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GENOTYPIC EFFECTS, OXIDATIVE STRESS AND TOLERANCE MECHANISMS INDUCED BY CADMIUM IN TWO *Lactuca sativa* CULTIVARS.

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ABSTRACT

A hydroponic experiment was carried out to investigate the effect of cadmium (Cd) on differential expression, oxidative stress and some antioxidant enzyme activities in two *Lactuca sativa* cultivars (Balady, related Romaine Group and Great leaks, related Crisp Group). Cd phytotoxicity was shown by growth retardation of Balady and Great leaks. Results showed that Great leaks showed more sensitivity to Cd toxicity than Balady cultivar. Increasing Cd supply markedly reduced the total chlorophyll, dry matter of both *Lactuca* cultivars and these decreases were more marked in great leaks. Increased Cd accumulation in various plant parts in both the *Lactuca* cultivars was observed as Cd concentration increased. Cd accumulated in the roots was much higher than in the shoot in the two cultivars, but more observed in the case of Balady cultivar. Balady cultivar had less uptake of Cd by shoot about two times than Great leaks shoot cultivar indicating that there are co-ordination of gene expression, regulation ion transport process operating in different root layer more efficient in Balady cultivar compared with Great leaks. Moreover, the induction of expression and activities of antioxidant enzymes and non protein thiol (NPT) increased in Balady cultivar more than Great leaks leading to H_2O_2 burst, lipid peroxidation, and growth inhibition. These gene expression and activities of antioxidant enzymes confer Balady cultivar some measure of Cd tolerance and presence of strong Cd-binding proteins in the roots. In conclusion, these results may be regarded as an indication of better tolerance mechanism of Balady cultivarmore than that of Great leaks to Cd contamination.

Keywords*:* *Lactuca sativa*, Cadmium Toxicity, Oxidative Stress, Biomarkers, Phytotoxicity.

INTRODUCTION

Contamination of soil and water by toxic heavy metals constitutes a major environmental hazard to human health. Cadmium (Cd), classified as a human carcinogen (Waisberg *et al.*, 2003), is released into the environment by anthropogenic activities such as mining, smelting, fuel composition, disposal of industrial effluents and sewage sludge as well as application of phosphate fertilizers (Clemens, 2006). It is a non-essential metal for the plants and humans, enters crops through roots, accumulates in plants and affects human health (Wagner, 1993). Plants exposed to Cd showed

reductions in photosynthesis, water and nutrient uptake (Sanità di Toppi and Gabbrielli, 1999). As a consequence, Cd-exposed plants showed various symptoms of injury such as chlorosis, accompanied by a lowering of photosynthetic rate growth inhibition, browning of root tips (Das *et al.*, 1997), disturbs cell proliferation (Rosas *et al.*, 1984), impedes respiration (Lee *et al.*, 1976), reduces mitochondrial electron transport (Miller *et al.*, 1973), induces high vacuolization in cytoplasm and nuclei, and increases disintegration of organelles (Liu and Kottke, 2003). With increased Cd dose in nutrient culture up to 10 mg L^{-1} causes yield reduction at 75 % for bean, 65 % for sugar beet, 60 % for turnip and 40 % for corn (Haktanır and Arcak, 1978). Tolerance indexes of tomato and corn plants changed in the range of 79.2-7.8 and 68.6-18 in response to (0.05-20 µg mL^{-1} Cd), respectively **(**Yildiz, 2005). Cd toxicity has been found to interfere with electron transport chains or block antioxidant enzymes structures, leading to accumulation of H_2O_2, and oxidative damage, membrane leakage and finally cell death (Schutzendubel *et al.*, 2002). Accumulation of Cd in plant cell generally results in functional alteration of the physiological pathways (Sanità di Toppi and Gabbrielli, 1999).

Approximately percent of the water that a plant absorbs from the soil is by evaporation from the leaves. Most transpiration occurs through the stomata, the numerous stomatal pores that are so effective in gas exchange for the photosynthesis also provide opening through which water vapor escapes living minerals required for the plant growth (Haktanir and Arcak, 1978). Harmful effects produced by Cd might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Fuhrer, 1982). In other words, toxicity of Cd may result from its binding to sulfydryl groups of proteins leading to inhibition of activity or disruption of structure, disturbance of cellular redox control (Schutzendubel *et al.*, 2002), and/or inducing the production of reactive oxygen species (Romero-Puertas *et al.*, 2004). Therefore, the present study aimed to evaluating the impact of different Cd concentration on lipid peroxidation and activity of some antioxidant enzymes, some nutrients uptake, cytotoxicity, phytotoxicity in addition to accumulation rate in *Lactuca sativa* cultivars. For this purpose, it is important to understand the mechanisms of Cd toxicity and tolerance mechanism in plants.

MATERIALS AND METHODS

Plant materials and experimental design

Hydroponic experiment was used to study phytotoxicity oxidative stress and differential expression and activities of antioxidant enzyme and concentration of cadmium (Cd) in different plant parts in two *Lactuca sativa* cultivar (Balady related Romaine Group and Great leaks, related Crisp Group). The experiment was carried out at the Faculty of Agriculture, Al-Azhar University, Cairo, Egypt during two seasons. The two *Lactuca sativa* cultivar were germinated in sand culture for 2 weeks. Then the plants were transferred to containers (7 liters per pot) having nutrient solution (stable water culture technique). The plants grown in Murashige and Skoog basal medium nutrient solution modified (containing: 1/4 MS) was used as growth

medium. After one week in the standard nutrient solution and adjusted pH to 6.0 .The nutrient solution was renewed twice a week and aerated continuously. The pots were randomly arranged several times during the growth period. Plants were grown under controlled climatic conditions and subjected to increasing Cd supply in the form of $CdCl_2$ (0, 16, 24, and 32 µmol/l)**.** After 10 days of treatment, the plants were harvested, washed twice with distilled water and divided into shoots and roots, then representative portions were taken for wet digested using a mixture of $HClO_4$ and H_2SO_4 at a rate of 1:1 to detriment some nutrients composition (Ca, Fe, Mn, Zn and Cd) by Inductively Coupled Plasma Spectrometer (ICP) plasma 400 (Page *et al.*, 1982).

Chlorophyll Content

Total chlorophyll was estimated according to the spectrophotometric method described by Hipkins and Baker (1986). Approximately 50 mg (fresh mass) of leaves was placed in 3 mL of 100% methanol in 5 mL vials. The vials were covered and incubated at 23 ^{o}C for 2 h in darkness. Each sample was mixed, the methanol fraction decanted, and the absorbance measured at 650 and 665 nm.

Tolerance indexes

The Cd tolerance indexes were measured according to the following equation of Das *et al.*, 1999.

$$\text{Tolerance indexes} = \frac{\text{Growth (dry matter) increase in Cd level}}{\text{Growth (dry matter) in nutrient solution without Cd}} \times 100$$

Tissue preparation for enzymatic antioxidants in Balady and Great leaks roots and shoot:-

Fresh root or shoot samples (0.5 g) were ground in liquid N_2 and homogenized in an ice-bath in 10 mL homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8). The homogenate was centrifuged at 8000 xg at 4 ^{o}C for 15 min and the resulting supernatant was used for enzyme assays.

Glutathione S-transferase (GST) determination in balady and great leaks roots and shoots

Glutathione S-transferase (GST, 2.5.1.18) activity was assayed according to Habig and Jacoby (1981). The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 6.5), 0.1 mM 1-chloro 2,4-dinitrobenzene (CDNB), 10 mM GSH and a suitable aliquot of enzyme extract. The CDNB conjugate formation was followed for 5 min at 340 nm. Specific activity of the enzyme was calculated using the extinction coefficient, 9.6 $mM^{-}1\ cm^{-}1$ and is expressed as units $mg^{-}1$ protein. The protein content in the supernatants was measured according to Lowry *et al.* (1951).

Catalase determination in Balady and Great leaks roots and shoot

Catalase (CAT, EC 1.11.1.6) activity was measured as disappearance of H_2O_2 at 240 nm (Cakmak and Marschner, 1992). A 2 ml of reaction mixture consisted of 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.2 ml of enzyme extract. Activity was calculated using an extinction coefficient of 39.4 $mM^{-1}\ cm^{-1}$ and expressed as enzyme unit g^{-1} FW. One CAT unit was defined as the enzyme amount that decomposes 1µM H_2O_2 min^{-1}.

Determination of MDA concentration in Balady and Great leaks roots and shoot

Root or shoot tissues (500 mg) were homogenized in 3 mL 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 2500 xg for 10 min and the supernatant was assayed for malondialdehyde (MDA) concentration with thiobarbituic acid (TBA) test using the method given by Heath and Packer (1968).

Determination of Hydrogen peroxide concentration (H_2O_2) in Balady and Great leaks roots and shoot

For determination of H_2O_2 concentration, root or shoot tissue (100 mg) was extracted with 5 ml TCA (0.1%, w/v) in an ice bath and centrifuged at 12,000 ×*g* for 15 min (Velikova *et al.*, 2000). An aliquot (0.5 ml) of supernatant was added to 0.5 ml of phosphate buffer (pH 7.0) and 1ml of 1M potassium iodide. The absorbance of the mixture was read at 390 nm. H_2O_2 content was determined using the extinction coefficient 0.28 μM^{-1} cm^{-1} and amount expressed as nmol g^{-1} FW.

Determination of NPT concentration in Balady and Great leaks roots and shoots

The concentration of non-protein thiols (NPT) was determined by measuring the absorbance at 412 nm following the method of Metwally *et al.* (2003). For this, 0.5 g of fresh root or shoot segments were homogenized in an ice-bath in 5mL of potassium phosphate buffer (pH 8.0), and the homogenate was centrifuged at 10,000 xg for 20 min. The supernatant was used for NPT assay using 5, 5-dithio-2, 2-dinitrobenzoic acid as a reagent.

Rapid extraction of proteins

This method is very fast and efficient for simple control of the protein profile on SDS-PAGE denaturing gel. The plant material fresh seedling ground in liquid N2 The products powder is weighed and taken up in an equal volume of 4x loading buffer (500 mM Tris-HCl pH 6.8, 12% SDS (w / v) glycerol 20% (v / v) 40 mM DTT, 20 mM bromophenol blue) The sample is denatured for 10 min at 100 ° C and then centrifuged 5 min at maximum speed (Eppendorf centrifuge 5417 R). The supernatant can then be used for electrophoreses separation.

Statistical analysis

The experiments were arranged in a randomized design. Differences among Cd concentrations and cultivars, as well as interactions between these variables, were tested using the SPSS statistical program. Statistical variance analysis of the data with three replicates was performed using ANOVA and compared with least significant differences (LSD) at the 5% level.

RESULTS

Effect of Cd treatment on chlorophyll content of two lactuca g cultivars.

Effect of Cd concentration on the chlorophyll content during seedlings development of two *lactuca* cultivars after 10 days of treatments was studied was shown in Table (1). Total chlorophyll decreased nearly linearly with increasing Cd in nutrient solution from 16-32 µmol/l. In seedlings treated with

32 µmol/L Cd, total chlorophyll was decreased by 53.02-63.09% in Balady and Great leaks, respectively as compared with control.

Table 1: Effect of cadmium treatment on chlorophyll content of Balady and Great Leaks cultivars.

Treatment	Balady	Great leaks	Percentage decrease in total chlorophyll	
	µg/g.D.W chlorophyll	µg/g.D.W chlorophyll	% Balady	% Great leaks
Control MS	1094	997	100	100
16 µmol	805	681	26.42	31.70
24 µmol	634	509	42.05	48.95
32 µmol	514	368	53.02	63.09

Effect of Cd treatment on dry matter and clerance index of two lactuca cultivars.

The results showed also differential disposition in both *Lactuca sativa* and variable decrease with increasing Cd concentration as compared with control. The highest yield was obtained in control. Dry matter production decreased dramatically with increasing concentrations of Cd (Table 2). However, dry matter of Balady decreased to 61.79 % at 32 µmol Cd applications and reached to 51.08 % in Great leaks at the same Cd levels. The yield reduction in two cultivars, Balady and Great leaks plants with tolerance index of 73.44 % and 70.88 % were approximately 26.56 and 29.12% decreased in dry matter at 16 µmol Cd, respectively. However, yield reduction of Balady and Great leaks were 38.21% and 49.92 at 32 µmol Cd, respectively (Table 2).

Table 2: Effect of cadmium treatment on dry matter and tolerance index of Balady and Great leaks cultivars

Treatment	g/plant D.M Balady	Tolerance index % Balady	g/plant D.M Great leaks	Tolerance index% Great leaks
control MS	0.351	100 %	0.3232	100 %
16 µmol	0.2578	73.44 %	0.2291	70.88 %
24 µmol	0.2517	71.70 %	0.2082	64.41 %
32 µmol	0.2169	61.79 %	0.1651	51.08 %

Effect of cadmium treatment on cadmium uptake and some nutrients content of two Lactuca cultivars.

Cd uptake and accumulation in various plant parts of both *Lactuca* cultivars was tested. Increased Cd accumulation two times in roots was observed with Cd application of 32 µmol/l in medium more than that of shoots (Tables 3 and 4). To furthet explore modifications induced by the Cd concentration on plants uptake of macro- and micronutrients, in the two *lactuca* cultivars. The level of nutrients absorbed by plants is related to the amount of available nutrients in the growth medium. Meanwhile, uptake of nutrients increased for some nutrients or decreased for the others depending on antagonistic or synergistic (interactions) effects among plant nutrients. Calcium content of the Great leaks was decreased in all Cd treatments, but it

increased with Balady cultivar especially in root parts at treatments Cd levels. Manganese content of Balady was not stable and did not show a clear trend and the same results in Great leaks roots, but it decreased in Great leaks shoots with increase the cadmium levels. Zn content of Great leaks was low in all treatments, except the control but with Balady cultivar, it increased in root and shoot parts. Fe content of Great leaks cultivar was low in all treatments, except the control but with Balady cultivar, it increased especially in root parts (Tables 3 and 4).

Table 3: Effect of cadmium treatment on cadmium uptake and some nutrient contents of Balady cultivars.

Treatments mg/l	Ions mg/g dry weight				
	Cd	Ca	Fe	Mn	Zn
Shoot Balady					
control MS	0.0025	9.090	2.549	0.234	0.714
16 µmol	0.3292	26.755	6.733	0.254	1.944
24 µmol	0.281	9.980	2.650	1.575	1.376
32 µmol	0.377	11.662	2.917	0.143	2.973
LSD 0.01	0.076	3.18	1.89	0.29	0.71
Root Balady					
control MS	0.001	5.560	4.013	0.173	0.444
16 µmol	0.556	25.714	12.841	0.185	4.495
24 µmol	1.220	12.983	11.031	0.156	1.274
32 µmol	1.959	14.288	9.777	0.173	2.206
LSD 0.01	0.314	3.46	2.41	0.13	0.93

Table 4: Effect of cadmium treatment on cadmium uptake and some nutrient contents of Great leaks cultivars.

Treatments mg/l	Ions mg/g dry weight				
	Cd	Ca	Fe	Mn	Zn
Shoot Great leaks					
control MS	0.0035	25.366	4.172	0.333	6.144
16 µmol	0.422	6.739	2.976	0.222	1.617
24 µmol	0.539	13.687	2.729	0.239	1.505
32 µmol	0.630	14.670	2.572	0.189	1.376
LSD 0.01	0.12	1.97	0.89	0.067	0.84
Root Great leaks					
control MS	0.0049	69.403	45.872	0.953	5.245
16 µmol	0.888	12.117	8.892	0.182	0.930
24 µmol	2.478	12.382	11.687	0.226	3.194
32 µmol	1.694	11.591	7.975	0.162	0.218
LSD 0.01	0.23	3.25	2.62	0.28	1.47

Lipid peroxidation

Lipid peroxidation in root and shoot tissue was determined by measuring malondialdehyde (MDA), a major thiobarbituric acid reactive species (TBARS) and product of lipid peroxidation. MDA content increased significantly ($p < 0.05$) after treatment with different concentrations of Cd (Table 5). The increase in MDA is more pronounced in Great leaks shoots and roots.

Table 5: MDA concentrations of roots and shoots in Great leaks and Balady cultivars exposed to different Cd concentrations

Cadmium concentrations				
Plant cultivars	**0 µmol**	**16 µmol**	**24 µmol**	**32µmol**
Shoot MDA				
Great leaks	30.51±0.87	36.22±0.55*	38.56±1.58*	42.49±1.09*
Balady	35.57±1.22	39.69±0.96	45.68±1.20*	49.05±1.13*
Root MDA				
Great leaks	38.44±0.73	46.43±1.68*	53.54±1.68*	57.91±1.47*
Balady	42.12±1.03	47.36±1.01	55.60±1.69*	60.09±1.76*

Values are means ± SEM of three different replicates.
MDA, malonaldehyde concentrations, nmole/g fresh weight
***Differences were significant at $p < 0.05$.**

H_2O_2 amount

Parallel to changes in MDA content, there was a significant increase in H_2O_2 amount in Great leaks and Balady shoots and roots after treatment with different concentration of cadmium as compared to untreated control (Table 6). The induction percent of H_2O_2 concentration is approximately the same in both cultivars.

Table 6: H_2O_2 levels of roots and shoots in Great leaks and Balady cultivars exposed to different Cd concentrations.

Cadmium concentrations				
Plant cultivars	**0 µmol**	**16 µmol**	**24 µmol**	**32µmol**
Shoot H_2O_2				
Great leaks	20.42±0.846	23.40±0.883*	25.81±0.382*	28.01±0.878*
Balady	23.23±0.723	26.84±0.792*	30.31±0.815*	32.16±0.774*
Root H_2O_2				
Great leaks	20.11±0.619	22.58±0.847*	26.28±0.714*	28.44±0.429*
Balady	20.82±0.912	24.28±0.664*	28.79±0.596*	30.65±0.603*

Values are means ± SEM of three different replicates.
H_2O_2, Hydrogen peroxide; nmole/g fresh weight.
***Differences were significant at $p < 0.05$.**

Assessment of antioxidant enzymes activities

There was a significant increase in activities of antioxidant enzymes (CAT and GST) in Great leaks and Balady roots and shoots exposed to Cd treatment (Table 7 and 8). In general, the induction of these enzymes at different concentration of Cd was more than untreated control. CAT activity was observed to be increased in Balady shoots and roots more than Great leaks. GST activity showed a concentration dependent response with a gradual increase up to 32µmol Cd however, the increase was more in Balady shoots and roots where the maximum activity (37.5 % higher than control) was noticed in Balady shoots.

Table 7: Catalase (CAT) activity of roots and shoots in Great leaks and Balady cultivars exposed to different Cd concentrations.

Cadmium concentrations				
Plant cultivars	**0 µmol**	**16 µmol**	**24 µmol**	**32µmol**
Shoot CAT				
Great leaks	19.67±0.463	23.35±0.615*	25.19±0.874*	26.93±0.979*
Balady	20.90±0.73	25.47±1.00*	28.21±0.64*	30.05±0.52*
Root CAT				
Great leaks	23.73±0.670	27.92±0.504*	29.76±0.665*	32.74±1.007*
Balady	23.73±0.670	24.10±0.988*	26.18±0.478*	28.77±0.850*

Values are means ± SEM of three different replicates.
CAT, catalase enzyme; CAT, EU/g fresh weight.
***Differences were significant at $p < 0.05$.**

Table 8: Glutathione S-transferase activity (GST) of roots and shoots in Great leaks and Balady cultivars exposed to different Cd concentrations.

Cadmium concentrations				
Plant cultivars	**0 µmol**	**16 µmol**	**24 µmol**	**32µmol**
Shoot GST				
Great leaks	21.8±0.46	24.0±0.78	25.5±1.07*	29.0±1.22*
Balady	22.5±0.45	24.7±1.00	27.6±1.61*	31.0±0.24*
Root GST				
Great leaks	24.9±0.36	27.3±0.44	28.6±0.41*	31.5±1.12*
Balady	25.2±0.43	28.3±0.24	31.0±0.91*	34.4±0.90*

Values are means ± SEM of three different replicates.
GST, glutathione S-transferase enzyme; units/ mg protein
***Differences were significant at $p < 0.05$.**

Assessment of non-protein thiols.

The concentrations of non-protein thiols (NPT) in the Cd-treated roots or shoots were significantly higher than those of the untreated controls. The maximum level of NPT was observed at 32 µmol Cd as compared with control (Table 9). The concentration of NPT increased significantly in Balady shoot more than Great leaks shoots.

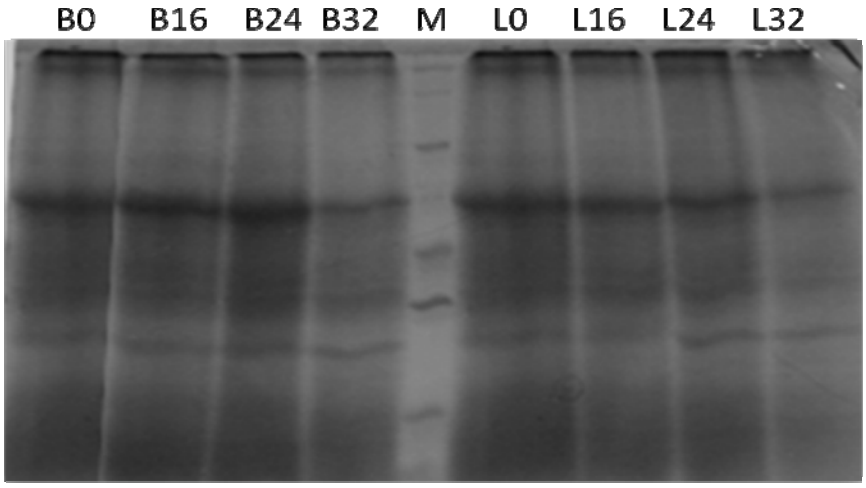
Table 9: Non protein thiols (NPT) concentrations of roots and shoots in Great leaks and Balady cultivars exposed to different Cd concentrations

Cadmium concentrations				
Plant cultivars	**0 µmol**	**16 µmol**	**24 µmol**	**32µmol**
Shoot NPT				
Great leaks	1.94±0.040	2.36±0.043*	2.65±0.061*	2.75±0.059
Balady	1.98±0.045	2.46±0.032*	2.79±0.056*	2.94±0.052*
Root NPT				
Great leaks	3.23±0.055	3.73±0.074*	3.92±0.023*	4.56±0.108*
Balady	2.54±0.041	2.99±0.049*	3. 32±0.055*	3.84±0.047*

Values are means ± SEM of three different replicates.
NPT, non-protein thiols; nmole/g fresh weight.
***Differences were significant at $p < 0.05$.**

Effect of cadmium on the protein profile of two *Lactuca sativa* cultivars

Figure 1 showes that the number and density of polypeptides gradually decreased as Cd concentration increased. The results showed that the Balady cultivar was more stable against protein damage at Cd concentration of 16 and 24 µmol than the Great leaks cultivar at the same concentrations of cadmium.



B=Balady L= Great leaks 0,16,24,32 µmol cadmium concentration

Figure 1: Effect of cadmium on total protein profile of Balady and Great leaks cultivars

DISCUSSION

The present results showed differential disposition in both *Lactuca sativa* and variable decrease as Cd concentration increased. Data showed that there was a relationship between dry matter decrease and mineral content in Balady and Great leaks. Tolerance indexes of Balady and Great leaks cultivars was changed in the range of 73.44 - 61.79 % and 70.88 -51.08 % in response to the concentrations 16 and 32 µmol Cd, respectively (Haktanır and Arcak, 1978; Yildiz 2005). In agreement with the present results, Prasad *et al.* (2004) and Abdel-Latif (2008) reported that high Cd inhibits the formation of chlorophyll by interfering with protochlorophyllide production.

The Cd uptake and accumulation in roots and shoots of both *Lactuca* cultivars were varied. The Cd content in roots and shoots were increased as Cd concentration increased. Cadmium ions were mainly accumulated in the roots, but small amounts of Cd were transferred to the shoots. It was observed that Balady shoots had less uptake of Cd than Great leaks cultivar (Lagriffoul *et al.*, 1998). These differences in root and shoot uptake might be explained by the fact that one of the normal functions of roots is to selectively acquire ions from soil solution, whereas shoot tissue does not normally play this role (Salt *et al.*, 1997). The accumulation of Cd decreased from

epidermis to inner parts of the root cortex. As the endodermis constitutes a barrier to ion transport, root cortex cells usually contain higher element concentrations than cells in the central vascular cylinder (Hagemeyer and Breckle, 1996). The induction in gene expression and activities of an antioxidant enzyme and non protein thiol confer Balady cultivar some measure of Cd tolerance in addition to the presence of strong Cd-binding proteins in the roots.

The effects of Cd on Ca, Fe, Mn and Zn concentration in both *Lactuca sativa* cultivars varied with the concentrations of Cd added. Ca, Fe, Mn and Zn uptake accumulation significantly decreased with increasing Cd ions in nutrient solution in both shoot and root tissues as compared to control plants (Table 3, 4), but the positive effect on Fe concentration was observed in the roots of Balady cultivar. In agreement with our results, Hernández *et al.*, (1998) found that Fe in pea plants treated with 50 µM Cd was higher than that recorded in the control ones.

The present study indicates that Cd exposure for different concentrations resulted in oxidative stress measured in terms of MDA content and H_2O_2 generation. MDA, a major TBARS, is an indicator of lipid peroxidation (Apel and Hirt, 2004) and links to peroxidation of polyunsaturated fatty acids in the membranes thereby releasing free radicals (Mustafa, 1990). The concentration of MDA was observed to be increased in Great leaks shoots than Balady roots. The same trend was observed in Great leaks roots that indicate that Cd absorption in Great leaks roots is more than Balady roots. This is can be explained by the increase in Cd accumulation in Great leaks shoots and roots. Also, Cd-induced lipid peroxidation has been reported in several plant species including pea (Sandalio *et al.*, 2001), sunflower (Gallego *et al.*, 1996), rice (Hsu and Kao, 2004), *Tagetes erecta* (Uraguchi *et al.*, 2006) and wheat (Singh *et al.*, 2008). In agreement with the present study, increased MDA content in response to Cd exposure is one of the mechanisms of Cd-toxicity (Dixit *et al.*, 2001; Smeets *et al.*, 2005; Garnier *et al.*, 2006; Rodrıguez-Serrano *et al.*, 2006; Chen *et al*, 2010). Cd-induced enhanced lipid peroxidation and altered electrolyte leakage suggests a negative impact on membrane integrity and thus membrane deterioration. Cd reportedly affects normal ion exchange capacity of plasma membrane and all the physiological activities linked to membrane functioning (Hernandez and Coke, 1997).

Furthermore, in the present study, Cd increased H_2O_2 content in both Great leaks and Balady shoots and roots as compared with control. It is similar to earlier reports indicating increased H_2O_2 content in response to Cd treatment under laboratory condition (Schutzendubel *et al.*, 2001; Romero-Puertas *et al.*, 2004; Rodrıguez-Serrano *et al.*, 2006). The observed changes in the contents of oxidative markers (MDA and H_2O_2) following Cd exposure indicates that Cd-induced oxidative stress in the Great leaks and Balady cultivars shoots and roots. The increase in H_2O_2 in response to Cd has also been reported in roots of bread wheat and it was correlated to oxidative stress in roots (Ranieri *et al.*, 2005). Increased levels of MDA and H_2O_2 indicated that Cd exposure results in generation of ROS, which are highly toxic molecules and cause cellular damage in plants (Apel and Hirt, 2004).

However, unlike other heavy metals, Cd being a non-redox metal, does not act through Haber–Weiss/Fenton reaction (Salin, 1988). It is parallel to a study by Garnier *et al.* (2006) who reported that Cd exposure to tobacco cells results in rapid O_2−generation that lead to oxidative damage.

An upregulation of scavenging enzymes CAT and GST to counter Cd-induced stress in Great leaks and Balady cultivars shoots and roots was observed. The observed enhancement in activities of CAT and GST in response to Cd exposure is in agreement with other reports (Dixit *et al.*, 2001; Schutzendubel *et al.*, 2001; Olmos *et al.*, 2003; Smeets *et al.*, 2005; Rodrıguez-Serrano *et al.*, 2006, Li *et al.*, 2011). The activity of CAT and GST enzymes (Tables 7-8) involved in GSH metabolism showed differential responses upon Cd exposure. The activity of CAT and GST showed a concentration dependent response with a gradual increase up to 32 µmol Cd treatment. GSTs catalyze GSH dependent detoxification of peroxides and xenobiotics and presumably heavy metals too (Moons, 2003). The observed induction in GST activity suggests its involvement in detoxification of Cd (Mishra *et al.*, 2008). Induction of various isoforms of GSTs in response to Cd has been reported in rice roots (Moons, 2003) and soyabean cells (Sobkoviak and Deckert, 2006).

Cellular non-protein thiol (NPT) increased significantly in shoots and roots of Cd-treated cultivars indicated their crucial role in ROS scavenging. NPT is an important antioxidant molecule for Cd detoxification by forming Cd bindings with their high affinity for SH groups (Pietrini *et al.*, 2003). In agreement with the present study, Mishra *et al.* (2009) observed a significantly high increase in the levels of thiols in response to Cd concentration. The maximum level of NP-SH was observed at 32 µmol Cd treatment, which was about 1.5-fold higher than control in both Great leaks and Balady cultivars shoots and roots (Table 9).

In conclusion, variation of total chlorophyll, tolerance indexes, cadmium accumulation, differential expression and antioxidant enzyme activities induced by Cd treatments revealed that Balady cultivar was more tolerant to Cd stress compared with Great leaks. Cd stress resulted in a reduction in photosynthetic efficiency and most nutrient uptake. The data indicated that there are co-ordination of gene expression, regulation ion transport process operating in different root layer more efficient in Balady cultivar compared with Great leaks, some protective mechanisms, such as activity of antioxidant enzymes may be protected from oxidative damage. In the case of Great leaks, Cd treatments possibly caused more oxidative and total protein profile damage than they did to Balady cultivar.

Analysis and evaluation of all parameters allowed classification of cultivars as tolerant (Balady) and less tolerant (Great leaks). Research must be expanded to prevent the risk of Cd uptake by crops in the food chain before the growth of Great leaks in Cd polluted regions.

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التأثير الوراثى، والإجهاد التأكسدى وآلية التحمل الناجمة عن الكادميوم فى إثنين من أصناف الخس المنزرعة.

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أجريت هذه التجربة فى المزارع المائية لدراسة تأثير الكادميوم على الإجهاد التأكسدى، والتعبير ونشاط بعض الإنزيمات المضادة للأكسدة فى إثنين من أصناف الخس المنزرعة (بلدى والجريت ليكس). وقد أظهرت النتائج تأخر في النمو لكلا الصنفين. وقد إتضح من النتائج أن الصنف الجريت ليكس كان أكثر حساسية لسمية الكادميوم من الصنف الآخر (الطراز الوراثى بلدى). وقد إنخفض ناتج ومحتوى الكلوروفيل الكلي والمادة الجافة انخفاضا شديدا فى كلا الطرازين الوراثيين مع زيادة التعرض للكادميوم، وكان ذلك أكثر وضوحا فى الطراز جريت ليكس. وقد لوحظ زيادة كبيرة فى تراكم الكادميوم في أجزاء النبات المختلفة في كلا الصنفين مع زيادة التركيز للكادميوم. وقد كان تراكم الكادميوم فى الجذور أعلى بكثير إذا ما قورنت قيمته بتراكمه فى المجموع الخضرى فى كلا الطرازين، ولكن لوحظ هذا أكثر فى الصنف البلدى. كما لوحظ أن امتصاص وتراكم الكادميوم فى المجموع الخضرى للطراز الوراثى البلدى كان أقل حوالي مرتين من إمتصاصه فى المجموع الخضرى للطراز الوراثى جريت ليكس، مما يشير إلى أن هناك تنسيقا في التعبير الجيني، وتنظيم عملية نقل الأيونات فى طبقات الجذرالمختلفة وكانت أكثر كفاءة فى التركيب الوراثى البلدى مقارنة بالصنف جريت ليكس. علاوة على ذلك فإن تعبير ونشاط الإنزيمات المضادة للأكسدة والثيول غير البروتينى قد زاد في التركيب الوراثي البلدى أكثر منه فى الجريت ليكس مما أدى إلى زيادة فوق أكسيد الهيدروجين وأكسدة الدهون، وتثبيط النمو. هذا التعبير والنشاط للإنزيمات المضادة للأكسدة يكسب الصنف الوراثي البلدى قدرا من التحمل للكادميوم وأيضا وجود البروتينات التى ترتبط بقوة بالكادميوم في الجذور. وعلى العموم قد تفسر هذه النتائج إمتلاك الخس آلية أفضل لتحمل التلوث بالكادميوم بالصنف البلدى عن الآلية الموجودة بالصنف الجريت ليكس.

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