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Araştırma Makalesi/*Research Article (Original Paper)*

Effect of Cadmium on Growth and Some Parameters of Oxidative Stress in Seedling Stage of Two Durum Wheat (*Triticum durum*) Genotypes

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Abstract: Seedlings of two durum wheat (*Triticum durum* L.) genotypes, sensitive and tolerant to cadmium, were treated at 3 to 4 leaves stage. The enzymic activities of catalase glutathione s-transferase (GST) and guaiacol peroxidase (GPX) in the case of sensitive genotype significantly increased compared to the control. The activity level of catalase (CAT) and ascorbate peroxidase (APX), however, did not show a significant change. Moreover, the enzymic activity of GPX, GST, CAT and also APX, in the case of cadmium utilization had not a significant change compared to the control. It is argued that the sum of activities of hydrogen peroxide (H_2O_2)-scavenging enzymes helped control of the amount of the pernicious compound (H_2O_2) at tolerant genotype. Cadmium stress led to the significant increase of malondialdehyde (MDA) in both durum wheat genotypes, although membrane damage and lipid peroxidation were more severe with regard to sensitive genotype. It follows that the augmentation of H_2O_2 and the drop in GST activity substantiate further increase in the amount of MDA in sensitive genotype. It is concluded that the activity of H_2O_2 scavenging enzymes and that of GST enzyme have a crucial role in detoxifying toxic compounds leading to more resistance against salt stress.

Keywords: Antioxidant enzymes, Cadmium, Defense mechanisms, Dry weight, Hydrogen peroxide

Kadmiyumun Fide Aşamasındaki İki Makarnalık Buğday (*Triticum durum*) Genotipinin Büyüme ve Bazı Oksidatif Stres Parametrelerine Etkisi

Özet: Kadmiyuma duyarlı ve tolerant iki makarnalık buğday (*Triticum durum* L.) genotipinin fideleri 3 ila 4 yapraklı aşamada uygulmaya maruz bırakılmıştır. Duyarlı genotipte katalaz glutatyon s-transferaz (GST) ve guaiacol peroksidaz (GPX) enzim aktiviteleri kontrol grubuna göre anlamlı olarak artmıştır. Bununla birlikte, katalaz (CAT) ve askorbat peroksidaz (APX) aktivite seviyelerinde önemli bir değişiklik gözlenmemiştir. Ek olarak, kadmiyum kullanımı durumunda, GPX, GST, CAT ve ayrıca APX'in enzimatik aktivitesi, kontrol ile karşılaştırıldığında önemli bir değişiklik göstermemiştir. Hidrojen peroksit (H_2O_2) temizleyici enzimlerinin toplam aktivitelerinin tolerant genotipte zararlı bileşim (H_2O_2) miktarının kontrol edilmesine yardımcı olduğu ileri sürülmektedir. Kadmiyum stresi, her iki buğday genotipinde malondialdehit (MDA) 'nın belirgin bir şekilde artmasına yol açmış; buna karşın membran hasarı ve lipit peroksidasyonu hassas genotip açısından daha şiddetli bulunmuştur. H_2O_2 temizleyici enzimlerinin artması ve GST aktivitesindeki düşüş, hassas genotipteki MDA miktarında daha fazla artışa yol açmıştır. H_2O_2 temizleyici enzimlerin ve toksik bileşiklerin detoksifikasyonunda önemli bir rolü olan GST enziminin aktivitesinin, tuz stresine karşı daha fazla dayanıklılık sağladığı sonucuna varılmıştır.

Anahtar kelimeler: Antioksidan enzimler, Kadmiyum, Savunma mekanizmaları, Kuru ağırlık, Hidrojen peroksit

Introduction

Cadmium as a heavy metal is one of important pollutant factors and very toxic for animals and plants. This element is increasing in fields by factory sewages (Soltani vd. 2006), irregular usage of insecticides and high application chemical fertilizers (Hafezi vd. 2009).

Cadmium is a nonessential element for plants and it had no known biological role (Soltani vd. 2006; Balsberg 1989), but because of high mobility of this element in soil, easily absorbed by plant roots and transferred into aerial organs (Lozano-Rodriquez vd. 1997). Accumulation this element in plants, causes morphological changes such as leaf area and dry mass loss (Oloumi and Manouchehri-Kalantari, 2003). Also cadmium causes changes in physiological and biochemical such as photosynthesis and respiration processes (Goncalvez vd. 2007; Saremi Rad vd. 2014). Subsequently, minus effects of cadmium on intracellular processes, active oxygen production increases in plant cells (Ammar vd. 2008). Reactive oxygen species have high affinity in reaction with bio molecules such as lipids, proteins and nucleic acids. Consequences of this phenomenon are lipid peroxidation, protein denaturation and mutation in DNA structure (Smeets vd. 2005; Esfandiari and Vahdati Rad 2012; Esfandiari and Javadi 2014). These bio molecules have key roles in cells. For example, lipids participate in formation of membrane structure as one of key points in metabolism regulation (Saremi Rad vd. 2014; Esfandiari and Mahboob 2014a). By oxidation of membrane lipids disrupted in permeability of membrane and produced toxic metabolite such as 4-hydroxynonenal. This metabolite is toxic for cell even at low amounts (10-20 μM) and activates other fatal metabolic pathways (Schneider vd. 2008). For example, increases endonuclease activity and releases cytochrome c-oxidase (Zarkovic 2003).

Plant cells confront negative effects of active oxygen and metabolic disorders through glutathione ascorbate, Mehler and xanthophyll cycles. This cycles are established with cooperation of antioxidant enzymes such as ascorbate peroxidase (Edreva, 2005; Martins vd. 2011).

In addition to the mentioned mechanisms, glutathione s-transferase is able to transfer extracellular toxic combinations such as cadmium and toxic metabolites such as 4-hydroxynonenal into vacuoles and decrease negative effects of them (Schneider vd. 2008). As mentioned before, more cadmium accumulation occurs in field soils than other heavy elements (Merrikhpour 2015) due to the fact that high application of phosphate fertilizers and its impurity with heavy metals. The high amounts of cadmium in field soils could decrease growth and development of durum wheat such other environmental stress and threaten human food security.

Due to the importance of wheat in human nutrition, knowledge from patterns of absorption and distribution in different organs of sensitive and tolerant genotypes and effect of high amounts of cadmium on growth and development of durum wheat is necessary. Thus, sensitive and tolerant genotypes of durum wheat genotypes were planted with aeroponics method and absorption and distribution patterns of cadmium were studied at high concentration level of cadmium and their effects on growth and development.

Cadmium (Cd) is a heavy metal with no known essential biological functions in higher plants, animals and humans. Like other heavy metals, cadmium becomes toxic at elevated concentrations. Toxicity levels depend on the organism, physiological conditions and environmental factors. The joint FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) expert committee on food additives (JECFA) has established a provisional tolerable level for cadmium at 25 μg cadmium intake per kg body weight per month (WHO 2015).

Cadmium is naturally released to the environment either due to volcanic activities on land or under the sea, weathering, erosion and river transport. More important than the natural sources of cadmium are the releases due to human activities. Such activities include mining, smoking, smelting and refining of non-ferrous metals, fossil fuel combustion, incineration of municipal waste, manufacture of phosphate containing fertilizers and the recycling of cadmium containing material. The remobilization of historic sources of cadmium such as the contamination of watercourses by drainage water from metal mines is also an important cadmium source. High levels of cadmium soil pollution are limited to areas with specific input histories such as mining or smelting. But low to medium levels of cadmium pollution in soil are a wide spread issue on agricultural soils. This pollution is mainly caused by the application of

cadmium containing phosphate and other fertilizers as well as low quality bio waste and by periurban atmospheric deposition. At these levels cadmium is not toxic to plants and does not hinder plant growth or soil fertility but may potentially transfer into humans with food or water. So in many countries, including Switzerland, cadmium is the most important metal pollutant in agricultural soil because of its toxicity risks for humans and its widespread distribution (WHO 2015).

Materials and Methods

The two genotypes of durum wheat, sensitive and tolerant to cadmium respectively, were selected from among 17 genotypes of durum wheat under study (Pourmohammad, unpublished data). Homogeneous seeds of these two genotypes were sterilized with 0.1 % SDS by stirring them for 20 minutes. Then the seeds were washed out several times with deionized water. The sterilized seeds germinated in 25 °C and dark conditions on filter paper for two days. Seedlings were planted as aeroponics method and experiment carried out by using factorial based completely randomized design with three replications in growth chamber and were grown inside the growth chamber in conditions of 16 hours of light, 8 hours of darkness, 25 °C, relative moisture of 65% and light intensity 6000 Lux. The source of light inside the growth chamber was a combination of yellow and white florescent lamps (Esfandiari vd. 2011). Seven and fourteen days after the germination of seeds, the tap water was replaced with one-half and full-strength Hoagland's solution (Hoagland and Arnon 1950), respectively. The composition of the nutrient solution was: 1 mM calcium nitrate [$Ca(NO_3)_2$, $4H_2O$]; 0.1 mM monopotassium phosphate (KH_2PO_4); 0.5 mM potassium sulfate (K_2SO_4); 0.5 mM magnesium sulfate ($MgSO_4$) and 10 µM boric acid (H_3BO_3); 20 µM manganese chloride ($MnCl_2$, $4H_2O$); 0.5 µM zinc sulfate ($ZnSO_4$, $7H_2O$); 1 µM copper sulfate ($CuSO_4$, $5H_2O$); 0.1 µM molybdenum trioxide (MoO_3), and 100 µM iron sulfate ($FeSO_4$, $7H_2O$). These solutions were continuously aerated by electrical pumps (Resun, AC 9904, China) and renewed every three days.

The pH of the nutrient solution was measured by a pH-EC meter (HANNA, HI9811, Hanna Instruments, Padova, Italy) and adjusted to 5.5 by adding 1 N sulfuric acid (H_2SO_4).

Once the seedling grew up to 4 to 5-leaf stage, salt stress was applied by using 200 mM NaCl. They were kept in stress conditions for 14 days. Then samples from mature and well-developed leaves were obtained and soaked in liquid nitrogen immediately. The samples were preserved in -20°C until the time for measuring physiological parameters.

Enzyme extraction

For CAT and GPX extraction, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay (Esfandiari vd. 2011).

For APX extraction, leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, 2mM ascorbate (AsA) and 5% polyvinylpyrrolidin (PVP) with pre-chilled pestle and mortar. The other stages were similar to the extraction of other enzymes (Esfandiari *vd.* 2011).

Enzyme activity assay

CAT activity was measured according to Aebi (1984). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 75 mM H_2O_2, enzyme extract and distilled water. Reaction started by adding H_2O_2, and the decrease in absorbance was recorded at 240 nm (ε= 36 $mM^{-1}cm^{-1}$) for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

APX activity was measured according to Yoshimura *vd.* (2000) by monitoring the rate of ascorbate oxidation at 290 nm (ε=2.8 $mM^{-1}cm^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH 7), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM reduced ascorbate (AsA) and the enzyme sample. No change in absorption was found in the absence of AsA in the test medium.

GPX activity was measured according to Panda vd. (2003). Reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, 5mM guaiacol, 15 mM H_2O_2 and enzyme sample. The enzyme produced a colorful product by using H_2O_2 and guaiacol as substrates. The absorbance of the product was monitored at 470 nm ((ε= 26.6 $mM^{-1}cm^{-1}$), and peroxidase activity was expressed as units/mg protein. min.

Extraction and activity measurement of GST

For GST extraction, leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 6.8) containing 0.4 mM EDTA, 0.5% (W/V) polyvinylpyrrolidin (PVP) and 1mM sodium metabisulfite with prechilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 21000×g. The supernatant was used for enzyme activity assay.

GST activity was determined with 1-chloro-2, 4-dinitrobenzene (CDNB) by a modified method of Carmagnol vd. (1981). The product of CDNB conjugation with reduced glutathione (GSH)-S-conjugation absorbed at wave length of 340 nm (ε= 9.6 mM-1cm-1). The reaction solution contained 100 mM potassium phosphate buffer (pH 6.25), 0.75 mM CDNB, 30 mM GSH and the enzyme extract (100µL). Enzyme activity was expressed as units/mg protein. min.

MDA and H_2O_2 determination

MDA was measured by colorimetric method. 0.5 g of leaf samples were homogenized in 5 ml of distilled water. An equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in an ice bath. The samples were then centrifuged at 10000×g for 30 min. The supernatant was removed, absorption read at 532 nm, and the amount of nonspecific absorption at 600 nm read and subtracted from this value. The amount of MDA present was calculated from the extinction coefficient (ε= 155 mM^{-1} cm^{-1}) (Stewart and Bewley 1980).

Hydrogen peroxide levels were determined according to Sergive vd. (1997). Leaf tissues (0.5g) were homogenized in ice bath with 5 ml 0.1% (w/v) TCA. The homogenate was centrifuged at 12000×g for 15 min and 0.5 ml of the supernatant was added to 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI. The absorbance of supernatant was read at 390 nm. The content of H_2O_2 was given on standard curve.

Statistical analysis

Enzyme activity, MDA and H_2O_2 content of samples were recorded with three replications. The data were analyzed with GenStat12 program and the means were compared through LSD method.

Data analysis carried out as factorial experiment based on completely randomized design with three replications. Before ANOVA, normality test did by Kolmogorov Smirnov (Steel and Torrie, 1980) for studied traits.

Results and Discussion

ANOVA of studied traits and parameters was shown in Table 1. In this study, seedling shoot dry weight in the case of tolerant genotype was not affected by cadmium. But in the case of sensitive genotype this amount is significantly decreased compared to the control parameters (Table 2). Dry weight of different parts of wheat seedlings, stabilization and reduction of carbon dioxide into carbohydrates happens during photosynthesis and converting carbohydrate to other biomolecules manufacturer structure of plants (Esfandiari and Mahboob 2014b). Therefore, any factor that could affect photosynthesis and biosynthesis of other biomolecules, causes growth reduction and development and production of dry matter. Cadmium is an element that might accumulate in the leaf, stomata closure and finally reduced stomatal conductance (Saremi Rad vd. 2014; Krantev vd. 2008; Wahid vd. 2008; Popova vd. 2008). The decrease in stomatal rate access reduces conductance to carbon dioxide in the photosynthetic cells, which is the outcome of the lack of metabolites required for the growth of plant cells. In addition, high levels of cadmium impair the

respiration process (Saremi Rad vd. 2014), energy and carbon skeletons production decreases for other cell biomolecules. Also, this element prevents the stabilization and reduction of nitrogen in the plant cells that, as a result, the conversion of α-steny to the amino acids, are limited (Esfandiari and Mahboob, 2014b). Therefore, cadmium by disrupting the above processes, prevented the adequate and timely supply of critical biomolecules of cell that the outcome of the seedling shoots dry weight loss in the case of tolerant genotype. In addition to the above mentioned items, cadmium by nutritional disorders disturbs the water balance of plant and then it may cause dry weight loss (Soltani vd. 2006). In this regard, Yang vd. (1996), reported decreased calcium absorption due to increased levels of cadmium in the environment can reduce the plant growth and dry matter production will be sought.

On the other hand, cadmium causes some changes in the structure of organelles inside the cell. In this regard Minoui vd. (2008) in a study of *Chlorophytum comosum* reported that the number of chloroplasts and its tilaclouid in amounts of 100 mg/liter of cadmium, decreased compared with the control. While no significant change happens in the structure of other organelles. But higher than this amount, the organelles structure become abnormal and changes which suggested that high levels of cadmium can cause decreased production of dry matter to be considered. Shoot dry weight loss at the high levels of cadmium reported by some researchers (Hafezi vd. 2009; Oloumi and Manouchehri-Kalantari 2003; Yang vd. 1996; Saremirad vd. 2014).

In each of two studied durum wheat genotypes, accumulated cadmium in leaves was significantly increased compared with control (Table 4). Although in the referred genotypes to the amount of cadmium in leaves there was a significant difference, the accumulated cadmium in the its leaves increased 148 and 23 percent in compared with the control, respectively (Table 4). Cadmium is an element that have high mobility in soil and easily absorbed by the roots of plants such as wheat and the accumulation in these organ can also be transmitted to the leaves. In this study, the accumulation of the element in the root and shoot of studied genotypes were significantly increased compared with control (Table 1).

Table 1- Analysis of Variance for Studied Traits and Parameters

Source	df	Mean Square								
		Leaf Dry Weight	Cadmium	H_2O_2	CAT	APX	GPX	GST	MDA	Shoot dry weight
Genotype	1	0.135**	0.746**	0.015*	1.021E-008^{ns}	6.984E-005^{ns}	0.655**	536066.471**	26.866^{ns}	0.015**
Cadmium	1	0.081**	1.507**	0.001^{ns}	6.674E-007*	0.001**	0.127*	4823334.486**	1684.818**	0.012**
Genotype*Cadmium	1	0.106**	0.482**	6.078E-006^{ns}	1.344E-007^{ns}	2.434E-005^{ns}	0.035^{ns}	321903.717**	767.940**	0.007**
Error	8	2.750E-006	0.001	0.003	8.071E-008	2.854E-005	0.015	25634.588	35.841	0.000

* P <0.05; ** P<0.01

Table 2. The comparison mean of treatments in the experiment

Treatment	Leaf Dry Weight	Cadmium	H_2O_2	CAT	APX	GPX	GST	MDA	Shoot dry weight	MDA
Tolerant- Control	0.5020a	0.2075d	0.2165a	0.0008a	0.0185a	0.1277a	1689.8311a	42.2308a	0.1677a	2.5332c
Tolerant- 250µg	0.1497b	0.5155b	0.2316a	0.0001a	0.0011a	0.0303a	94.2805c	2.5332c	0.0567b	29.2239b
Sensitive-Control	0.1017d	0.3052c	0.2887a	0.0005a	0.0205a	0.7034a	1784.9785a	29.2239b	0.0490b	21.5251b
Sensitive-250µg	0.1257c	1.4149a	0.3010a	0.0003a	0.0088a	0.3891a	844.5652b	21.5251b	0.0343b	42.2308a
Total	0.2198	0.6108	0.2594	0.0004	0.0122	0.3126	1103.4138	23.8782	0.0769	

Differences between means of each column with common characters are not significant at probability level of 5%

Table 3- The cadmium effect on studied traits and parameters

cadmium level	leaf dry weight	cd	h_2o_2	cat	apx	gpx	gst	mda	shoot dry weight
control	0.301833a	0.256383b	0.252606a	0.000665a	0.019468a	0.415563a	1737.404811a	12.029126b	0.1083a
250µm	0.137666b	0.965233a	0.266280a	0.000193b	0.004973b	0.209675b	469.422886b	35.727353a	0.0455b
total	0.2197500	0.6108083	0.2594433	0.0004292	0.0122208	0.3126192	1103.4138492	23.8782400	0.0769

Differences between means of each column with common characters are not significant at probability level of 5%

Cadmium is toxic to plant cells and accordingly plants are attempting to use strategies such as connecting it to the cell wall (Gong vd. 2003), storage in the vacuole (Lozano-Rodriquez vd. 1997) and the chelated by fitoclatin (Gong vd. 2003) stabilize this element in the root and by reducing the toxicity of this

element, causes the more accumulation of cadmium in the roots and low transfer it to aerial parts (El-Beltaghi *vd.* 2010; Kalantari and Oloumi 2005).

Table 4- The genotype effect on studied traits and parameters

Genotype	Leaf Dry Weight	H_2O_2	CAT	APX	GPX	GST	MDA	Shoot dry weight
Tolerant	0.32583a	0.22402b	0.00046a	0.00981a	0.07898a	892.05585b	22.38198a	0.11217a
Sensitive	0.11367b	0.29487a	0.00040a	0.01463a	0.54626b	1314.77185a	25.37450a	0.04167b
Total	0.21975	0.25944	0.00043	0.01222	0.31262	1103.41385	23.87824	0.07692

Differences between means of each column with common characters are not significant at probability level of 5%

Due to the significant increase of cadmium in leaves compared with control, it can be said that the mechanisms used in root of durum wheat genotypes to prevent the transfer of cadmium to shoots has not been effective. Accumulation of cadmium in all parts of plants such as leaves, with higher levels of this element in the root zone by Lozano-Rodriquez vd. (1997), Liu vd. (2006), Izadiyar and Yargholi (2010) and Saremi Rad vd. (2014) has been reported.

The results showed that the presence of cadmium in the environment, hydrogen peroxide as one of the most toxic and harmful compounds to vital biomolecules, only in leaf cells sensitive genotype significantly increased in compared to control (Table 4) and 31 percent was for control one. Also, lipid peroxidation following the addition of cadmium into the environment rises only in the leaf of genotype - and reaches to - percent of controls.

In this study, enzymic activities of catalase, ascorbate peroxidase and guaiacol peroxidase as the most important enzymes involved in collecting hydrogen peroxide were evaluated. The results showed that in the presence of cadmium in the environment, catalase activity in leaves of sensitive genotype was significantly decreased at the 5% and reached – percent of control. But catalase activity did not change significantly in the leaf tolerant genotype (Table 3).

Guaiacol peroxidase enzymic activity showed no significant change in presence of cadmium in leaves of durum wheat genotypes compared to the control. But the enzyme activity decreases in the presence of cadmium in sensitive genotype compared with the control. In addition, the results showed that, ascorbate peroxidase activity was significantly decreased in 250 µM cadmium (Table 3). Also, glutathione s transferase activity in the presence of cadmium in leaves of durum wheat genotypes were significantly decreased compared to control (Table 1).

Due to shoot dry weight loss the in presence of cadmium (Table 1) and the accumulation of this element in the leaves of sensitive genotype as well as the negative effects of cadmium on critical processes, such as lack of consumer optical products of photosynthesis in the Calvin cycle in terms of reduced stomatal conductance (Saremi Rad vd. 2014s), it was expected that the production of reactive oxygen species in the cells of the leaves had a rising trend and in both genotypes studied, the rate of hydrogen peroxide and lipid peroxidation increased in comparison with controls and oxidative stress occurs. Increased production of reactive oxygen species and oxidative stress in high levels of cadmium have also been reported by many researchers (Oloumi and Manouchehri-Kalantari, 2003; Cho and Seo, 2005; Kalantari and Oloumi, 2005; Unyayar vd. 2006; Martins vd. 2011; Shan vd. 2012).

In this study, reduction in dry matter production in the presence of cadmium in sensitive genotype represents degeneration Calvin cycle or other metabolic pathways that convert carbohydrates from Calvin cycle drawn to other biomolecules, which can reduce the dose of cadmium dry matter, indicating the sensitivity of this durum wheat genotypes under study. By reducing the activity of Calvin cycle by blockage of chloroplast electron transport chain, increased production of reactive oxygen species in the chloroplast (Edreva 2005). While on the contrary, the results showed that the presence of cadmium in the environment, hydrogen peroxide as one of the forms of reactive oxygen species and one of the most toxic compounds harmful to vital biomolecules, only in leaf cells of sensitive genotype increased compared to control (Table 4). Also, lipid peroxidation after adding cadmium to the environment leaves only sensitive genotype significantly increased compared to control (Table 1).

In the case of sensitive genotype, reduced activity of catalase, guaiacol peroxidase and ascorbate peroxidase (Table 1) in the presence of cadmium, the reasons for the increased production of hydrogen peroxide, can be considered. Also, in this genotype, glutathione s transferase in the presence of cadmium was reduced compared to control and among the genotypes studied, the highest concentration of cadmium was observed.

In the case of sensitive genotype, reduction on the activity of peroxidase, guaiacol peroxidase, catalase and glutathione s transferase with reduced enzymic activity (Table 1) can cause an increase in lipid peroxidation in this genotype to be considered, because the ascorbate peroxidase in cycles of glutathione-ascorbate and Mehler as the final enzyme plays an important role in collecting hydrogen peroxide (Mittler 2002; Edreva 2005). It should be noted that, contrary to Mehler cycle that runs only in chloroplasts, glutathione-ascorbate cycle runs in other organelles such as mitochondria and cytosol (Edreva 2005). Increased activity of the mentioned cycles can prevent the accumulation of hydrogen peroxide and lipid peroxidation (Edreva 2005). Also, glutathione s-transferase is capable to move cadmium and toxic metabolite 4-Hydroxynonenal into the vacuole and to minimize toxicity (Schneider vd. 2008) after which the negative effects on the key points of metabolism will be the lowest. Cho and Seo (2005), Unyayar vd. (2006) and Shan vd. (2012) argue that the production of reactive oxygen species such as hydrogen peroxide effect of cadmium on the mechanisms, they have overcome aggregator and increased damage to the membranes and oxidative stress.

In the case of sensitive genotype, cadmium accumulation in leaves was significantly increased compared with control. Contrary to expectations this could not reduce durum wheat seedling shoot dry weight compared with the control (Table 1). In this genotype, ascorbate peroxidase and guaiacol peroxidase activity decreased, but the activity of glutathione s-transferase compared to sensitive genotype was higher and was able to leaf cadmium accumulated in the vacuoles. This change was the result of the accumulation of hydrogen peroxide and lipid peroxidation that does not happen compared to the control.

It seems that the glutathione s-transferase was able to detoxify cadmium prevents damage to key sectors such as membranes of cells, yielding a reduction in the volume of hydrogen peroxide and lipid peroxidation (Table 2). In other words, because of the ability of these genotypes in isolation and maintenance of cadmium in areas such as the vacuole, despite reduced activity of the Calvin cycle and the drop in production of dry matter, due to increased production of reactive oxygen species is not necessary to increase the activity of antioxidant enzymes. Cho and Seo (2005) and Zhenng vd. (2010) also mentioned that increased antioxidant activity and lack of accumulation of hydrogen peroxide as their major cause of resistance to cadmium. Martins vd. (2011) also reported increased activity of the guaiacol peroxidase by adding cadmium to the environment in leaf tobacco, have stated that this enzyme plays an important role in controlling and collecting hydrogen peroxide and copes with oxidative stress due to high levels of cadmium.

Increased activity of the glutathione s-transferase and collecting enzymes of hydrogen peroxide, by the effect of adding cadmium to the environment by El-Beltaghi vd. (2010) have also been reported and they believe that the high activity of these enzymes reduces the negative effects of cadmium and decreased lipid peroxidation and oxidative stress. Thus, plant cells are in stable condition and maintained their metabolic activity (El-Beltaghi vd. 2010).

Conclusion

I can be generally concluded that although cadmium is toxic for the cell leaves, cadmium tolerant wheat genotypes can accumulate this toxic element in sectors with glutathione s transferase. As a consequence of this event key parts of cells such as membranes are prevented, so that reason this type of genotype will be resistance to high concentrations of cadmium.

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