The effect of cadmium and mercuric chlorides on some physiological traits in two cultivars of wheat

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Abstract

One of the important abiotic stresses that negatively affect cereals such as wheat is heavy metals. Soil pollution with heavy metals has become one of the major environmental concerns resulting from the industrial development and use of fertilizers containing heavy metals. One way to counteract the negative effects of heavy metals in plants which produce reactive oxygen species is the activation of antioxidant systems in plant cells. In order to study the physiological traits involved in resistance to these stresses, a factorial experiment was conducted in a completely randomized design with three replications. The first factor was wheat cultivars including Gonbad (tolerant to Fusarium) and Tajan (susceptible to Fusarium). The second factor was spraying heavy metals (mercuric chloride at concentrations of 5, 10, 15, and 20 µM, cadmium chloride at concentrations of 0.25 and 0.5 mM and control) and the third factor was sampling times after treatments (after 8 and 16 hours). The results showed that the total protein and soluble sugar contents increased with cadmium and mercuric chloride treatments. Furthermore, with increasing concentration of mercuric chloride, the catalase, peroxidase, and polyphenol oxidase activities decreased while the activities of these enzymes increased with increasing concentration of cadmium chloride. Thus, this study showed that spraying of cadmium and mercuric chloride at micro- and nano-molar concentrations can stimulate and strengthen plant antioxidant system and make plants more tolerant to heavy metals stresses.

Key words: antioxidant enzymes; cadmium chloride; mercuric chloride; physiological traits; wheat

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Introduction

Abiotic stresses such as heavy metals can cause great damages to wheat. Industrialization of societies has led to releasing a lot of toxic compounds in biosphere. Among heavy metals, cadmium and mercury are considered as the most toxic metals in the environment. These metals are the most important environmental pollutants and their toxicity causes great problems regarding ecological, evolutionary, nutritional, and environmental considerations (Amani, 2008). Heavy metals may deactivate key enzymes by replacing them with active cations (Koocheki et al., 2005).

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Following the cell growth arrest caused by toxic metals, a variety of mechanisms may occur for cell coordination and balance including 1) higher activity of some enzymes involved in catabolism regulation to maintain cellular energy production, 2) the use of low-energy transmission systems required to insert amino acids and other carbon sources, 3) facilitating the synthesis of amino acids and other carbon-metal bonds, and 4) reinforcement of metal transition system for the regulation of intracellular metals (Isarankura et al., 2009).

Cadmium, as one of the most toxic metals, creates complexes with organic compounds such as proteins, prevents the vital activities of cell, increases lipid peroxidation, and produces oxygen species which cause membrane deterioration. Also, cadmium has a strong affinity to sulfhydryl and hydroxyl groups and nitrogen-containing ligands. Therefore, this element deactivates most important enzymes and reduces CO_2 fixation through inhibition of Rubisco activity in the plants, and as a result, reduces photosynthesis. It also leads to impairment of respiration and other metabolic processes in plants (Wang et al., 2009). Cadmium, with production of various forms of reactive oxygen species (ROS), causes toxic reactions and damages to proteins, carbohydrates, and DNA and ultimately results in oxidative stress (Zhang et al., 2010). Reactive oxygen species also affect expression of genes and induce changes in many processes such as growth, cell cycle, programmed cell death, and response to abiotic stress (Singh Gilles and Tuteja, 2010). Glutathione and Phytochelatin strongly react with cadmium and reduce free cadmium in leaf cytosol and therefore limit toxicity of cadmium (Skrinder et al., 2009).

Another toxic metal, mercury, through accumulation in cell wall and entry into cytoplasm causes oxidative stress followed by production of reactive oxygen species in plants. This process causes significant damages to membrane lipids structure and disrupts cell metabolism. It also stops mitochondrial activity (Zhou et al., 2007). Mercury toxicity during addition of this element to plant growth medium causes reduction in water potential, plant nutrition disturbance, alteration in cell membrane permeability, root and shoot growth arrest, and reduction in protein production and germination (Zhao et al, 2008).

Carbohydrates are a group of organic compounds that contain carbon, hydrogen, and oxygen. The simplest carbohydrates which are the most characteristic of this group include three-carbon sugars (triose) like glyceraldehyde and dihydroxyacetone (Hopkins and Huner, 2008). Furthermore, increase in carbohydrate content acts as a metabolic signal which enhances the expression of defense-related genes and reduces photosynthesis (Kocal et al., 2008). Among plant responses to such stresses are activities of enzymes such as catalase (CAT) and peroxidase (POX), which lead to neutralizing reactive oxygen species activity produced in cells. Production of reactive oxygen species in plant cells stimulates and enhances the activity of the aforementioned enzymes (Mishra et al, 2009).

The aim of this study was to evaluate the effects of different concentrations of mercury and cadmium on total protein and soluble sugar contents as well as activities of enzymes involved in plant stress tolerance at three-leaflet stage.

Materials and Methods

The study was performed in the greenhouse at the University of Mohaghegh Ardabili, Ardabil in 2012. A factorial experiment based on completely randomized design with 3 replications was conducted. The first factor was wheat cultivars including Gonbad (resistant to Fusarium) and Tajan (susceptible to Fusarium). The second factor was related to heavy metals at seven levels (mercuric chloride at 5, 10, 15, and 20 µM, cadmium chloride at 0.5 and 0.25 mM, and control), and the third factor was sampling time after 8 and 16 hours of heavy metal treatment.

Seeds (Gonbad and Tajan cultivars) were prepared from research center of Moghan, sterilized, and planted in square filter papers and placed in a germinator for 2 days in order to germinate. Eventually, the seedlings from each cultivar were transferred to greenhouse to culture. Spraying with cadmium chloride and mercuric chloride was performed at three-leaf stage. After 8 and 16 hours of spraying, sampling from control and treated plants was performed

and samples were immediately transferred into liquid nitrogen and stored at -70 °C.

Measuring the biochemical parameters

To determine protein concentration, Bradford method (1976) was used spectrophotometrically at 595 nm wavelength.

Study of enzymatic activity of the protein extract

To extract protein, 0.2 g of frozen samples were ground with porcelain mortar in 2 ml of buffer including 0.5 mM of HCL-Tris (pH = 7.5). The resulting homogenate was transferred to centrifuge tubes and centrifuged at 13000 g for 20 min at 4 °C (Sudhakar et al., 2001). At the end of centrifugation, the supernatant (containing protein extract) was used to study the activities of catalase, peroxidase, and polyphenol oxidase.

Measurement of catalase activity

Kinetic activity of catalase enzyme was measured using Chance and Maehly method (1955) with some modifications. For this purpose, 2.5 ml of Tris buffer with 0.3 ml of hydrogen peroxide and 60 µL of enzyme extract were mixed in the ice bath. The curve of absorbance changes was read at 240 nm wavelength using spectrophotometer.

Measurement of peroxidase activity

To study the activity of peroxidase, Kar and Mishra method (1976) was used with some modifications. For this purpose, the solutions of Tris buffer 1 M, hydrogen peroxide 50 mM, and pyrogallol 100 mM were prepared and then 10 ml of each solution was taken and the resulting solution was reached to 100 ml and finally 2.5 ml of this solution was mixed with 50 ml of the enzyme extract. Curve of absorbance changes was read using spectrophotometer at wavelength of 425 nm.

Measurement of polyphenol oxidase

The activity of polyphenol oxidase was investigated by Mishra method (1976) with some modifications. To do this, 1.5 ml of Tris buffer was mixed with 0.4 mL pyrogallol and 0.1 mL of enzyme extract and placed in water bath for 5 minutes at 25 °C. The curve of absorbance changes was read with spectrophotometer at 420 nm.

The activity of each enzyme was calculated according to change in absorbance unit per minute per mg protein.

Measurement of total sugars in leaves

The extraction of total sugars from the youngest leaves was performed using Omokolo and colleagues method (1996). For this purpose, 0.1 g of leaf sample was ground in porcelain mortar with 5 mL of ethanol 80% and resulting extract was centrifuged at 10,000 rpm for 10 minutes. Measuring of sugar content was performed using Mc-Cready et al method (1950) and soluble sugar content was measured using the spectrophotometer at wavelength of 620 nm.

Statistical analysis

Data analysis was performed using SAS9.1 and SPSS18 softwares and the means were compared as completely randomized factorial design using LSD test at 1% probability level.

Results

The results of variance analysis (Table 1) showed that mutual tripartite effects of heavy metal × cultivar × time on total protein and soluble sugar contents as well as activity of catalase, peroxidase, and polyphenol oxidase in wheat cultivars under study (Gonbad and Tajan) were significant ($p \leq 0.01$). So cut method was used to investigate the mutual two-sided effects (Tables 2, 3, and 4).

Mean squares of mutual effect for cultivar × time which cut for soluble sugars content determination in Gonbad 8 hours after spraying, as well as the amount of enzyme activity of peroxidase and polyphenol oxidase in Tajan (8 and 16 h) were not statistically significant, so the comparison of their means was ignored.

Table 1
Variance analysis of characteristics involved in resistance to metals in wheat leaves and activity of antioxidant enzyme

SOV	df	Mean of square				
		carbohydrates	total protein	polyphenol oxidase	peroxidase	catalase
cultivar	1	0.0024 **	0.067*	6.70**	4.38**	0.007^{ns}
Heavy metal	6	0.0025 **	0.059**	3.14**	1.95**	0.202**
Time	1	0.0004 *	0.116**	3.27**	2.93**	0.440**
cultivar× Heavy metal	6	0.0040 **	0.032**	2.11**	1.96**	0.022**
cultivar× Time	1	0.0068 **	0.003^{ns}	0.541^{ns}	0.54**	0.004^{ns}
Heavy metal × Time	6	0.0021 **	0.017**	0.548**	0.68**	0.076**
Time× heavy metal ×Cultivar	6	0.0034 **	0.026**	0.642**	0.66**	0.016**
Error	56	0.00007	0.002	0.167	0.082	0.002
Coefficient of variation		4.4	14.4	21.8	17.2	4.7

Ns: non-significant, * and **: significant at 5% and 1 % probability level, respectively

Table 2
Cut mean comparison of tripartite effects of heavy metal × cultivar × time by cultivar × time factor for soluble sugar contents

Trait		Average Yield						
		Mercuric Chloride Concentrations (µM)					Cd Concentrations (mM)	
	Time (hours) × Cultivar	0	5	10	15	20	0.25	0.5
Soluble Sugar Content (mg/g leaves wet weight) LSD: 0.158	Gonbad*16	1.437^{abc}	1.363^{abcd}	1.307^{abcd}	1.242^{d}	1.378^{abcd}	0.558^{gh}	0.370^{i}
	Tajan *8	1.383^{abcd}	1.403^{abc}	1.403^{abc}	1.461^{a}	1.451^{ab}	0.913^{e}	1.299^{bcd}
	Tajan *16	1.383^{abcd}	0.854^{ef}	1.415^{abc}	1.308^{abcd}	1.332^{abcd}	0.491^{hi}	0.702^{fg}

Means with same letters statistically have no significant differences at the probability level of T-test (LSD: 0.158)

Comparison of means for mutual effects of cultivar × time for soluble sugar content (Table 2) in Gonbad cultivar (16 hours after spraying) showed that there is no significant difference between increased concentrations of mercuric chloride and control, but with increasing concentrations of cadmium chloride, soluble sugar content decreased significantly compared to control. In Tajan (8 and 16 h), there was no significant difference in soluble sugar content with increasing concentrations of mercuric chloride compared to control, except for concentration of 5 mM (16 hours), in which soluble sugars content was significantly reduced compared to control. The soluble sugars level showed significant differences with increasing concentrations of cadmium chloride compared to control, so that the soluble sugar contents increased with increasing concentrations of cadmium chloride.

The means comparison of total protein contents in Gonbad cultivar (8 and 16 h) showed significant increase in mercuric chloride and cadmium chloride levels as compared to control (Table 3). In Tajan (8 hours) significant difference was observed in total protein content with increasing mercuric chloride and cadmium chloride concentrations, compared to control. But significant decrease in total protein content was observed 16 hours after increasing concentration of mercuric chloride and cadmium chloride compared to control. The highest and lowest total protein contents were observed in Tajan at 20 mM (0.349 g g^{-1} FW) after 8 hours and control (0.021 g g^{-1} FW), respectively.

The results of means comparison for catalase activity (Table 3) in Gonbad cultivar (8 hours) showed significant difference when levels of mercuric chloride and cadmium chloride increased compared to control, but after 16 hours, except for concentration of 5 mM of mercuric chloride which was not significantly different from control regarding catalase activity, in the other concentrations (10, 15 and 20 mM) significant difference was observed compared to control.

Table 3: Comparison of cut mean of mutual effect of heavy metal × cultivar × time by cultivar × time factor for total protein characteristic and catalase activity

Traits		Average Yield						
		Mercuric Chloride Concentrations (µM)					Cd concentrations (mM)	
	Time (h) × Cultivar	0	5	10	15	20	0.25	0.5
Total Protein (g per gram of leaves wet weight) LSD: 0.048	Gonbad*8	0.021^{l}	0.052^{kl}	0.271^{b}	0.146^{efgh}	0.206^{cd}	0.124^{fghi}	0.154^{efg}
	Gonbad*16	0.021^{l}	0.030^{l}	0.118^{fghi}	0.026^{l}	0.230^{bc}	0.105^{hij}	0.049^{kl}
	Tajan *8	0.126^{fghi}	0.110^{ghi}	0.118^{fghi}	0.123^{fghi}	0.349^{a}	0.165^{def}	0.180^{de}
	Tajan *16	0.126^{fghi}	0.163^{def}	0.103^{hij}	0.085^{ijk}	0.058^{jkl}	0.185^{cde}	0.104^{hij}
Catalase (Changes in mg absorption of protein) LSD: 1.694	Gonbad*8	10.50^{a}	5.12^{bc}	0.86^{h}	1.71^{fgh}	1.48^{fgh}	1.64^{fgh}	1.49^{fgh}
	Gonbad*16	10.50^{a}	6.23^{b}	2.11^{efgh}	10.83^{a}	1.39^{gh}	2.26^{efgh}	4.15^{cd}
	Tajan *8	2.46^{defgh}	3.09^{def}	2.78^{defg}	2.24^{efgh}	1.02^{h}	1.19^{gh}	0.92^{h}
	Tajan *16	2.46^{defgh}	1.47^{fgh}	2.51^{defgh}	3.46^{cde}	5.96^{b}	0.90^{h}	1.96^{efgh}

Means with same letters statistically have no significant differences at the probability level of T-test.

Table 4: Comparison of cut mean of tripartite effect of heavy metal ×cultivar × time by cultivar × time for peroxidase and polyphenol oxidase activities

traits		Average yield						
		mercuric chloride concentrations (µM)					cadmium concentrations (mM)	
	Time (h) × cultivar	0	5	10	15	20	0.25	0.5
Peroxidase (absorbance changes in mg of protein) LSD: 2.980	Gonbad*8	13.98^{a}	7.89^{bc}	1.12^{g}	2.20^{fg}	1.57^{fg}	2.46^{efg}	1.84^{fg}
	Gonbad*16	13.98^{a}	9.95^{b}	2.43^{efg}	10.53^{b}	1.35^{g}	2.95^{efg}	6.23^{cd}
Polyphenol Oxidase (absorbance changes in mg of protein) LSD: 7.631	Gonbad*8	30.12^{a}	16.43^{cd}	2.34^{g}	4.25^{fg}	3.01^{g}	5.10^{efg}	3.96^{fg}
	Gonbad*16	30.12^{a}	20.60^{bc}	5.40^{efg}	24.26^{ab}	2.75^{g}	6.02^{efg}	12.28^{de}

Means with same letters statistically have no significant differences at the probability level of T-test.

In Tajan (8 hours) with increasing concentrations of mercuric chloride and cadmium chloride, no significant difference was observed in catalase activity level compared to control, but 16 hours after mercuric chloride and cadmium chloride treatments, a significant difference was observed in catalase activity compared to control.

In Gonbad and Tajan (8 hours) cultivars, with increasing cadmium chloride concentrations catalase activity first decreased and then (after 16 hours) increased. The highest and lowest catalase activities were observed at 15 mM (16 h) and 10 mM (8 hours) concentrations of mercuric chloride, in Gonbad cultivar, respectively.

According to Table 4, means comparison of peroxidase activity showed that in Gonbad cultivar, except for cadmium chloride treatment (16 hours), with increasing concentrations in treatment conditions, significant decrease was observed. The highest and lowest levels of peroxidase activities in Gonbad cultivar were related to control and 10 µM concentration of mercuric chloride, respectively.

Means comparison of polyphenol oxidase activity (Table 4) showed that in Gonbad cultivar (8 hours) there was a significant difference with increasing concentrations of mercuric chloride and cadmium chloride compared to control. In Gonbad cultivar (16 hours), there was no significant difference in polyphenol oxidase activity under treatment of 5 and 10 mM of mercuric chloride compared to control. But, at 15 and 20 mM concentrations of cadmium chloride, polyphenol oxidase activity significantly decreased compared to control. In Gonbad cultivar, the highest and lowest levels of polyphenol oxidase activities were related to control and 10 µM concentration of mercury chloride (8 h).

Discussion

Total protein and soluble sugar contents

Plants can hold their carbohydrate store in an optimal level with increasing soluble sugars, in order to maintain cell basic metabolism in environmental conditions under stress. Heavy metals cause the loss of chlorophylls in leaves, as observed when monitoring surface characteristics of wheat. This is why in seedlings treated with cadmium and mercury, increase in concentrations of cadmium and mercury led to leaf chlorosis, i.e., the leaves turned into yellowish-green, and thus reduction in carbohydrate contents and activation of some resistance genes in cell (Bolton, 2009). This is consistent with results of the present study in Gonbad cultivar (16 hours). The reduction in sugar contents is probably due to consumption of sugar for synthesis of proteins and polypeptides such as phytochelatins and glutathione. Many studies have shown that by entry of Cd^{+2} cations into leaf cells, the respiratory rate decreases and therefore, the ultra-structural changes occur in cell organelles. Also, the behavior of key enzymes in several metabolic pathways changes. The presence of Cd^{+2} in the cytosol of leaf cells causes the increase of activity of enzymes degrading insoluble sugars as well as sucrose synthase and acid invertase (Verma and Dubey, 2001). Increased soluble sugar content when plants are treated with cadmium chloride has been reported in canola, safflower and lentils by other researchers (Benavides et al., 2005; Karimi and Nojavan, 2007; Noorani Azad and Kafilzadeh, 2011). In order to cope with heavy metals stress, plants start to synthesize defensive proteins and to do so, they recruit metabolites and enzymes presented in protein structure. In Gonbad cultivar, increased expression of these proteins in wheat leaves caused by mercury is probably due to increased synthesis of certain enzymes such as antioxidant enzymes, as well as synthesis of proteins and polypeptides involved in cellular defense system against ions (metallothionins and phytochelatins). Karimi and Nojavan (2007) showed that with increasing cadmium concentration, the soluble proteins content was increased in lentil leaves. This increase may reflect an increase in enzymes involved in defense mechanisms of plant and antioxidant polypeptides. These findings are consistent with the results of the present study on Gonbad and Tajan cultivars (8 hours after spraying) treated with mercuric chloride. Cadmium is very similar to nitrogen and sulfur ligands and this is why cadmium, by binding to sulfhydryl groups, causes inhibition and structural destruction of proteins and controlling redox state of cell as well as destruction of ion channels and ions leak (Mishra et al., 2009). Reduced amount of storage proteins can be attributed to destruction of protein structures and presence of free radicals which is consistent with our results in Gonbad and Tajan cultivars (16 hours).

Antioxidant enzymes activity (catalase, peroxidase and polyphenol oxidase)

Cadmium, by damage to membrane lipids, alters cell membrane function and this could affect enzymatic activities associated with membranes such as H^{+}-ATPase. Also, at cellular level, cadmium, by decreasing cell wall elasticity, decreases turgor pressure and inhibits cell growth (Aina et al., 2007). Cadmium, unlike metals such as copper and iron which cause oxidative stress through redox cycles like Fenton or Haber-Weiss reactions, cause damage to cell through indirect mechanisms such as interference with defense systems, destruction of electron transport chain, and induction of lipid peroxidation (Benavides, et al., 2005). High concentrations of cadmium cause toxicity and therefore oxidative stresses in plants. Oxidative stresses, by producing free oxygen radicals such as superoxide radicals (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (^{-}OH) damage plant cells. Free hydroxyl radicals are initiators of reactions that cause lipid peroxidation (Chen et al., 2007). To reduce and eliminate different active oxygen species and avoid oxidative damages to plants, activities of antioxidant enzymes such as catalase, superoxide dismutase, and peroxidase increase (Smith et al., 2008). Oxidative stress caused by mercury ions is followed by increased activity of antioxidant enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase, and catalase and production of these enzymes show the extent of oxidative stress (Zhou et al., 2007). This is

consistent with our results about Tajan cultivar (16 hours after spraying).

Catalase is a common enzyme found in nearly all living organisms in the presence of oxygen. One molecule of catalase can change millions of hydrogen peroxide molecules to water and oxygen per second (Chelikani et al., 2004). Catalase and peroxidase enzymes are responsible for removing excess reactive oxygen species and involving in cell regulation which, in this field, the role of catalase is more prominent. The role of catalase has been demonstrated in the defensive system and aging in plants (Mura et al., 2007). Since catalase helps to maintain homeostasis of reactive oxygen in biotic and abiotic stresses, its activity increases in stress conditions (Magbanua et al., 2007). Imbalance in produced and induced H_2O_2 leads to accumulation of large amounts of H_2O_2 when treated with cadmium, and presence of enzymes such as catalase and peroxidase prevents H_2O_2 accumulation and thus increases stress tolerance in plants (Zhang et al., 2010). In the present study, catalase activity was significantly increased in cadmium-treated cultivars, which probably shows the destruction of toxic hydrogen peroxide (H_2O_2) when accumulation of cadmium are done by catalase and it can cause, in turn, reduction in lipid peroxidation by free radicals during cadmium toxicity.

Pourakbar and Ashrafi (2011) showed that cadmium increases the activity of catalase, ascorbate peroxidase, lipid peroxidation, hydrogen peroxide accumulation, and cell death in corn. Increased catalase activity in Gonbad and Tajan cultivars (16 hours after spraying) is consistent with the results of aforementioned research. With increasing catalase activity, the excess hydrogen peroxide in cells disappears and to cope with accumulation of free oxygen radicals, the plant increases the activity of this enzyme.

Cadmium causes reduction in seed germination and seedling growth, activity inhibition of some enzymes, sedimentation of essential nutrients or metabolites, and cell destruction (Ghosh et al., 2005). Sandalio et al. (2001) in their study showed that H_2O_2 induced by accumulation of cadmium in pea leaves results in activity inhibition of catalase and peroxidase. Decrease in catalase activity has been reported as a general response in many environmental stresses such as severe salinity, drought, cold, and heavy metal stress, which is probably due to inhibition of enzyme synthesis or changes in assembly of enzyme subunits (Shah et al., 2001). This can be a reason for reduction in catalase activity under cadmium chloride treatment in Gonbad and Tajan cultivars (8 hours after spraying). Hameed et al. (2011) showed that cadmium chloride and mercuric chloride caused decreased catalase activity in okra plant that is presumably due to enzyme synthesis inhibition, consistent with the results of the present study. Noorani Azad et al. (2010) showed that mercuric chloride increases the activity of catalase and ascorbate peroxidase in dill leaves. Increased antioxidant activity in leaves and activation of antioxidant defense can be one of the mechanisms to tolerate toxicity of mercury, which is consistent with the present study on Tajan cultivar (16 hours after spraying). The amount of cadmium uptake by plant and its concentration in plants depend on environmental and physiological conditions as well as biochemical factors. Plants, to cope with damages caused by oxidative stress and free radical production and in particular antioxidant hydrogen peroxide, apply complex antioxidant system whose elementary components include carotenoids, ascorbate, glutathione, and tocopherole. Antioxidant enzymes include superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione peroxidase, and enzymes involved in ascorbate-glutathione cycle such as glutathione reductase (Baby and Gini, 2011).

It seems that peroxidase acts generally as an enzyme to detoxify reactive oxygen species, since hydrogen peroxide is a substance that acts as an electron acceptor for a wide range of peroxidase-dependent reactions. Meanwhile, peroxidase breaks H_2O_2 through several different mechanisms (Kawano, 2003). Therefore, it can be concluded that spraying of heavy metals results in high activity of peroxidase and breaks down hydrogen peroxide in cells and thus prevents production of reactive oxygen species. Thus, plants are less invaded by reactive oxygen species with increasing activity levels of this enzyme, primarily because catalase and peroxidase are recognized as the main enzymes involving in

removing H_2O_2 (Tewari et al., 2005). Peroxidase plays a very important role in response to various stresses. It is responsible for removing excess hydrogen peroxide and is among proteins induced by host plant as a defense mechanism against stress. Peroxidase belongs to a large family of multigenes and is involved in a wide range of physiological processes such as lignin and suberin formation, phytoalexins synthesis, and metabolism of reactive oxygen species (Almagro et al., 2009). Superoxide dismutase is the first enzyme involved in detoxification and causes the conversion of O_2^- to hydrogen peroxide, and thus the accumulation of hydrogen peroxide by catalase and peroxidase, and finally reduction in the amount of free radicals in cell organelles. This enzyme also causes the conversion of hydrogen peroxide into oxygen and water (Zhang et al., 2009). Poly phenol oxidase, found in higher plants and recognized as catecholase and tyrosinase, catalyzes two reactions in the presence of oxygen, first hydroxylation of mono phenol compounds into quinine compounds, and second and the main role is catalyzing a kind of quinone from phenols in the presence of molecular oxygen. Among the main roles of this enzyme, is to affect formation of adventitious roots and organization of root development (Ylimaz et al., 2003).

Conclusion

Altogether, study of total protein and soluble sugars concentrations showed that plants in order to cope with all different kinds of stresses use all their energy to synthesize factors involved in defense mechanisms, so that when treated with cadmium chloride and mercuric chloride, total protein and soluble sugar contents, which play key roles in plant defense mechanisms increase. Also, the data obtained on the activity of antioxidant enzymes of catalase, polyphenol oxidase, and peroxidase showed that activity of these enzymes decreased with increasing concentrations of mercuric chloride and in contrast, the activity of these enzymes increased with increasing concentrations of cadmium chloride.

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