American-Eurasian Journal of Toxicological Sciences 3 (1): 41-46, 2011
ISSN 2079-2050

Antioxidative Response in Tomato Plants *Lycopersicon esculentum* L. Roots and Leaves to Zinc

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Abstract: This work aims to evaluate the response of tomato plants (*Lycopersicon esculentum L*. var. Rio Grande) to treatment with zinc and accumulation (trace element) in the roots and leaves of young plants. This is done by analyzing the effects of zinc on the rate of chlorophyll and enzyme activity involved in the antioxidant system (CAT, GST, APX). Plants previously grown on a basic nutrient solution is treated by increasing concentrations of $ZnSO_4$ (0, 50, 100, 250, 500 microM) for 07 days. The results show that Zn does not affect the amount of chlorophyll at 50 and 100 microns, while it seems to inhibit the higher concentrations (250 and 500 microns). On the other hand, treatment with zinc induced the activity of enzymes studied, namely (CAT, APX, GST) especially for higher concentrations. Finally, the determination of zinc in the roots and leaves of tomato shows a greater accumulation in the roots compared to leaves.

Key words: Zinc · Chlorophyll · Enzyme activities · Oxidative stress · *Lycopersicon esculentum*

INTRODUCTION

Many parts of the world, especially near urban and industrial areas, are heavily polluted by heavy metals generally from human activity. Of all these heavy metals: Cd, Cu, Hg, Ni, Pb and Zn are the most dangerous. Nowadays, the use of mineral fertilizers and pesticides and the use of sludge and wastewater are among the main sources of contamination of cultivated soils by cadmium [1, 2].

Under conditions of metal stress, one of the remarkable features of some higher plants is their ability to concentrate excess metals absorbed in certain organs [3-5]. Inside the cell, excess metals may be absorbed from an association with organic ligands in order to maintain these ions at a particularly low level of activity in the cytosol. The association with organic or inorganic acids or sequestration in specialized organelles appears to be among the means used by intracellular detoxification in higher plants [1, 6-9]. Other methods of protection is also included organic synthesis of peptides of stress metal "as well as phytochelatins that can ensure the transport of these metals to the vacuolar compartment [5, 10, 11]. However, these elements can also be transported to the leaves via the xylem where they will also be accrued and (or) redistributed to the youth or vegetative reproductive organs and back to the roots [12-14].

To counter this toxicity, cells contain antioxidants such as α-tocopherol, β-carotene, glutathione (GSH) and ascorbate in addition to other antioxidant enzymes: Superoxide Dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) involved in eliminating reactive oxygen species [15, 16].

Since zinc is a metal trace elements considered essential for normal functions of the plant, we sought in this work to analyze the behavior of tomato plants exposed to different concentrations of zinc. We wanted to know what was its amount and its distribution at these roots and leaves. In addition, we analyzed the impact of pollution on the defense system "antioxidant" of the plant by measuring several enzyme activities play a major role in this defense.

MATERIAL AND METHODS

Culture Conditions: The seeds of tomato (*Lycopersicon esculentum* Var. Rio Grande) are disinfected with a solution of hydrogen peroxide at 10% (v/v), rinsed thoroughly with distilled water and then germinated in Petri dishes lined filter paper soaked in distilled water.

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Germination was conducted in the dark at 25±1°C. The 7-day old seedlings were then transplanted and maintained on nutrient solutions Hoshang base, whose pH is maintained between 5.5 and 6.5. The composition of culture medium is KNO3: 100.1 mg / l, KH_2PO_4: 136.09 mg / l iron citrate: 38.91 mg / l $ZnSO_4$: 0.28 mg/l H_3BO_3: 1.85 mg/l; $MgSO_4$: 246.4 mg/l, $CaCl_2$: 147 mg/l, $CuSO_4$, 0.25 mg/l $MnSO_4$, 0.84 mg/l.

After 12 days of culture, seedlings were transferred on the same medium supplemented or not with different concentrations of $ZnSO_4$ (0, 50, 100, 250, 500 microns) for 7 days. The different nutrient media are continuously aerated by bubbling compressed air. Crops are produced in the laboratory hood.

Analytical Techniques

Rate of Chlorophylls (a and b): The extraction of chlorophyll is measured by the method of [17], which consists of a maceration of the plant in acetone. Sample processing is as follows: 1g of leaves were weighed, cut into small pieces and crushed in a mortar with 20 ml of 80% acetone and about 100 mg of calcium bicarbonate ($CaCO_3$). After grinding all, the solution is then filtered and placed in black boxes to prevent the oxidation of chlorophyll by light. The reading is done at two wavelengths 645 nm and 663 nm, after calibration of the device with the control solution of 80% acetone. The equation that allows us to calculate the values of chlorophylls [18] is:

Chl. a = 12.70 x DO (663) – 2.69 x DO (645)
Chl. b = 22.90 x DO (645) – 4.60 x DO (663)

Enzyme Assays: After 12 days of treatment, the fresh roots (1g) were ground in a cold mortar with 5 ml of phosphate buffer (80mm, pH7.5). The homogenate is then filtered through a cloth properly before centrifugation at 12000g for 20min Cold (centrifuge Sigma 3-16K). The supernatant obtained was used as enzyme extract for determination of different enzyme activities.

Determination of Catalase Activity (CAT): The spectrophotometric determination of catalase activity (CAT) was made following the method [19]. The decrease in absorbance was recorded for three minutes (spectrophotometer Jenway 6300) for a wavelength of 240 nm and a molar extinction coefficient $\varepsilon = 39400\ M^{-1}.cm^{-1}$.l To reach a final volume of 3 ml, the reaction mixture contains: 100 µl of the crude enzyme extract, 50 µl of hydrogen peroxide to 0.3% H_2O_2 and 2850 µl of phosphate buffer (50 mM, pH7.2). The calibration of the device is in the absence of the enzyme extract. The reaction is initiated by the addition of hydrogen peroxide. The catalase activity is expressed as nmol /min/mg of protein.

Determination of Glutathione S-Transferase activity (GST): The determination of glutathione S-transferase is produced by the method of [20] the samples were homogenized in phosphate buffer at pH 6.5 and 100 mM than centrifuged at 9000g for 30 min. The method involves reacting the mixture on a GSTS CDNB (20 mM)-GSH (100 mM) the change in optical density due to the appearance of CDNB-GSH complex was measured every 15 seconds for 2 minutes at 340nm. Concentrations of GST are expressed in nmol /min/mg of protein.

Determination of the Activity-ascorbate Peroxidase (APX): The spectrophotometric determination of ascorbate peroxidase activity is carried out following the procedure adopted by [21]. The final reaction volume of 3ml contains: 100µl of enzyme extract, 50µl of 0.3% H_2O_2 and 2850µl phosphate buffer NaK-Ascorbate (50 mM NaK, 0.5 mM ascorbate, pH7.2). The calibration of the device is in the absence of the enzyme extract. Lecture is performed at 290 nm (spectrophotometer GeneSys 8) for 1min and for a linear molar extinction coefficient $\varepsilon = 2800\ M^{-1}.cm^{-1}$. APX activity is expressed as nmol/min/mg of protein.

Analysis of Metals: The root system and aerial part previously dried in an oven at 70°C until a constant weight (between 24 and 72) are individually subjected to a hot mineralization with nitric acid HNO3 70% [22]. After complete evaporation of the mixture and obtaining a dry residue of whitish color, a standard volume (20 ml) of nitric acid solution 1% (v / v) was added to dry residue. The dosage is made on minerals extracted nitric using an atomic absorption spectrophotometer.

RESULTS

Impact of Zinc Treatment on the Synthesis of Chlorophyll: From Figure 1, we observed that the presence of Zn (essential trace element in various functions of the plant) increases the rate of chlorophyll "a" for small doses (50 and 100 microns). Beyond these concentrations, the rate decreases to a minimum value (8.43 mg / g FM) to 500 microns, equivalent to 30% reduction compared to the control while the rate of chlorophyll "b" increases for the different concentrations of zinc. The ratio a / b follows the same variation in the rate of chlorophyll a.

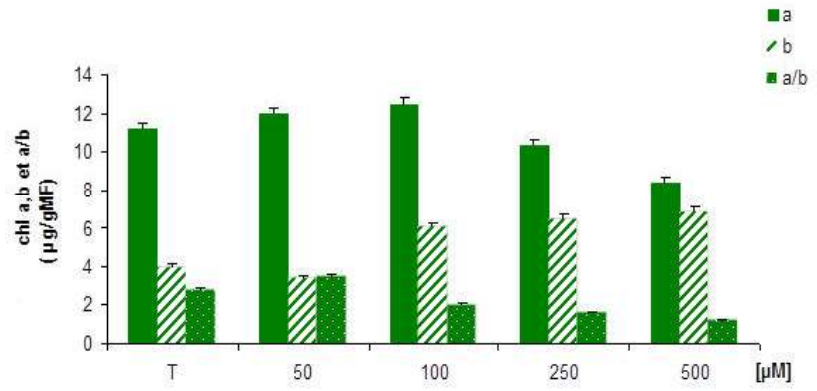

Fig. 1: Effect of Zn on chlorophyll a, b and c / b in tomato treated for 7 days

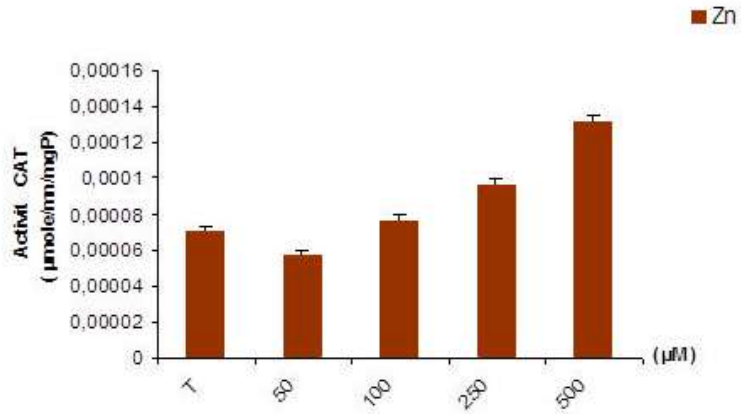

Fig. 2: Effect of Zn on the catalase activity in tomato roots treated for 7 days.

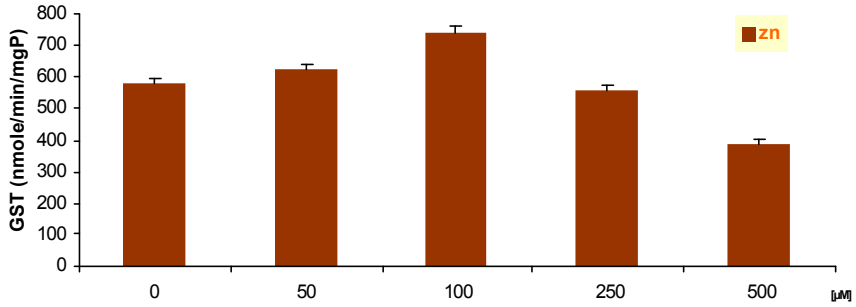

Fig. 3: Effect of Cd, Zn and interaction Cd / Zn activity GST in tomato roots treated for 7 days.

The statistical study revealed highly significant differences (**) the rate of chlorophyll a and b as functions of increasing concentrations of the metal.

Analysis of Enzyme Activities in Tomato Roots Treated with Zn for 7 Days

The Effect on Catalase Activity (CAT): Catalase is mainly localized in the peroxisome, is involved in degradation of H_2O_2 generated by cadmium [23].

From Figure 2 we observed that treatment with zinc stimulates the activity of CAT enzyme in higher concentrations (250 and 500 microM), whereas this activity was inhibited for concentrations (50 and 100 microns).

The analysis of variance controlled reveals highly significant differences (**) of catalase activity as a function of increasing concentrations of the metal.

The effect on the activity glutathione S-transferase (GST)

From Figure 3, analysis of GST activity in tomato plants treated with zinc showed a total inhibition of this activity at different concentrations with a slight stimulation at 100 microns equivalent to 738.09 nmol / min / mg protein.

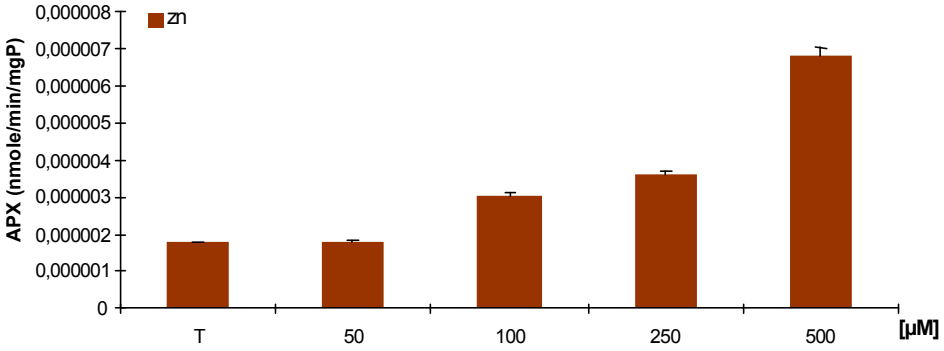

Fig. 4: Effect of Zn on APX activityin tomato roots treated for 7 days.

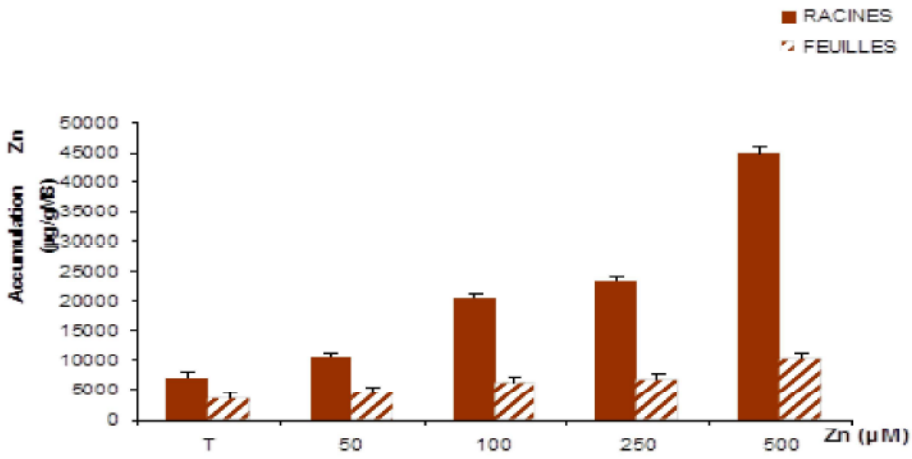

Fig. 5: Zn accumulation in roots and leaves after 7 days.

This was confirmed by analysis of variance with two factors controlled who found highly significant differences (**) of the activity of GST depending on the concentrations of the metal.

The Effect on the Activity Ascorbate Peroxidase (APX): The induction of APX activity, an enzyme important in the defense system is a response to oxidative stress in plants. The APX protects the cell against oxidative damage by H2O2 toxicity.

The results obtained and shown in Figure 4 show that APX activity is inhibited at 50 microns, while a Zn induction is observed at higher concentrations to reach a maximum at 500 microns (1,7316.10-6 nmol / min / mgProt). This was confirmed by analysis of variance showed highly significant differences (**) APX activity as a function of increasing concentrations of the metal.

Analysis of Metals (Zn assay): Figure 5 shows that the accumulation of zinc in roots and leaves increased with increasing concentrations of metal (Zn) except that this accumulation is higher in roots than in leaves where it reaches a maximum are (49.7, 20.5 mg / g DM) at the highest concentration. The amount of zinc retained in the leaves varies between 35% and 30%, whereas it is 65% and 70% in roots, relative to total Zn absorbed by the plant.

The analysis of variance with two factors control revealed very highly significant differences between (***) accumulation of Zn in roots and leaves and depending on the metal concentrations increased.

DISCUSSION

Our results show that the presence of zinc in the culture medium involves its passage from the roots to different parts of the plant and that most of the absorbed zinc is accumulated in the roots of tomato. Our results are consistent with those obtained by [24] which show a high accumulation of Zn in both organs (roots and leaves) this is due to the role played by the latter in controlling the permeability of water in the cell. All of these observations allows us to confirm the hypothesis that the roots of some higher plants can serve as a body trap interposed export of metal pollutants towards the top, sites of various physiological processes [9, 13, 25, 26].

The Determination of chlorophyll in leaves of tomato exposed to high concentrations of zinc showed a decrease in its amount compared with the control which confirms the results of [9, 27-30] who report the frequent degeneration chlorophyll and carotenoids in plants exposed to different concentrations of heavy metals. [31, 32] explain this regression by the existence of oxidative damage and disruption of membrane potential and electrochemical ($\Delta\mu H$ + and $\Delta\Psi$) induced by the accumulation of heavy metals. While zinc stimulates the biosynthesis of chlorophyll at low concentrations.

Biochemical analysis of GST activity (glutathione S-transferase) in the presence of zinc in its nature as trace element essential functions of the plant did not induce oxidative stress in roots where inhibition seen at 100 microns except where there was a slight stimulation.

The CAT activity in tomato roots treated with zinc has an inhibitory function in increasing concentrations of the metal with a slight induction at 500 microns. This is consistent with the results of [33, 34, 35] showing that high concentrations of Zn induce severe toxicity that can destabilize the metabolic balance.

Finally, treatment with Zn does not appear to have any stress to the cell and at low levels that this element is essential to the functioning of the cell. High doses of Zn generate severe toxicity resulting in an establishment in the cells of plants treated with a defense system that is manifested by stimulation of APX activity, which clearly demonstrates its role in the elimination of hydrogen peroxide (H_2O_2) formed through the accumulation of Zn in the roots of tomato [36].

REFERENCES

1. Wagner G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health, Adv. Agron., 51: 173-212.
2. Ravera O., 2001. Monitoring of the aquatic environment by species accumulator of polluants. J. Limnol., 60: 63-78.
3. Hardiman R.T., B. Jacoby and A. Benin, 1984. Factors affecting the distribution of cadmium, copper and lead and their effect upon yield and zinc content in bush beans (*Phaseolus vulgaris L.*), Plant Soil, 81: 17-27.
4. Hart J.J., R.M. Welch, W.A. Norvell and L.V. Kochian, 2002. Transport interaction between Cd and Zn in roots of bread wheat and durum wheat seedling. Physiol Plant, 116(1): 73-78.
5. Ammar W.B., I. Nouari, M. Zarrouk, M.H. Ghorbel and F. Jemal, 2008. Antioxydative response to cadmium in roots and leaves of tomato plants. Biologia Plantarium, 52(4): 727-731.
6. Van Balen, E., S.C. Van de Geijn and G.M. Desomet, 1980. Autoradiographie evidence for the incorporation of cadmium into calcium oxalate crystals. Z. Pflanzenphysiol., 97: 123-133.
7. Godbold D.L., W.J. Host, J.C. Collins, D.A. Thurmann and H. Marshner, 1984. Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitose*.
8. Verkleij, J.A.C., P.C. Lolkema, A.L. De Needling and H. Harmens, 1991. Heavy metal resistance in higher plants: biochemical and genetic aspects. in Ecological reponses to environmental stresses. Kluwer Academc Publisher, Pays-Bas., pp: 8-19.
9. Zoghlami Boulila L., W. Djebali, W. Chaib and M.H. Ghorbel, 2006. Modification physiologiques et structurales induites par l'interaction cadmium-calcium chez la tomate *(Lycopersicon esculentum)*. C.R Biologies, 329: 702-711.
10. Neumann, D., O. Lichtenberger, D. Gunther, K. Tschiersch and L. Nover, 1994. Heat-shock proteins induce heavy-metal tolerance in higher plants. Planta, 194: 360-367.
11. Scheller, H.V., B. Huang, E. Hatch and P.B. Goldsbrough, 1987. Phytochelatines Synthesis and Glutathione levels in reponse to heavy metals in tomato cells. Plant Physiol., 85: 1031-1035.
12. Cataldo, D.A., R.E. Wildung and T.R. Garland, 1987. Speciation of trace inorganic contaminants in plants and bioavailability to animals: An overview. J. Environ. Qual., 16: 289- 295.
13. Djebali W., W. Chaibi and H.W. Ghorbel, 2002. Croissance, activité peroxydasique et modification ultrastructurales induites par le cadmium dans la racine de tomate, Can. J. Bot., 80 : 942-953.
14. Young Yang, Fu-Suo Zhang, Hua-Fen Li and Rong-Feng Jiang, 2009. Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. J. Environmental Management, 90: 1117-1122.
15. Razinger, J., M. Dermastia, L. Drinovec, D. Drobne, A. Zrimec and J.D. Koce, 2007. antioxidative responses of duckweed *(Lemna minor L.)* to short-term copper exposure. Environ. Sci. Pollut. Res. 14 (3): 194-201. doi:10.1065/espr2006.11.364.

16. Mediouni, C., W. Ben Ammar, G. Houlné, M.E. Chabouté and F. Jemal, 2009. Cadmium and copper induction of oxidative stress and antioxydative reponse in tomato (*Solanum lycopersicon*) leaves. Plant Growth Regul., 57: 89-99.
17. Holden, M., 1975. Chlorophylls in chemistry and biochemistry. 2nd Ed. Ed Academy press, New york. pp: 133.
18. Arnon, D.I., 1949. Cooper enzymes in isolated chloroplasts polyphenoloxydase in Betavulgaris. Plant physical., 24: 1-25.
19. Cakmak, I. and W.J. Horst, 1991. Effect of aluminium on lipid peroxidation, superoxidase dismutase, catalase and peroxidase activities in root tips of soybean *(Glycine max*). Physiol. Plant, 83: 463-468.
20. Habig W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathion S-Transferases, the First enzymatic step in mercapturic acid formation. The J. Biological Chemistry, 249: 7130-7139.
21. Nakano, Y. and K. Azada, 1987. Purification of ascorbate peroxidase in spinach chloroplasts : its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol., 28: 131-140.
22. Van Assche, F., C. Cardinaels and H. Clijsters, 1988. Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose-response relations in *Phaseolus vulgaris L.,* treated with zinc and cadmium, Environ. Pollut., 52: 103-115.
23. Foyer, C.H., M.L. Lelandais and K.J. Kunert, 1994. Photo-oxydative stress in plants, Physiol. Plant, 92: 696-717.
24. Rygal, J., W.M. Arnold and U. Zimmermann, 1992. Zinc and salinity effects on membrane transport in *Chara conivens*. Plant Cell Environ, 15: 11-23.
25. Jarvis, S.C., L.H.P. Jones and M.J. Hopper, 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant. Soil., 44: 179-191.
26. Dong, J., F. Wu and G. Zhang, 2006. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedling (*Lycopersicon esculentum*), Chemosphere, 64: 1659-1666.
27. Gallego, S.M., M.P. Benavides and M.L. Tomaro, 1996. Effect of heavy Metal ion excess on sunflower leaves: evidence for involvement of an oxidative stress. Plant Sci., 121: 151-159.
28. Moustakas, M., T. Lanaras, L. Symeonidis and S. Karataglis, 1997. Growth and some photosynthetic characteristics of field grown *Avena sativa* under copper and lead stress. Photosynthetica, 30: 389-396.
29. Singh, K.P., D. Mohan, S. Sinha and R. Dalwani, 2004. Impact assessments of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural and environmental quality in the wastewater disposal area. Chemosphere, 55: 227-255.
30. Sbartai, H., R. Rouabhi, I. Sbartai, H. Berrebbah and M.R. Djebar, 2008. Induction of anti-oxidative enzymes by cadmium stress in tomato (*Lycopersicon esculentum*). African J. Plant Sci. 2(8): 72-76.
31. Clijsters, H., A. Cuypers and J. Vangronsveld, 1999. Physiological responses to heavy metals in higher plants; defence against oxidative stress, Z. Naturforsch, 54c: 730-734.
32. Sanità di Toppi, L., M. Lambardi, L. Pazzagli, M. Cappugi, M. Durante and R. Gabrielli, XXXX. Reponse to cadmium in carrot *in vitro* plants and cell suspension cultures, Plant Sci., 137: 119-129.
33. Cakmak, I., R.M. Welch, B. Erenoglu, V. Romheld, W.A. Norvell and L.V. Kochian, 2000. Influence of varied Zn supply n re-translocation of Cd (^{109} Cd) and Rb (^{86} Rb) applied on mature leaf of durum wheat seedling. Plant Soil, 219: 279-284.
34. Abmann, S., K. Sigler and M. Hofer, 1996. Cd^{+2} induced damage to yeast plasma membrane and its alleviation by Zn^{+2}: studies on *schizosaccharomycetes* pompe cells and reconstituted plasma membrane vesicles. Arch Microbiol., 165: 279-284.
35. Zhao, Z.Q., Y.G. Zhu and Y.L. Cai, 2005. Effect of zinc on cadmium uptake by spring wheat (*Triticum aestivum* L): long-time hydroponic study and short-time 109 Cd tracing study, J. Zhejiang Univ. SCI, 6A(7): 643-648.
36. Chaoui, A., S. Mazhoudi, M.H. Ghorbal and E. El Ferjani, 1997. Cadmium and Zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean, Plant Sci., 127: 139-147.