-1})	1.73 ± 0.05^{a}	1.82 ± 0.06^{a}	1.48 ± 0.04^{b}	1.32 ± 0.03^{c}	1.16 ± 0.08^{d}

Data are expressed mean+SE of three experiments, performed in duplicate. $P < 0.05$

of APx was increased. But this level of Cd contamination is satisfactory and the plant is resistant to it. Fortunately, Cd-contamination levels in the soil of the Iran and even in many parts of the world are less than this amount.

By increases in soil cadmium level-up to 75 mg L^{-1}, peroxidase activity was raised, but at higher concentrations of cadmium, enzyme activity was reduced by comparing to controls. This phenomenon appears to be due to the induction of toxicity and stress due to the presence of high concentrations of cadmium. This means that soil cadmium contamination level-up to 75 mg L^{-1} for the *V. radiata* is tolerable (increase in POD), but higher concentrations can be stressful to the plant. Further, by increases in the concentration of

cadmium in root, stem, and the total plant, the peroxidase activity was slightly decreased. These results are in agreement with other findings of Somushicariyh et al. (1992), Nooraniazad and Kafil Zadeh (2010, es high, the peroxidase activity is reduced, and vice versa, only in the treatment of 100 mg L^{-1} activity of both enzymes was very low and close to each other. On the other hand, according to this result, APx at lower concentrations (25 mg L^{-1}) and POD at higher concentrations (75 mg L^{-1}) have had the highest level of activity. It seems that since both of these enzymes are antioxidant enzymes with similar functions, in stress conditions, complementary working and functional impairment compensate for each other.

ROS generation in effect, biological and abiotic stress, such as O^{-2}, and OH^{-} are lead to oxidative stress, damage to DNA, proteins, pigments, and lipid peroxidation that may eventually be conducted to cell death. ROS in mitochondria and chloroplasts formed through electron transfer reactions can be quickly converted to H_2O_2 (Hassibi 2007). Also, in cells, H_2O_2 is caused by reactions that are enzymes mediated such as glycolate oxidase in photorespiratory. Oxalate oxidase converts oxalate and oxygen to H_2O_2, and Co_2. Moreover, O^{-2} has produced the transfer of electrons from NADPH to oxygen, by NADPH oxidase, later be converted to H_2O_2. It seems that the ROS, H_2O_2 for signaling is better than others because it has higher stability and half-life. The production of such species is a common response to both biological and non-biological stress. These tensions are included pathogens,

Table 3. Correlation matrix of parameters (Pearson correlation coefficients (r) per parameter).

	Soil Cd	Root Cd	Stem Cd	Leaf Cd	Sheath Cd	Shoot Cd	Total Cd (Root + Shoot)	Soluble Proteins	Ascorbat Peroxidase	Peroxidase	Total Capacity Of Antioxident	Hydrogen peroxidase
Soil Cd	1											
Root Cd	0.651**	1										
Stem Cd	0.183	0.522*	1									
Leaf Cd	0.676**	0.689**	0.693**	1								
Sheath Cd	0.251	0.052	-0.35	-0.079	1							
Shoot Cd	0.586*	0.699**	0.833**	0.924**	0.021	1						
Total Cd (Root + Shoot)	0.504	0.914**	0.726**	0.743**	-0.039	0.797**	1					
Soluble Proteins	-0.32	-0.118	0.109	-0.143	-0.11	-0.036	-0.079	1				
Ascorbate Peroxidase	-0.223	-0.339	-0.429	-0.493	0.3	-0.434	-0.332	-0·543*	1			
Peroxidase	0.517*	-0.121	-0.313	0.2	0.221	0.045	-0.271	-0.332	0.218	1		
Total Capacity Of Antioxidant	0.075	-0.197	-0.258	0.086	0.038	-0.063	-0.197	-0.373	0.204	0.14	1	
Hydrogen Peroxide	-0.634*	-0.696**	-0.622*	-0.832**	0.151	-0.794**	-0.689**	0.029	0.45	-0.311	0.305	1

In terms of statistical, significance at $p \leq 0.01$ with ** and significant at $p \leq 0.05$ are shown by *. + Sign above numbers indicate that there is a positive correlation between the two traits. – Sign above numbers indicate the reverse correlation exists between the two traits. Increasing one will decrease the other.

elicitors (irritants), heat, cold, UV light, and ozone. Hence the H_2O_2 is a sign molecule in plants.

As shown in Table 2 and Figure 2, the maximum and minimum activity of hydrogen peroxide has been observed in the treatment of 25 and 75 mgCd/L, respectively. The correlation result (Table 3), shows that, by the increase of cadmium in soil, roots, stems, leaves, and biomass, the hydrogen peroxide concentration was dose-dependently decreased. These results disagreed with the reported results of Pourakbar and Ashrafi (2011), Behtash et al. (2010), and Rahimi and Ronaghi (2012). In fact, the increase in soil cadmium level-up to 75 mg L^{-1}, caused to increase in the activity of POD and the generation of hydroxyl radicals in a plant, but during the enzymatic reaction hydrogen peroxide was disintegrated, and its level in the plant decreased. Then, by increase cadmium level to 100 mg L^{-1}, peroxidase activity also decreased (Table 2; Figure 2). So, where the amount of peroxidase is greater, hydrogen peroxide will be less (Table 3). However, with increase cadmium level, the contamination of soluble proteins, and conversely the amount of peroxidase was increased. It appears that, in the stress conditions, plants have a higher generation rate of enzymatic protein such as peroxidase. This mechanism was increased the plant resistance to oxidative damage caused by hydrogen peroxide. Probably one of the most important strategies of *V. radiata* to cope with the stress of cadmium is upregulating the peroxidase enzyme.

CONCLUSIONS

The plant response to cadmium stress is a complex multidisciplinary interaction among plant processes and their intrinsic properties. The result of the present study reveals that the increase in soil cadmium has no effect on the antioxidant capacity of *V. radiata* and this plant could be resisted against the toxicity of cadmium. Based on a range of natural antioxidant levels in *V. radiata*, tolerance to high cadmium levels, and less body absorbance of cadmium, we suggested that plantings of *V. radiata* in addition to producing a source of beneficial food can be a solution for environmental and agricultural management in polluted areas. These results may contribute to improving mung bean productivity through soil Cd stress management.

ACKNOWLEDGMENTS

We thank Shahid Chamran University of Ahvaz for the technical/scientific supports and scholarships granted.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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