J. Agr. Sci. Tech. (2012) Vol. 14: 929-937

Cadmium-induced Stress and Antioxidative Responses in Different *Brassica napus* Cultivars

T. Touiserkani[1], and R. Haddad[1*]

ABSTRACT

To estimate plant resistance to Cadmium Chloride ($CdCl_2$) stress for phytoremediation purposes, the effect of cadmium (Cd) phytotoxicity was assessed on total soluble protein, chlorophyll (Chl) content and antioxidant enzymes in the leaves of three different *Brassica napus* (*B. napus*) cultivars; Mohican, Reg.Cob and Okapi. Plants were exposed to three levels of $CdCl_2$ (0.75, 1.5 and 2.25 mM) in irrigation water. A reduction in protein and Chl content was noted for all treatments in the three cultivars. Generally, superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were increased with 0.75 mM $CdCl_2$ and then decreased at higher concentrations. SOD activity was enhanced up to 1.5 mM $CdCl_2$ concentration in Mohican cultivar. Moreover, APX activity of Okapi cultivar was increased at a much higher rate of $CdCl_2$ levels compared to Mohican and Reg.Cob cultivars. Different concentrations of $CdCl_2$ induced a reduction in the catalase (CAT) activity of Mohican and Reg.Cob. However, this activity was increased with 0.75 mM $CdCl_2$ in Okapi and then decreased with higher concentrations. These results indicate that *B. napus* cultivars have different tolerances to $CdCl_2$ stress and in consequence, different phytoremediation efficiencies. Moreover, because Okapi possesses a higher antioxidant enzyme activity than the other two cultivars, it is suggested that it is probably the most tolerant cultivar to $CdCl_2$ stress.

Keywords: Antioxidant enzymes, *B. napus*, Cadmium Chloride, Cultivars, Stress.

INTRODUCTION

Heavy metal contamination is responsible for limiting crop productivity in agricultural lands (Smith, 2009). Current remediation methods to lower the impacts of heavy metals are expensive and environmentally invasive. A low cost remediation technique and safe to human health and environment is the use of plants species to remove heavy metals from soil by phytoextraction. Currently, it is believed that there are around 400 plant species from a number of different families such as the Asteraceae, Brassicaceae, Caryophyllaceae, Poaceae, Violaceae and Fabaceae that possess the ability to tolerate very high levels of heavy metals in the soil (Poschenrieder *et al.*, 2006; Matthew *et al.*, 2008). The Brassicaceae is the best represented among these metal-hyperaccumulator families with 87 Brassica species classified as metal hyperaccumulators. First coined by Brooks *et al.* (1977), metal hyperaccumulators are plants that are able to accumulate metals in their above-ground tissues to very high concentrations (approx. 100 times that of a nonaccumulator plant species). Cd is the fourth most toxic element to vascular plants (Qadir *et al*., 2004). One of the major consequence of such stresses is enhanced production of reactive oxygen species (ROS) which usually damage cell membrane by inducing lipid peroxidation (Ünyayar *et al*.,

[1] Department of Agricultural Biotechnology, Imam Khomeini International University, Qazvin, Islamic Republic of Iran.
[*] Corresponding author; e-mail: raheemhaddad@yahoo.co.uk

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

2006; Gonçalves *et al*., 2007), various portions of photosynthetic apparatus in plant such as chloroplast ultrastructure, organization of thylakoids and the activity of photosynthetic enzymes, biosynthesis of Chl pigment (Singh and Tewari, 2003) and also various enzymes of carbon metabolism (Amani, 2008). Evidence from different plants shows that heavy metal including Cd may interfere with the activity of enzymes involving in removal of ROS. To repair the damage caused by ROS, plants have evolved complex antioxidant activities (both enzymatic and non-enzymatic) in the tissue cells (Arrora *et al.,* 2002; Farhoosh *et al.,* 2004; Rajaei *et al.,* 2008; Sadeghi *et al.,* 2009). Enzymatic systems such as SOD and CAT are involved in the detoxification of O_2^- and H_2O_2 respectively. Similar to CAT, APX is another antioxidant component of the ascorbate-glutathion cycle which is responsible for the removal of H_2O_2 (Mishra *et al*., 2006). Non-enzymatic scavengers including ascorbate, tocopherol and glutathione remove H_2O_2 in plants cell, thus reducing the accumulation of the free radicals of oxygen (Prochazkova *et al*., 2001).

Phytoremediation is described as the use of plants to remove pollutants from the environment or to render them harmless. Therefore, the present study was initiated to evaluate the effects of several CdCl2 levels on B. napus to analyze tolerance by some of the biochemical indices.

MATERIALS AND METHODS

The research project was carried out at the Agricultural Biotechnology Department of Imam Khomeini International University in IR of Iran during October 2005–May 2006. Seeds of oilseed rape (*B. napus* var. Mohican, Reg.Cob and Okapi) were used as Cd accumulation plants. As there were no preliminary data on these three oilseed plants for cadmium tolerance, cultivars were selected according to the primary field experiment characters between 15 planted cultivars (Data not shown). Seeds of such cultivars (Table 1) were cultivated in pots approximately 18 cm wide and 21 cm deep (1 seed per each pot) with the soil mixture of 3/8 field soil, 3/8 compost, 1/8 vermiculate, and 1/8 rice bran. The plants were grown in greenhouse under controlled light (14 hour photoperiods) and temperature (25/20°C, day/night). The pots were watered with treated tap water to approximately 60% of their holding capacity every 2 days. The control plants were watered with the same amount of water. Different concentrations of $CdCl_2$ (0.75, 1.5 and 2.25 mM) were prepared by dilution in irrigation water (Baryla *et al*., 2001; Hayat *et al*., 2007; Mobin *et al*., 2007; Qadir *et al*., 2004). The plants were exposed to $CdCl_2$ 72 hours before sampling in each step including: (1) germination (8 days after cultivation); (2) rosette (88 days after cultivation); (3) budding (132 days after cultivation); (4) flowering (175 days after cultivation); (5)-ripening (202 days after cultivation), and (6) senescence (233 days after cultivation).

Each experiment was set out using a completely randomized design with three replicates per treatment and was analyzed on a factorial experiment. The collected data were subjected to analysis of significant differences using a one way analysis of variance and Duncan's multiple range test of SPSS 10.0.1 software (SPSS Inc., Chicago, USA). Data were presented as means with standard error.

Table 1. Some characters of the cultivars selected for the research project.

No.	Cultivar name	Yield (Kg/h)	1000 kernel weight (g)	Plant height (cm)	Plant dry weight (g)	Oil percentage
1	Mohican	2665	4.47	94.7	20.98	48.6
2	Reg.Cob	2436	4.8	97.7	24.92	47.05
3	Okapi	2683	4.27	97.4	29.52	48.05

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

In order to extract total soluble protein, 1 gr fresh weight (FW) leaf tissue, as experimental sample, was homogenized in 4 ml of extraction buffer including 50 mM phosphate buffer (pH 7.0) and 1 mM sodium metabisulfate containing 100 mg insoluble polyvinyl pyrolidone (PVP). The homogenate was centrifuged at 15,000 rpm for 30 minutes (Beckman Coulter Allegra 64R centrifuge, USA) and the supernatant was used as the source of enzyme assays. All the extraction steps were carried out at 4°C. The enzyme activity was measured spectrophotometrically at lab temperature (25°C). The supernatants were used for the determination of protein by method of Bradford (1976). Bovine serum albumin (BSA, Sigma, USA) was used to draw standard curve.

Leaf Chl was extracted in 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm. The values were placed in the following formula proposed by Arnon (1949) to compute Chl content: *Total chlorophyll= 20.2(A645)+8.02 (A663)*.

CAT (EC 1.11.1.6) activity was determined by measuring hydrogen peroxide consumption at 240 nm for 3 minutes according to Aebi (1987) method by UV-Vis spectrophotometer (UV-Vis double beam PC8 scanning auto cell UVD-3200, USA) and was expressed in enzyme units (mg protein^{-1}) by using the extinction coefficient (ε) of 39.8 mM^{-1} cm^{-1}. The reaction solution contained 70 mM H_2O_2 (soluble in 100 mM potassium phosphate buffer (pH 7.0)), 100 mM potassium phosphate buffer (pH 7.0), ddH_2O and 0-60 µl enzyme extract. One unit of enzyme is the amount necessary to decompose 1 µM of H_2O_2 per minute at 25°C.

APX (APX EC 1.11.1.11) was assayed following the procedure described by Nakano and Asada (1981) APX (APX EC 1.11.1.11) was assayed following the procedure described by Nakano and Asada (1981). Enzymatic oxidation was performed by reduction in absorption at 290 nm in 3 minutes.APX activity was calculated by using the coefficient (ε) of 2.8 mM^{-1} cm^{-1} and expressed in enzyme units (mg protein^{-1}). The reaction solution contained 0.5 mM ascorbate soluble in 100 mM potassium phosphate buffer (pH, 7.0), 2 mM H_2O_2 and 0-60 µl enzyme extraction. One unit of enzyme is the amount necessary to decompose 1 µM of substrate per min at 25 °C.

SOD (EC 1.15.1.1) activity was measured by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) as the method described by Beauchamp and Fridovich (1971) and was expressed in unit of the enzyme (mg^{-1} protein h^{-1}). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 74 mM NBT, 2 mM riboflavin, 0.1 mM EDTA and 0.60 µl enzyme extract and was placed under a 15 W florescent lamp for 10 min. One enzyme unit was considered for almost %50 inhibition reduction of NBT by light.

RESULTS

A reduction in protein content was observed in all cultivars treated with different $CdCl_2$ concentration over controls (Figure 1). The changes in total soluble protein were statistically significant among the three cultivars.

Cd addition suppressed Chl content in all cultivars (Figure 2). The changes in Chl content were statistically significant between cultivars. Moreover, the greatest Chl concentration was observed in Mohican.

APX activities were increased significantly in all cultivars with 0.75 mM $CdCl_2$ treatment as compared to the control and then decreased at higher $CdCl_2$ levels (Figure 3). On average, the APX activity of cultivar Okapi was enhanced at a much higher rate than the other two cultivars at the same $CdCl_2$ concentration. The changes in APX activity were also statistically significant among the three cultivars.

SOD is a key enzyme in protecting cells against oxidative stress. In the present work, SOD activities in Reg.Cob and Okapi were increased up to 0.75 mM $CdCl_2$, while such activity was declined at higher

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

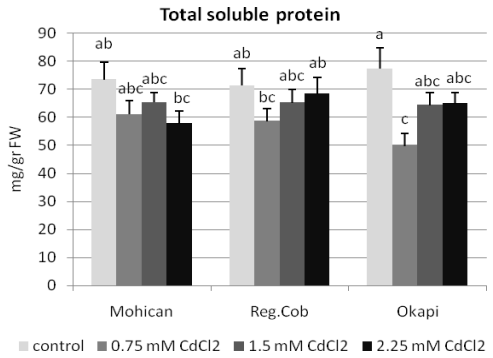

Figure 1. Total soluble protein of three *B. napus* cultivars as affected by $CdCl_2$ treatments.

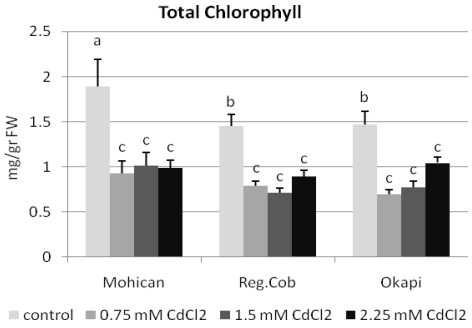

Figure 2. Changes in Chl concentration of three *B. napus* cultivars as affected by $CdCl_2$ treatment

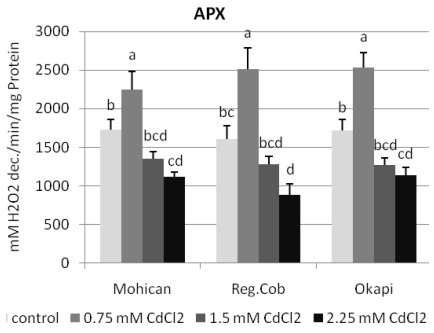

Figure 3. APX activity of three *B. napus* cultivars as affected by $CdCl_2$ treatments.

concentrations of $CdCl_2$ (Figure 4). Mohican cultivar showed increased SOD activity up to 1.5 mM $CdCl_2$. Furthermore, the changes in SOD activity were statistically significant among all cultivars.

CAT activity in Mohican and Reg.Cob was declined at all $CdCl_2$ treatments compared with the controls (Figure 5). In contrast, Okapi showed an increase in CAT activity in the plants exposed to 0.75 mM $CdCl_2$ which was then declined with increasing $CdCl_2$ concentration. CAT may be an important enzyme responsible for Okapi tolerance to $CdCl_2$ stress. This means that the changes in CAT activity were significantly different between these cultivars.

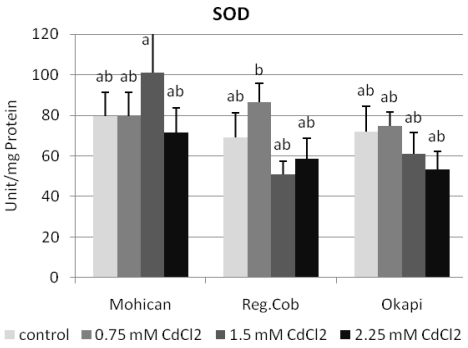

Figure 4. SOD activity of three *B. napus* cultivars as affected by $CdCl_2$ treatments.

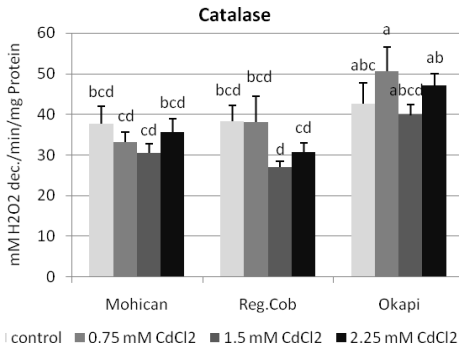

Figure 5. CAT activity of three *B. napus* cultivars as affected by $CdCl_2$ treatments.

DISCUSSION

The reduction in total soluble protein under $CdCl_2$ stress could be due to protein reaction with oxygen free radicals resulting in a change in specific amino acid, polypeptide splitting and increased

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

susceptibility of protein to proteolysis. The functionality of protein can also be affected by ROS either by oxidation of amino acid side chains or by secondary reaction with aldehydes products of lipid peroxidation (Romero-Puertas *et al*., 2002, Ayoughi *et al*., 2011). Similar observations based on protein decline have been reported in *Brassica juncea* (Mobin and Khan, 2007).

Cd appears to reduce Chl levels in various manners. Cd seems to inhibit Chl biosynthesis through a reaction with protochlorophyllide reductase and synthesis of 5-aminolevulinic acid (Guillermo *et al.,* 2007). Moreover, this element interacts with the sulfhydryl requiring enzymes such as δ-aminolevulinic acid dehydratase and porphobilinogen deaminase (Walley, 2005). Cd causes a degradation of Chl and carotenoid as well as inhibition of their biosynthesis (Otero *et al.,* 2006) which can disturb the electron transport rates of PSI and PSII, leading to the generation of oxygen free radicals. Zengin and Munzuroglu (2005) found a decrease in Chl content under lead, copper, mercury and Cd stress in bean (*Phaseolus vulgaris* L.) seedlings which is consistent with the present study.

Increased activity of the APX under $CdCl_2$ stress suggests its role in the detoxification of H_2O_2 (Weckx and Clijsters, 1996). H_2O_2 plays a role as the signal for the regulation of enzymes (Noctor and Foyor, 1998). Accumulation of H_2O_2 has also been observed in plants exposed to high temperature (Zhao *et al*., 2011), heat (Gao *et al*., 2010), pathogens (Jia *et al*., 2010) and chilling (Liu *et al*., 2011). Also, H_2O_2 is a systematic signal for the induction of APX (Morita *et al*., 1999; Nakano and Asada 1981). In mustard (Ahmad *et al.,* 2011) and *B. juncea* (Qadir *et al*., 2004) Cd treatment resulted in a significant increase in APX activity.

SOD activity was higher in Mohican and Reg.Cob than Okapi cultivar. SOD activity has been enhanced under a variety of stress conditions including Cu, Al, Mn, Fe and Zn toxicities (Prasad *et al*., 1999). Cd may induce an oxidative burst (Piqueras *et al*., 1999). Reactive oxygen species of O_2^- and H_2O_2 have been considered as the central components of signal transduction which triggers the defense genes responsible for oxidant enzymes, such as SOD (Arrora *et al*., 2002; Liu *et al*., 2011). Conversely, the increased enzyme activity contributes to the removal of O_2^- (Alvarez and Lamb, 1997; Liu *et al*., 2011). The increase in SOD activity may be the consequence of de novo synthesis of enzymatic proteins (Slooten *et al*., 1995; Allen *et al*., 1997) or the changes in gene expression (Qadir *et al*., 2004). Enhancement of SOD activity has also been reported in mustard (Ahmad *et al.,* 2011) and *B. juncea* (Qadir *et al*., 2004), while such activity has been reduced in rice (Huang *et al.,* 2008). In the leaves of rice, SOD activity was declined for Cd treated plants from the tillering to the jointing stages. In the similar results of Chen *et al*. (2010), barley plants exhibited different changes for SOD activity under Cd stress. Leaf SOD illustrated lower activity in some barley genotypes than other genotypes. Moreover, this enzyme exhibited various levels of activity in different barley tissues and also days after Cd treatment. In the present research project, the decreased activities of SOD in leaves under Cd stress were probably due to the harmful effect of overproduction of ROS or its poisonous ROS derivatives or could itself be attributed to Cd-induced inhibition of protein synthesis (Chen *et al.,* 2010).

CAT activity in Mohican and Reg.Cob was reduced with all $CdCl_2$ applications compared to the controls. Luna *et al*. (1994) and Mazhoudi *et al*. (1997) reported that Cu^{+2} may replace Fe^{+2} in the enzyme resulting in enzyme reduction. It is possible that CAT in Mohican and Reg.Cob is more sensitive to excess Cd^{+2} than Okapi. Similar observations have been reported in *Phaseolus aureus* (Shaw, 1995), *Helianthus annuus* (Gallego *et al*., 1996), and *Phaseolus vulgaris* (Chaoui *et al*., 1997). Enzymatic antioxidants demonstrated different responses based on cultivars and Cd addition

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

stress. CAT activity was reduced in *B. juncea* (Qadir *et al*., 2004) and rice (Huang *et al.,* 2008), but it was increased in mustard (Ahmad *et al.,* 2011) under Cd stress. In the present paper, CAT activity in Okapi increased with 0.75 mM $CdCl_2$. Plants exposed to heavy metals have also exhibited similar responses (Hayat *et al.*, 2007; Zawoznik *et al.*, 2007).

In conclusion, the data reported in the present study suggests that Cd at the levels used in the present study may adversely affect metabolism, although treated plants did not exhibit acute toxic symptoms. Moreover, the metal activates the cell system (Zengin and Munzuroglu, 2005), which in turn may improve the resistance capacity of plant to the stress (Poschenrieder *et al.*, 2006), and therefore different plant species show different tolerances to Cd stress. One can also conclude that *B. napus* genotypes differ in the relative tolerance to Cd stress. With regard to the superiority of these cultivars as phytoremediators and also based on the APX and Catalase activities, Okapi seems to be one of the candidates in this study, over the other two cultivars. SOD removes superoxide anion free radicals accompanying the formation of H_2O_2, which are then detoxified by CAT and APX (Sudhakar *et al*., 2001; Arrora *et al.,* 2002). Recently, similar to our result, this cultivar has also been introduced as one of the salinity resistant plants (Bybordi, 2010). The molecular details regarding the exact cause of changes response to the Cd toxicity are merits to be studied in details.

REFERENCES

1. Aebi, H. E. 1987. Catalase *In Vitro*. *Methods Enzymol.,* **105**: 121-126.
2. Ahmad, P., Nabi, G. and Ashraf, M. 2011. Cadmium-induced Oxidative Damage in Mustard [*Brassica juncea* (L.) Czern. and Coss.] Plants Can Be Alleviated by Salicylic Acid. *S. Afr. J. Bot.*, **77(1)**: 36-44.
3. Allen, R. P., Webb, R. P. and Schake, S. A. 1997. Use of Transgenic Plants to Study Antioxidant Defenses. *Free Radic. Biol. Med.,* **23**: 472–479.
4. Alvarez, M. E. and Lamb, C. 1997. Oxidative Burst Mediated Defense Responses in Plant Disease Resistance. In: "*Oxidative Stress and the Molecular Biology of Antioxidant Defenses*", (Ed.): Scandalios, J. G.. Cold Spring Harbor Laboratory Press, New York, PP. 815-839.
5. Amani, A. F. 2008. Cadmium Induced Changes in Pigment Content, Ion Uptake, Proline Content and Phosphoenolpyruvate Carboxylase Activity in *Triticum aestivum* Seedlings. *Aust. J. Basic. Appl. Sci.,* **2(1)**: 57-62.
6. Arnon, D. I. 1949. Copper Enzymes in Isolated Chloroplast: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.,* **24**: 1-15.
7. Arrora, A., Sairam, R. K. and Srivastava, G. C. 2002. Oxidative Stress and Antioxidant System in Plants. *J. Plant Physiol.,* **82**: 1227–1237.
8. Ayoughi, F., Marzegar, M., Sahari, M. A. and Naghdibadi, H. 2011. Chemical Compositions of Essential Oils of *Artemisia dracunculus* L. and Endemic *Matricaria chamomilla* L. and an Evaluation of Their Antioxidative Effects. *J. Agric. Sci. Tech.*, **13(1):** 79-88.
9. Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C. and Havaux, M. 2001. Leaf Chlorosis in Oilseed Rape Plants (*Brassica napus*) Grown on Cadmium-polluted Soil: Causes and Consequences for Photosynthesis and Growth. *Springer Berlin, Planta,* **212**: 696-709.
10. Beauchamp, C. and Fridovich, F. 1971. Superoxide Dismutase: Cd Assay and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.,* **44**: 276-27.
11. Bradford, M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.,* **72**: 248-254.
12. Brooks, R. R., Lee, J., Reeves, R. and Jaffre, T. 1977. Detection of Nickeliferous Rocks by Analysis of Herbarium Specimens of Indicator Plants. *J. Geochem. Explor.,* **7**: 49–58.
13. Bybordi, A. 2010. Effects of Salinity on Yield and Component Characters in Canola (*Brassica napus* L.) Cultivars. *Notulae Scientia Biologicae,* **2(1)**: 81-83.

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

14. Chaoui, A., Mazhouri, S., Ghorbal, M. H. and Ferjani, E. 1997. Cadmium Affects on Lipid Peroxidation and Antioxidant Enzyme Activities in Bean (*Phaseolus vulgaris* L.). *Plant Sci.,* **127**: 139-147.
15. Chen, F., Wang, F., Wu, F., Mao, W., Zhang, G. and Zhou, M. 2010. Modulation of Exogenous Glutathione in Antioxidant Defense System against Cd Stress in the Two Barley Genotypes Differing in Cd Tolerance. *Plant Physiol. Biochem.,* **48(8)**: 663-672.
16. Farhoosh, R., Purazrang, H., Khodaparast, M. H. H., Rahimizadeh, M. and Seyedi S. M. 2004. Extraction and Separation of Antioxidative Compounds from *Salvia leriifolia* Leaves. *J. Agric. Sci. Technol.,* **6**: 57-62.
17. Gallego, S. M., Benavides, M. P. and Tomaro, M. L. 1996. Effect of Heavy Metal Ion Excess on Sunflower Leaves: Evidence for Involvement of Oxidative Stress. *Plant Sci.,* **121**: 151-159.
18. Gao, Y., Guo, Y. K., Lin, S. H., Fang, Y. Y. and Bai, J. G. 2010. Hydrogen Peroxide Pretreatment Alters the Activity of Antioxidant Enzymes and Protects Chloroplast Ultrastructure in Heat-stressed Cucumber Leaves. *Sci. Hortic.*, **126**: 20-26.
19. Gonçalves, J. F., Becker, A. G., Cargnelutti, D., Tabaldi, A. L., Pereira, L. B., Battisti, V. I, Spanevello, R. M., Morsch, V. M., Nicoloso, F. T. and Schetinger, M. R. C. 2007. Cadmium Toxicity Causes Oxidative Stress and Induces Response of the Antioxidant System in Cucumber Seedlings. *Braz. J. Plant Physiol.,* **19(3)**: 24-26.
20. Guillermo, O. N., Karina, B. B., Alcira, B. and Maria, L. T. 2007. Cadmium Induced Oxidative Stress in Soybean Plants also by the Accumulation of δ-aminolevulinic Acid. *Bio Metals.,* **20**: 841-851.
21. Hayat, S., Ali, B., Aiman, H. S. and Ahmad, A. 2007. Brassinosteroid Enhanced the Level of Antioxidants under Cadmium Stress in *Brassica juncea. Environ. Exp. Bot.,* **60**: 33-41.
22. Huang, D. F., Xi, L. L., Yang, L. N., Wang Z. Q. and Yang J. C. 2008. Comparison of Agronomic and Physiological Traits of Rice Genotypes Differing in Cadmium-tolerance. *Acta Agron. Sci.*, **34(5)**: 809-817.
23. Jia, Z., Zou, B., Wang, X., Qiu, J., Ma, H., Gou, Z., Song, S. and Dong, H. 2010. Quercetin-induced H_2O_2 Mediates the Pathogen Resistance against *Pseudomonas syringae* pv. Tomato DC3000 in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.,* **396**: 522-527.
24. Liu, Y., Jiang, H., Zhao, Z. and An, L. 2011. Abscisic Acid is Involved in Brassinosteroids-induced Chilling Tolerance in the Suspension Cultured Cells from *Chorispora bungeana*. *J. Plant Physiol.*, **168(9)**: 853-862.
25. Luna, C. M., Gonzalez, C. A. and Trippi, V. S. 1994. Oxidative Damage Caused by an Excess of Cu in Oat Leaves. *Plant Cell Physiol.,* **35**: 11–15.
26. Matthew, J. M. and Leon, V. K. 2008. Investigating Heavy-metal Hyperaccumulation Using *Thlaspi caerulescens* as a Model System. *Ann. Bot*., **102**: 3-13.
27. Mazhoudi, S., Chaoui, A., Ghorbal, M. H. and Ferjani, E. E. 1997. Response of Antioxidant Enzymes to Excess Copper in Tomato (*Lycopersicon esculentum*, Mill). *Plant Sci.,* **127**: 129-137.
28. Mishra, S., Srivastava, S., Tripathi, R. D., Govindarajan R., Kuriakose, S. V. and Prasad M. N. V. 2006. Phytochelatin Synthesis and Response of Antioxidants during Cadmium Stress in *Bacopa monnieri* L. *Plant Physiol. Biochem.,* **44**: 25-37.
29. Mobin, M. and Khan, N. A. 2007. Photosynthetic Activity, Pigment Composition and Antioxidative Response of Two Mustard (*Brassica juncea*) Cultivars Differing in Photosynthetic Capacity Subjected to Cadmium Stress. *J. Plant Physiol.,* **164**: 601-610.
30. Morita, S., Kaminaka, H., Masumura, T. and Tanaka, K. 1999. Induction of Rice Cytosolic Ascorbate Peroxidase mRNA by Oxidative Stress Signaling. *Plant Cell Physiol.,* **40**: 417–422.
31. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.,* **22**: 867-880.
32. Noctor, G. and Foyer, C. H. 1998. Ascorbate and Glutathione: Keeping Active Oxygen under Control. *Annu. Rev. Plant Physiol.,* **49**: 249-279.
33. Novakova, M., Matejova, E. and Sofrova, D. 2004. Cd^{2+} Effect on Photosynthetic Apparatus in *Synechococcus elongates* and Spinach (*Spinacia oleraceae* L.). *Biomed. Life Sci.,* **42**: 425-430.

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

34. Otero, S., Núñez-Olivera, E., Martínez-Abaigar, J., Tomás, R., Arróniz-Crespo, M. and Beaucourt, N. 2006. Effects of Cadmium and Enhanced UV Radiation on the Physiology and the Concentration of UV-absorbing Compounds of the Aquatic Liverwort *Jungermannia exsertifolia* subsp. *Cordifolia Photochem. Photobiol. Sci.,* **5**: 760-769.
35. Piqueras, A., Olmos, E., Martinez-Solano, J. R. and Hellin, E. 1999. Cd Induced Oxidative Burst in Tobacco BY-2 cell: Time- course, Subcellular Location and Antioxidant Response. *Free Radic. Res.,* **31**: S33-S38.
36. Poschenrieder C., Tolrà, R. and Barceló J. 2006. Can Metals Defend Plants against Biotic Stress? Review Article. *Trends Plant Sci.*, **11(6)**: 288-295.
37. Prasad, K. V. S. K., Saradhi, P. P. and Sharmila, P. 1999. Concerned Action of Antioxidant Enzymes and Curtailed Growth under Zn Toxicity in *Brassica juncea*. *Environ. Exp. Bot.,* **42**: 1–10.
38. Prochazkova, D., Sairam, R. K., Srivastava, G. C. and Singh, D. V. 2001. Oxidative Stress and Antioxidant Activity as the Basis of Senescence in Maize Leaves. *Plant Sci.,* **161**: 765-771.
39. Qadir, S., Qureshi, M. I., Javed, S. and Abdin, M. Z. 2004. Genotypic Variation in Phytoremediation Potential of *Brassica junceae* Cultivars Exposed to Cd Stress. *Plant Sci.,* **167**: 1171-1181.
40. Rajaei, A., Barzegar, M. and Sahari, M. A. 2008. Comparison of Antioxidative Effect of Tea and Sesame Seed Oils Extracted by Different Methods. *J. Agric. Sci. Technol*, **10**: 345-350.
41. Romero-Puertas M. C., Palma, J.M., Gómez, M., Del Río, L. A. and Sandalio L. M. 2002. Cadmium Causes the Oxidative Modification of Proteins in Pea Plants. *Plant, Cell Environ.,* **25(5)**: 677-686.
42. Sadeghi, N., Jannat, B., Oveisi, M. R., Hajimahmoodi, M. and Photovat, M. 2009. Antioxidant activity of Iranian Pomegranate (*Punica granatum* L.) Seed Extracts. *J. Agr. Sci. Tech,* **11**: 633-638.
43. Shaw, B. P. 1995. Effect of Mercury and Cd on the Activities of Antioxidative Enzymes in the Seedling of *Phaseolus aureus. Biol. Plants.,* **37**: 587-596.
44. Singh, P. K. and Tewari, R. K. 2003. Cadmium Toxicity Induced Changes in Plant Water Relations and Oxidative Metabolism of *Brassica junceae* Plants. *Environ. Biol.,* **24**: 107-112.
45. Slooten, L., Capiiau, K., Van Camp, W., Van Montagu, M., Sybesma, C. and Inze, D. 1995. Factors Affecting the Enhancement of Oxidative Stress Tolerance in Transgenic Tobacco Overexpressing Mn-SOD in the Chloroplast. *Plant Physiol.,* **107**: 737–750.
46. Smith, S. R. 2009. A Critical Review of the Bioavailability and Impacts of Heavy Metals in Municipal Solid Waste Composts Compared to Sewage Sludge: Review Article. *Environ. Int.,* **35:** 142-156.
47. Sudhakar A., Lakshmi S. and Giridarakumar, A. 2001. Changes in the Antioxidant Enzyme Efficacy in Two High Yielding Genotypes of Mulberry (*Morus alba* L.) under NaCl Salinity. *Plant Sci.,* **161**: 613–619.
48. Ünyayar, S., Çelik, A., Özlem Çekiç, A. and Gözel, A. 2006. Cadmium-induced Genotoxicity, Cytotoxicity and Lipid Peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis,* **21(1)**: 77-81.
49. Walley, J. 2005. The Effects of Low-level Cadmium Toxicity on Field and Greenhouse Grown Soybean (*Glycine max*). MSc. Thesis, Department of Botany, Miami University, Oxford, Ohio,
50. Weckx, J. E. J. and Clijsters, H. M. M. 1996. Oxidative Damage and Defense Mechanisms in Primary Leaves *Phaseolus vulgaris* as a Result of Root Assimilation of Toxic Amounts of Copper. *Physiol. Plant,* **96**: 506–512.
51. Zawoznik, M. S., Groppa, M. D., Tomaro, M. L. and Benavides, M. P. 2007. Endogenous Salicylic Acid Potentiates Cadmium-induced Oxidative Stress in *Arabidopsis thaliana*. *Plant Sci.,* **173**: 190-197.
52. Zengin, F. and Munzuroglu, O. 2005. Effects of Some Heavy Metals on Content of Chlorophyll, Proline and Some Antioxidant Chemicals in Bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biol. Cracov. Ser. Bot.,* **47(2)**: 157–164.
53. Zhao, X., Nishimura, Y., Fukumoto, Y. and Li, J. 2011. Effect of High Temperature on Active Oxygen Species, Senescence and Photosynthetic Properties in Cucumber Leaves. *Environ. Exp. Bot.,* **70**: 212-216.

[DOR: 20.1001.1.16807073.2012.14.4.18.4]

تنش ایجاد شده توسط کادمیوم و واکنش‌های ضد اکسایش در ارقام مختلف کلزا (*Brassica napus*)

ط. تویسرکانی، و ر. حداد

چکیده

برای تخمین میزان مقاومت گیاه به کادمیوم جهت پاکسازی محیط زیست، اثر سمیت کادمیوم بر میزان پروتئین محلول کل، کلروفیل و فعالیت آنزیم‌های ضد اکسنده در برگ‌های سه رقم مختلف کلزا شامل (Mohican، Reg.Cobو Okapi) مورد ارزیابی قرار گرفت. گیاهان از طریق آبیاری در معرض مقادیر مختلف کادمیوم (۰/۷۵، ۱/۵ و ۲/۲۵ میلی مولار) قرار گرفتند. بر اثر تیمارهای اعمال‌شده میزان کلروفیل و پروتئین محلول کل در کلیه ارقام کاهش یافت. به طور کلی، میزان فعالیت آنزیم‌های آسکوربات پراکسیداز و سوپراکسید دیسموتاز در تمام ارقام در غلظت ۰/۷۵ میلی مولار تیمار نسبت به شاهد افزایش و سپس کاهش پیدا کرد. در رقم Mohican میزان فعالیت آنزیم سوپراکسید دیسموتاز تا غلظت ۱/۵ میلی مولار کادمیوم افزایش یافت. علاوه برآن آنزیم آسکوربات پراکسیداز در رقم Okapi فعالیت بیشتری نسبت به ارقام Mohican و Reg.Cob در غلظت‌های بالاتر کادمیوم نشان داد. میزان فعالیت آنزیم کاتالاز در ارقام Mohicanو Reg.Cob در اثر تیمار با غلظت‌های مختلف کادمیوم کاهش داشت. با این وجود، در رقم Okapi این فعالیت تا غلظت ۰/۷۵ میلی مولار کادمیوم افزایش و سپس کاهش پیدا کرد. نتایج به‌دست‌آمده نشان می‌دهد که ارقام مختلف کلزا در میزان مقاومت نسبت به کادمیوم و در نتیجه توانمندی پاکسازی محیط زیست متفاوتند. علاوه بر آن، از آنجا که رقم Okapi فعالیت ضد اکسنده بیشتری نسبت به دو رقم دیگر نشان داد، این رقم نسبت به تنش کادمیوم مقاومتر می باشد.

[DOR: 20.1001.1.16807073.2012.14.4.18.4]