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Evaluation of cadmium tolerance in oxidative stress in wheat cultivars

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Abstract

Wheat is the world's most important cereal crop in terms of both area cultivated and amount of grain produced. Pot experiments were conducted to evaluation of xidative stress in cadmium tolerance in wheat cultivars. This experiment was conducted in Varamin zone at Iran during 2012. In this respect, Research was conducted with complete randomized block experimental design with factorial arrangement with four replications. In this experiment, Cadmium tolerance levels 1- Control (A_1), 2- 50 mg/lit Cadmium (A_2), 3- 100 mg/lit Cadmium, (A_3), 4- 150 mg/lit Cadmium(A_4). And Wheat cultivar on four levels: 1- Pishtaz (B_1),2- Dena(B_2),3- Ariya (B_3) and 4- Parsi(B_4).Results of the study showed that Cadmium tolerance decreased the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in wheat cultivars. There were significant differences among genotypes for antioxidant enzyme activity. Also, Cadmium tolerance × cultivar interactions showed significant difference on GPX activities. Results of the study also indicated that Cadmium tolerance causes production of reactive oxygen species(ROSs),which results in greater membrane permeability, i.e. malondialdehyde (MDA) content and oxidative stress in the plants. Moreover, genotypes having greater levels of antioxidants showed better resistance to Cadmium tolerance.

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Introduction

A biotic stress is the main factor negatively affecting crop growth and productivity worldwide. The heavy metal, Cd is commonly released into the arable soil from industrial processes and farming practices and has been ranked No. 7 among the top 20 toxins. Even at low concentrations, Cd is toxic for most of the plants at concentrations greater than 5–10 μg Cd g–1 leaf dry weight, except Cd-hyper accumulators which can tolerate Cd concentrations of 100 μg Cd g–1 leaf dry weights (Gill and, Tuteja, 2011).

Cereals are the main source of food in many countries. Among them, wheat *(Triticum aestivum)* is one of the most consumed and spread (Tejera *et al*, 2013). Wheat was a key factor enabling the emergence of city-based societies at the start of civilization because it was one of the first crops that could be easily cultivated on a large scale, and had the additional advantage of yielding a harvest that provides long-term storage of food (Palmer & John, 2001). Significant efforts have been expended during recent years to evaluate the transfer of elements from source to plants (Robards & Worsfold, 2011). On the other hand, cereals do also contain heavy metals which, on the contrary, are not essential for the organism, and penetrate through the ground, the air and the water (Golia *et al*, 2008). Accumulation of cadmium soils and plants is determined by the subsoil type. Cadmium is easily taken up by plants and translocated to different plant parts, while high Cadmium accumulation in plants causes a potential hazard to human health through the food chain (Jackson & Alloway, 1992). Cadmium is extremely readily taken up by plants, both by their roots and leaves, usually in proportion to the cadmium content in the environment (Friesl- Hanl *et al*, 2009). Cadmium may interfere with nutrient uptake by affecting the permeability of plasma membranes. According to Jalil *et al.* (1994), Cadmium addition decreased the concentration of K, Zn and Mn in wheat root and shoot, while Fe and Cu concentrations in shoot and root were not affected. Alternatively, Cd-caused induction of enzymes of S assimilation pathway has been reported in many plants(Khan *et al*, 2007). However, Cd is a non-redox active metal, but it induces the generation of reactive oxygen species (ROS) including superoxide radical (O_2^+), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-)(Gill and Tuteja 2010) which has to be kept under tight control because the presence of Cd lead to excessive production of ROS causing cell death due to oxidative stress such as membrane lipid per oxidation, protein oxidation, enzyme inhibition and damage to nucleic acid (Mishra *et al*, 2008). Therefore, the objective of this study was to evaluate the cadmium tolerance influence on antioxidant enzymes and destruction biomarkers in wheat cultivars under oxidative stress.

Materials and methods

This study was conducted in a greenhouse at the Agricultural College. Pot experiments were carried out in order to investigate effect of oxidative stress in cadmium tolerance in wheat (*Triticum aestivum*) cultivars in Varamin, Iran. This experiment was conducted in Varamin zone at Iran during 2012. In this respect, Research was conducted with complete randomized block experimental design with factorial arrangement with four replications. In this experiment, Cadmium tolerance levels 1- Control (A_1), 2- 50 mg/lit Cadmium (A_2), 3- 100 mg/lit Cadmium (A_3), 4- 150 mg/lit Cadmium (A_4). And Wheat cultivar on four levels: 1- Pishtaz (B_1), 2- Dena (B_2), 3- Ariya (B_3) and 4- Parsi (B_4). The research was compared 40 cm with a diameter of pots.

The site is located at 35:20"N latitude, 51:31"E longitude, with an altitude of 1050 m above sea level. The soil consisted of 10% clay, 20% silt and 70% sand.

Pots were arranged to 142 pots at four replication in the greenhouse, so that treatment was constituted a 3 pot. After settling each pot was covered with soil. Cadmium was added to the soil. Measured parameters where the amount of biochemical characters [Super Oxide Dismuatase (SOD), Catalase (CAT) and Glutathione per Oxidase (GPX), and destruction biomarkers (MAD), DT and D-OH-dG).

Sampling

After cadmium tolerance treatment, four leaves of each plant were removed. The samples were put then frozen in liquid N_2 and then stored at -80°C pending biochemical analysis (Lowry, 1951).

Preparation of extracts

Leaf sample was homogenized in a mortar and pestle with 3 mL ice-cold extraction buffer. The homogenate was centrifuged at 18000 g for 30 min at 48°C and then supernatant was filtered through paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity. All operations were carried out at 48°C.

Assay of antioxidant enzymes

Catalase activity was estimated by the method of Cakmak and Horst (De Boot, 1990). The reaction mixture contained 100 crude enzyme extract, 500 µL 10 mM H2O2 and 1400 µL 25 mM sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min by spectrophotometer, model Cintra 6 GBC. CAT activity of the extract was expressed as CAT units per milligram of PROT(Paglia, 1997).

Superoxide dismutase activity

(SOD) was defined with the reaction mixture contained 100 µL 1 µM riboflavin, 100 µL 12 mM L-methionine, 100 µL 0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na2 CO3 (pH 10.2) and 100 µL 75 µM Nitroblue Tetrazolium (NBT) in 2300 µL 25 mM sodium phosphate buffer (pH 6.8), 200 µL crude enzyme extract in a final volume of 3 mL.

Superoxide dismutase activity (SOD) was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amountof enzyme activity that was able to inhibit by 50% the photo reduction of NBT to blue formazan. The SOD activity of the extract was expressed as SOD units per milligram of PROT (Misra and Fridorich, 1972).

Peroxidase

activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm[Hernandez *et al*, 2000]. The reaction mixture contained 100 µL crude enzyme, 500 µL H2O2 5 mM, 500 µL guaiacol 28 mM and 1900 µL potassium phosphate buffer 60 mM (pH 6.1). POX activity of the extract was expressed as POX units per mg (Tohidi-Moghadam *et al.*, 2009).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Statistical analysis System (SAS, 1988) and followed by Duncan's multiple range tests. Terms were considered significant at P< 0.05. And graphs drawing by Excel software.

Results

Total metal concentration in soil is usually not found to be a good predictor of the metal uptake by the plants since only a fraction of the total metal concentration is available for uptake. Results of ANOVA and comparison of the means for cadmium tolerance in wheat cultivars study are presented in Table.1, Table.2and Table.3 respectively. Results showed significant differences ($P \leq 0.05$) for GPX activities in cadmium tolerance in wheat cultivars treatments (Table-1). However, were not significant in SOD and CAT characteristics (Table 1). Results of analysis of variance indicated that the interaction in cadmium tolerance in wheat cultivars treatments on DT was significant at the 5% level and were not significant in MAD and D-OH-dG characteristics (Table 1).

These results are in agreement with findings of Habibi *et al,* (2004) and Tohidi-Moghaddam *et al*, (2009). H_2O_2 can be harmful because of its oxidative and destructive effects on the metabolism of plants. In organisms, H202 can be destroyed by catalase and glutathion peroxidace. Catalase protects cells from the effects of H202. Under normal condition catalase

for some cells has an important role in increasing the resistance to oxidative stress. The reaction of free and semifree radicals of oxygen can be seen in destructive functions such as senescence (Ames *et al.*, 1993). The mutual action of CAT and SOD converts the toxic and H_2O_2 into water and molecular oxygen, preventing the cellular injure under drought stress (Manivannan *et al.*, 2007).

Table 1. Mean squares of some biochemical parameters.

SOV	df	SOD	CAT	GPX	MDA	DT	D-OH-dG
Replication	3	2.149^{ns}	2.806^{ns}	3.46^{ns}	1.877^{ns}	13.249^{ns}	30.805^{ns}
Cadmium(A)	3	59.565**	3.771^{ns}	31.606**	300.216**	228.994**	92.958**
Cultivar(B)	3	994. 159**	51.678**	51.213**	327.08**	458.051**	123.512**
cadmium × cultivar(AB)	9	2.081^{ns}	5.847^{ns}	2.194*	19.572^{ns}	14.557*	5.684^{ns}
Error	45	3.182	3.119	1.028	10.063	5.99	4.903
CV (%)	-	10.24	9.14	10.05	9.01	7.5	8.45

*.** Means significant in 0.05 and 0.01 Level of probability respectively and ns: non-significant.

In this investigation, the lowest of SOD activity, CAT activity and GPX activity were observed on C_4V_1 (150 mg/lit Cadmium & Pishtaz) treatment with average (5.472 U/g protein), (6.795 U/g protein) and (5.145 U/g protein), respectively. The results showed that cadmium treatments and Variety of Wheat significant affect on GPX activity5% level. So that the highest SOD activity, CAT activity and GPX activity obtained from in C_1V_4 (Control & Parsi) treatment (16.49 U/g protein), (13.06 U/g protein) and (13.2 U/g protein), respectively.

Table 2. Means comparison for different Cadmium treatments and Variety on Wheat.

Treatment	SOD (U/ protein)	CAT (U/g protein)	GPX (U/g protein)	MDA (nM/g protein)	DT (nM/g protein)	D-OH-dG (nM/g protein)
C_1	13.4^{a}	9.787^{a}	9.492^{a}	30.78^{d}	29^{c}	23.64^{c}
C_2	13.12^{a}	9.448^{a}	8.198^{b}	33^{c}	30.4^{c}	25.06^{c}
C_3	10.98^{b}	8.977^{a}	7.16^{c}	36.39^{b}	33.63^{b}	26.97^{b}
C_4	9.306^{c}	8.696^{a}	6.219^{d}	40.73^{a}	37.51^{a}	29.19^{a}
V_1	7.348^{c}	7.569^{c}	9.014^{c}	40.34^{a}	39.25^{a}	29.95^{a}
V_2	14.63^{a}	9.063^{b}	7.234^{b}	37.28^{b}	27.07^{d}	25.87^{b}
V_3	10.4^{b}	8.561^{bc}	7.563^{b}	33.25^{c}	34.42^{b}	25.8^{b}
V_4	14.45^{a}	11.76^{a}	10.26^{a}	30.02^{d}	29.81^{c}	23.23^{c}

Control (C_1), 2- 50 mg/lit Cadmium (C_2), 3- 100 mg/lit Cadmium, (C_3), 4- 150 mg/lit Cadmium(C_4). And Wheat cultivar on four levels: 1- Pishtaz (V_1), 2- Dena(V_2), 3- Ariya (V_3) and 4- Parsi(V_4).
Means with the same letter in each column have not statistically significant difference.

H_2O_2 can be removed using the ascorbate-glutathione cycle [ascorbic acid (ASA) (GSH cycle) which GPX activity and SOD activity are the key enzymes in this cycle (Pasternak *et al.*, 2005).

Hydrogen peroxide is converted to oxygen and water by CAT activity and GPX activity which use ascorbate as the hydrogen donor (Maldonado-Rodriguez, 2002). Many researchers have also suggested that Cadmium tolerance is frequently associated with a more inefficient antioxidative system (Farooq *et al.*, 2009). These comments are consistent with the results of this study. In addition, the minimum antioxidant enzymes activities were found in Cadmium tolerance. In our study, Cadmium tolerance decreased the activity of these enzymes maybe by elimination of free radicals (Tohidi-Moghadam *et al.*, 2009).

Table 3. Effects of interactions on Cadmium tolerance and in wheat variety on antioxidant enzymes and destruction biomarkers.

Treatment		SOD (U/ g protein)	CAT (U/g protein)	GPX (U/g protein)	MDA (nM/g protein)	DT (nM/gprotein)	D-OH-dG (nM/g protein)
	V_1	9.185^{de}	8.083^{de}	7.148^{efg}	34.16^{defg}	33.47^{def}	26.2^{cd}
C_1	V_2	15.23^{ab}	10.51^{abcd}	8.37^{cde}	30.12^{ghi}	26.26^{ij}	22.5^{ef}
	V_3	12.72^{bc}	7.54^{de}	9.25^{bc}	31.16^{fghi}	30.84^{fgh}	24.2^{def}
	V_4	16.49^{a}	13.06^{a}	13.2^{a}	27.7^{i}	25.45^{ij}	21.64^{f}
	V_1	8.337^{e}	8.708^{cde}	6.315^{gh}	36.55^{de}	36.21^{cd}	29.26^{bc}
C_2	V_2	16.35^{a}	9.007^{cde}	7.217^{defg}	14.36^{def}	25.12^{j}	24.15^{def}
	V_3	12.45^{bc}	7.585^{de}	8.793^{cd}	30.87^{ghi}	32.16^{fg}	26.03^{cde}
	V_4	15.35^{ab}	12.49^{ab}	10.47^{b}	28.43^{hi}	$8.12\ h^{ij}$	20.78^{f}
	V_1	6.398^{ef}	6.738^{e}	5.448^{h}	42.15^{bc}	40.22^{b}	30.58^{b}
C_3	V_2	14.1^{abc}	9.135^{cde}	7.265^{defg}	38.66^{cd}	27.77^{hij}	27.65^{bcd}
	V_3	9.153^{de}	8.89^{cde}	6.675^{fgh}	33.57^{defgh}	36.19^{cd}	25.5^{de}
	V_4	14.28^{abc}	11.15^{abc}	9.252^{bc}	31.17^{fghi}	30.33^{fgh}	24.15^{def}
	V_1	5.472^{f}	6.795^{e}	5.145^{h}	48.51^{a}	47.1^{a}	33.75^{a}
C_4	V_2	12.83^{bc}	7.600^{de}	6.082^{gh}	44.19^{ab}	29.14^{ghi}	29.2^{bc}
	V_3	7.262^{ef}	10.05^{bcd}	5.532^{h}	37.41^{cde}	38.49^{bc}	27.47^{bcd}
	V_4	11.66^{cd}	10.34^{abcd}	8.113^{cdef}	32.79^{efghi}	35.32^{cde}	26.34^{cd}

Control (C_1), 2- 50 mg/lit Cadmium (C_2), 3- 100 mg/lit Cadmium, (C_3), 4- 150 mg/lit Cadmium(C_4). And Wheat cultivar on four levels: 1- Pishtaz (V_1), 2- Dena(V_2), 3- Ariya (V_3) and 4- Parsi(V_4)

Means with the same letter in each column have not statistically significant difference.

Data presented in Table 3 indicated that increasing Cadmium significantly increased destruction biomarkers. The most of MDA, DT and D-OH-dG have assigned C_4V_1 (150 mg/lit Cadmium & Pishtaz) treatment with average (48.51 nM/g protein),(47.1 nM/g protein) and (33.75 nM/g protein), respectively. And the lowest of MDA, was observed on C1V4 (Control & Parsi) treatment (27.7 nM/g protein). And DT obtained from in C_2V_2 (50 mg/lit Cadmium& Dena) treatment (25.12 nM/g protein) and D-OH-dG has assigned on C_2V_4 (50 mg/lit Cadmium& Parsi) treatment (20.78 nM/g protein), There was not significant with C_1V_4 (Control & Parsi) treatment.

Conclusion

It seems that destruction biomarkers and Antioxidant Enzyme Activities are correlated with each other. These comments are consistent with the results of this study According to these results it can be suggested that Cadmium was harmful per plant and reduction this element can reduce the harmful effects of ROS and improves plant yield.

References

Ames BN, Shigena MK, Hegen TM. 1993. Oxidants, antioxidants and the degenerative disease of aging. Proceedings of the National Academy of Sciences of the United States of America **90,** 7915-7922.
http://www.pnas.org.

DeBoot M. 1990. Application of Polymeric Substances as Physical Soil conditioners. In: Soil Colloids and their Association in Soil Aggregates, De Boodt, M. *et al.* (Ed.). Planum Publishing Corporation, London, New York, ISBN: 0306434199, 580-592 p.
http://www.wajae.org.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA. 2009. Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development **299,** 185-212.
http://dx.doi.org/10.105/agro.2008021.www.agronomy-journal.org.

Gill SS, Tuteja N. 2010. Reactive oxygen species and anti-oxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem **48,** 909–930. http://dx.doi.org/10.1016/j.plaphy.2010.08.016.

Gill SS, Tuteja N. 2011. Cadmium stress tolerance in crop plants. Plant Signaling & Behavior **6(2),** 215–222. http://dx.doi.org/10.4161/psb.6.2.14880

Golia EE, Dimirkou A, Mitsios IK. 2008**.** Influence of some soil parameterson heavy metals accumulation by vegetables grown inagricultural soils of different soil orders. Bulletin of Environmental Contamination and Toxicology **81,** 80-84. http://dx.doi.org/10.1007/s00128-008-9416-7.

Habibi D, Boojar MMA, Mahmoudi A, Ardakani MR, Taleghani DF. 2004**.** Antioxidative enzymes in sunflower subjected to drought stress. Proceeding of the 4th International Crop Science Congress, 26 September-10 October, Brisbane, Australia. http://www.ijpbs.net

Hernandez JA, Jimenez A, Mullineaux Pand Sevilla F. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell Environment **23,** 853- 862. http://cat.inist.fr/?aModele=afficheN&cpsidt=14603 06

Jackson AP, Alloway BJ. 1992. The transfer of cadmium from agricultural soils to the human food chain. In: Adriado, D.C.(Ed.), Biogeochemistry of Trace Metals. Lewis Publishers, Boca Raton, FL, 109–158 p. www.paper.edu.cn.

Jalil A, Selles F, Clarke JM. 1994. Effect of cadmium on growth and the uptake of cadmium and other elements by durum wheat. Journal Plant Nutrition **17,** 1839–1858. http://dx.doi.org/10.1080/01904169409364851

Khan NA, Samiullah Singh S, Nazar R. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. Journal of Agronomy and Crop Science **193,** 435–444. http://onlinelibrary.wiley.com/doi/10.1111/j.1439-037X.2007.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Fooling phenol reagent. Journal Biological Chemistry **193,** 265-275. http://www.jbc.org/content/193/1/265.citation

Maldonado-Rodriguez R. 1999-2002. Biolyzer Software. Laboratory of Bioenergetics, University of Geneva, Switzerland, 457 p. http://www.bio21.bas

Manivannan P, Jaleel CA, Somasundaram R, Panneerselvam R. 2008. Osmoregulation and antioxidant metabolism in drought-stressed Helianthus annuus under triadimefon drenching**.** Comptes Rendus Biologies **331,** 418-425**.** http://www.ncbi.nlm.nih.gov/pubmed/18510994. 10.1016/j.crvi.2008.03.003.

Misra HP, Fridovich I. 1972. The Generation of super oxide radical during oxidation. Journal Biological Chemstry **285,** 339-346. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2101 79

Mishra S, Srivastava S, Tripathi RD, Dwivedi S, Shukla MK. 2008. Responses of antioxidant enzymes in coontail (*Ceratophyllum demursum* L.) plants under cadmium stress. Environmental Toxicology. **23,** 294–301. http://www.ncbi.nlm.nih.gov/pubmed/18214904 http://dx.doi.org/10.1002/tox.20340.

Paglia D. 1997. Studies on the quantitive trait Dase. Journal Laboratory medicine **70,** 158-165.13.

Palmer J. 2001. How to Brew. Defenestrative Publishing *Co*. 233 p. ISBN 0-9710579-0-7. www.pkhope.com/wheat-production-varieties-and-management-practice

Pasternak T, Rudas V, Potters Gand Jansen MAK. 2005. Morphogenic effects of a biotic stress: Reorientation of growth in Arabidopsis thaliana seedlings. Environmental and Experimental Botany**. 53,** 299-314. http://cat.inist.fr/?aModele=afficheN&cpsidt=16710563.

Raquel L, Tejera G, Dailos González-Weller L, José M, Caballero Ángel J, Gutiérrez Carmen R, Hardisson A. 2013**.** Metals in wheat flour; comparative study and safety control. Nutrition Hospital **28(2),** 506-513. http://www.nutricionhospitalaria.com/mostrarrevista. http://dx.doi.org/10.3305/nh.2013.28.2.6287

Robards Kand Worsfold P. 2011.Cadmium: toxicologyand analysis. Analyst, **168,** 325–329. http://pubs.rsc.org. http://dx.doi.org/**10**.1039/AN9911600549

Tohidi-Moghadam HR, Shirani-Rad AH, Nour-Mohammadi G, Habibi D, Mashhadi-Akbar-Boojar M. 2009**.** Effect of Super Absorbent Application on Antioxidant Enzyme Activities in Canola (*Brassica napus* L.) Cultivars under Water Stress Conditions. American Journal of Agricultural and Biological Sciences **4(3),** 215-223. http://thescipub.com. http://dx.doi.org/10.3844/ajabssp.2009.215.223