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Full Length Research Paper

Induction of anti-oxidative enzymes by cadmium stress in tomato *(Lycopersicon esculentum)*

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In the present study, the cadmium's effect on anti-oxidative enzymes in tomato (*Lycopersicon esculentum*) plants was evaluated. Plants treated with increasingly $CdCl_2$concentrations (0, 20, 40, 80, 100 and 200 µM respectively) were grown in a basic nutrient solution for 7 days. APX (Ascorbate Peroxidase) and GPX (Guaïgol Peroxidase) activities showed to increase below 100 µM concentration after treatment. In excessive concentration however, more than determined level, a significant decrease in the enzymes activities was determined. The increase in enzymatic activity can be associated with induction of oxidative stress by cadmium treatment. Excessive calcium displayed also low APX and GPX activities, which were virtually the same activity levels of control plants. However, it should be note that concentration more than 80 µm of Cd resulted in decreasing of APX and GPX activity. The enzymatic activity of GSH followed in the presence of Cd showed a significant increase by 80 µM of Cd then the activity showed decreasing. Calcium treatment resulted in impairing of GSH stimulation in the presence of Cd conditions. Eventually, Cd treatment resulted in decline in GST activity that was significantly increased in the presence of calcium, suggesting stimulation of antioxidant enzymatic activities. Therefore, accumulation of Cd can be associated with oxidative stress, which may be inhibited and reduced its adverse effects by calcium treatment in the cell culture.

Keywords: Cadmium, Calcium, APX, GPX, GSH, GST, *Lycopersicon esculentum,* enzymatic activity.

INTRODUCTION

Many parts of the world, especially near urban and Industrial areas, are highly polluted by heavy metals resulted from human activity. Among all these heavy metals: Cd, Cu, Hg, Ni, Pb and Zn are the most dangerous. Now the use of mineral fertilizers, pesticides, sludge and sewage are among the main sources of soil contamination by cadmium (Wagner, 1993; Ravera, 2001).

Cadmium is relatively rare in the ecosphere (McBride, 1995). It is one of the most toxic pollutants and more mobile in the soil-plant system. Therefore, its assimilation and accumulation in the tissues of plants can be animal or human source of contamination by consumption Grant and Bailey (1995). Indeed, the Cadmium is an oxydoreductor toxic heavy metal not known to interact with living organisms. It can increase the formation of intracellular Reactive Oxygen Species (ROS) and generates an oxidative stress (Lagadic et al., 1997).

As a defence mechanism, the plant cells involve in enzymatic system consisting special antioxidants such as α-tocopherol, β-carotene, Glutathione-S-Transferase (GSH) and ascorbate. In addition to other antioxidant enzymes, Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT) and Glutathione reductase (GR), were involved in the eradication of reactive species with oxygen against this toxicity (Rosen, 2002).

Tripeptide glutathione consists of glutamate cysteine and glycine; it plays a crucial role in defending against free radicals (AOS) in plants that are under the oxidative stress conditions (De Vos et al., 1992; Ranieri et al., 1993). It is also, the precursor of phytochelatines that hold heavy metals in plants (Rosen, 2002).

In this study, we investigated in the first hand the behaviour of plants treated with a xenobiotic (cadmium) compound, and the antioxidantive protection system components in which GST (Glutathione-S-Transferase)

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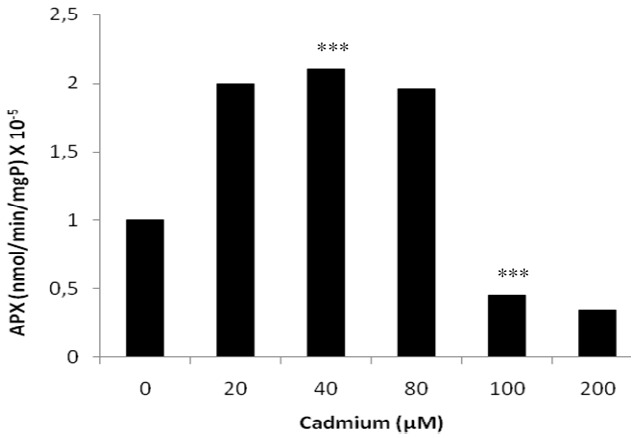

Figure 1. APX Activity of Tomato roots treated with different concentrations of Cd ($p<0.001$).

reduced glutathione (GHS), Ascorbate Peroxidase (APX) and Guaïgol-peroxidase (GPX) activities that have been associated with the possible role of calcium against cadmium in tomato (*Lycopersicon esculentum)* (Kauss, 1987; Hagemeyer and Breckle, 1996; Kinraide, 1998).

MATERIAL AND METHODS

Conditions of culture

The tomato seeds (*L. esculentum*, Rio Grandy Variety) were disinfected with a solution of peroxide hydrogen at 10% (V/V), rinsed thoroughly with distilled water, and then were placed into Petri plates and the sprout were grown on filter paper soaked in distilled water.

Germination was done in the dark at 25 ± 1°C. Seedlings older than 7 days were transplanted into nutrient solutions (Zoghlami-Boulila et al., 2006). pH was between 5.5 and 6.5. The composition of the culture medium was KNO_3: 100.1 g/l; KH_2PO_4: 136.09 g/l; Citrate iron: 38.91 g/l; $ZnSO_4$: 0.28 g/l; H_3BO_3: 1.85 g/l; $MgSO_4$: 246.4 g/l; $CaCl_2$: 147 g/l; $CuSO_4$: 0.25 g/l; $MnSO_4$: 0.84 g/l.

After 12 days in culture, the seedlings were transferred in the same medium consisting $CdCl_2$ in different doses (0, 20, 40, 80, 100 and 200 µM) or control without $CdCl_2$ for 7 days. In order to investigate cadmium-calcium interaction, calcium $CaCl_2$ (1mM) was added to the basic solutions containing different concentrations of $CdCl_2$.

The different nutritional mediums were continuously aerated with a bubbling compressed air pump under the hood in Laboratory.

Analytical techniques

Enzymatic extraction

Tomato roots were extracted according to Loggini (1999). The extract was used for the estimation of the activity of ascorbate peroxidase (APX), Guaicol-peroxidase (GPX), and Glutathione-S-Transferase (GST).

At 12 days of treatment, 1 gram of roots sample was cooled, and crushed cold in a mortar with 5ml of phosphate buffer (pH 7.5). The homogenate was properly filtered before making any cold centrifuge 4°C at 12000 g for 20 min (Sigma 3-16K). The obtained supernatant was used as the extract for the determination of the different enzymatic activities.

Determination of APX

Ascorbate Peroxidase activity was measured according to the protocol of Azada and Nakano (1987). The final reaction volume of 3ml contains 100µl of enzyme extract, 50 µl of H_2O_2 in 0.3% and 2.85 ml of NaK-ascorbate buffer (50 mM of NaK, 0.5 mM of ascorbate, pH7.2). The absorbance was measured by spectrophotometer at 290 nm for 1min, for a coefficient of linear molar extinction $\varepsilon = 2800\ M^{-1}.cm^{-1}$. The APX activity was expressed as nmol/min/mg of protein.

Determination of GPX

The guaïacol-peroxidase (GPX) activity is determined by colorimetric method (spectrophotometer Jenway 6300) at 470 nm (Chaoui et al., 1997) the coefficient of linear molar extinction is $\varepsilon = 2470\ M^{-1}cm^{-1}$. The final volume of 3 ml contains 100 µl of enzyme extract, 50 µl of H_2O_2 in 0.03% and 2.85 ml of phosphate-Guaïacol buffer (50 mM of NaK, 8 mM of gaïacol, pH 7.2). The reaction was started by the addition of peroxide hydrogen. GPX activity is expressed as nmol/min/mg of protein.

Determination of GSH

The enzyme extract was homogenized in a solution of 0.02 M EDTA, then, it undergoes a disinfecting, moistening by sulfo-Salicylic acid 0.25%. After centrifugation at 2000 g for 10min, supernatant was used for the spectrophotometric assay at 412 nm with the reagent DTNB at 0.01 million. The GSH activity was expressed in µM/mg of protein (Anderson, 1985).

Determination of the GST and total protein

Glutathione-S-Transferase level was estimated using Habig (1974) method. Samples were homogenized in a 100 mM of phosphate buffer in pH 6.5, and then it was centrifuged at 9000 g for 30 min. The method was based on the reaction of the GSTS in a mixture of CDNB (20 mM) and GSH (100 mM), the change in optical density is due to the emergence of complex CDNB-GSH that measured every 15 seconds for 2 min at 340 nm. Concentration of the GST was expressed in nmol/min/mg of protein. The total protein level was measured according to Bradford (1976) method.

RESULTS AND DISCUSSION

In despite of their positive role in several metabolic processes, heavy metals cause severe cellular damage (Kyunghee and Junghoon, 2001). The molecular mechanisms by which cells defend against stress generated by heavy metals are an important focus of research.

The results show a strong stimulation of the APX and GPX enzymatic activity in treated plants roots with low concentrations of Cd (Figure 1 and 3), this result shows accordance with the results of Chaoui et al. (1997).

Meanwhile, the increased activity of GSH were observed in our study (Figure 5), suggesting the involvement of this enzyme in the detoxification of (RSO) and free radicals (Asada and Takahashi, 1987). Moreover, compared to enzymatic activity recorded in control plants

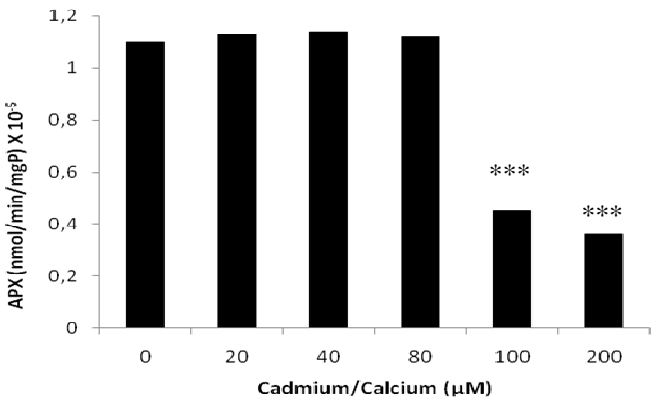

Figure 2. APX Activity of tomato roots treated with different concentrations of Cd and combined with Ca (Cd/Ca) ($p>0.05$), with 100 and 200 µM ($p<0.001$).

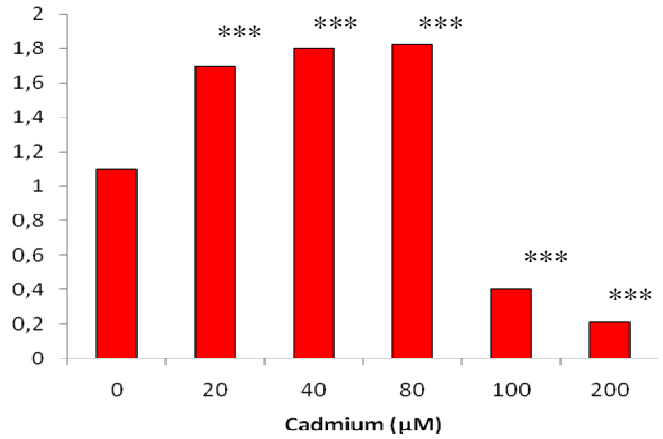

Figure 3. GPX activity of tomato roots treated with Cd ($p<0.001$).

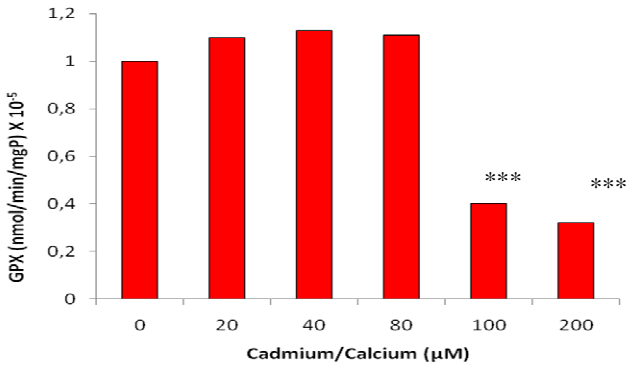

Figure 4. GPX Activity of Tomato roots treated with different concentrations of Cd and combined with Ca (Cd/Ca) ($p>0.05$).

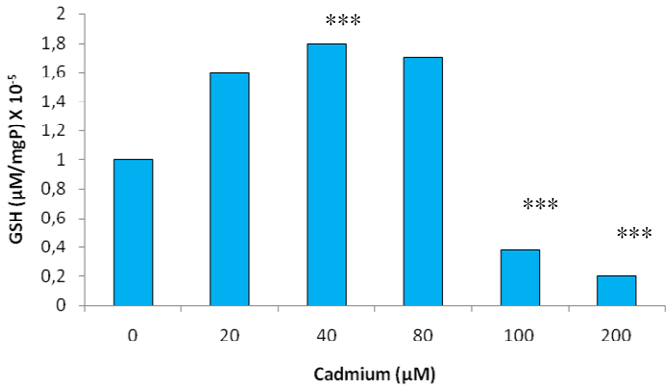

Figure 5. GSH Activity of Tomato roots treated with Cd ($p<0.001$).

and those processed by the low concentrations of Cd, the obtained results in treatment with 100 and 200 µM of Cd clearly put in evidence the toxic effect of this metal. This is fully consistent with the reported observations on *Brassica* by Zhu et al. (1999), *Pteris vitata* by Cao et al. (2004) and *Tlaspi caerulescens* by Freeman et al. (2004), where the standard of GSH is directly linked with increased tolerance to the accumulation of Cd at concentrations below 100 µM.

Likewise, the obtained results on *Scenedesmus bijugatus* by Prasad et al. (1999) and *Helianthus annuus* by Gallego et al. (2002) where the level of GSH decreases in response to stress produced by Cd and Cu at concentrations above 100 µM.

Variations of registered GST show that cadmium tends to stimulate this activity slightly at low concentrations (20 to 80 µM). Beyond these concentrations the toxic effect of this xenobiotic is clearly established (Figure 7). This result clearly highlights the role of this enzyme in the tolerance of tomato plants to cadmium.

The addition of 1mM of calcium raises significantly the inhibitory effect observed in the presence of cadmium for all measured enzymes (Figure 2, 4, 6 and 8). This effect is due to directly entering of the calcium ion in competition with cadmium for the same sites thereby blocking the influx of the metal within the cells. These results are compared with those obtained by Bernal and Ruvalcaba (1995) and Fellenberg (2000).

In conclusion, the results suggest that treatment with low concentrations of cadmium (under 100 µM) generates hydrogen Peroxide and low ROS accumulation (Un-Haing and Nam-Ho, 2004). This low accumulation may boost a range of defence systems in cell damage, these systems may lead to stimulation of antioxidant enzymatic activities (APX, GPX, GSH, GST), playing a decisive role in the detoxification of cells which are involved in (Chaffei et al., 2003).

The combined treatment Cd/Ca showed to measured activity level of the enzymes as in the cells of control plants. The addition of calcium lift the inhibition of enzymatic activity induced by cadmium treatment was as suggested in previous studies (Parameswaran and Majeti, 2003; Romero-Puertas et al., 2007).

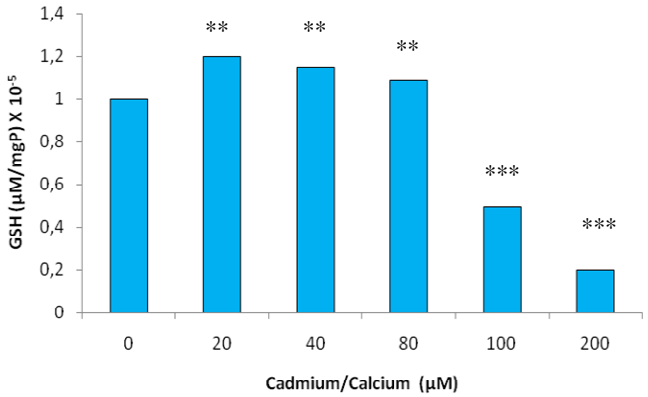

Figure 6. GSH Activity of Tomato roots treated with different concentrations of Cd and combined with Ca (Cd/Ca) (p<0.01).

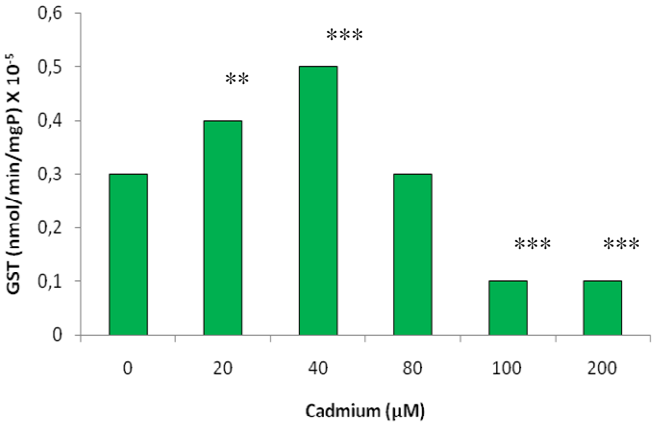

Figure 7. GST Activity of Tomato roots treated with Cd (p<0.001).

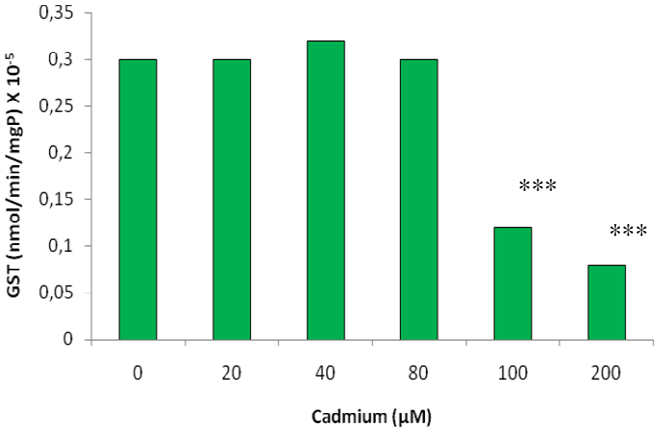

Figure 8. GST Activity of Tomato roots treated with different concentrations of Cd and combined with Ca (Cd/Ca) (p<0.001).

REFERENCES

Anderson ME (1985). Determination of glutathione and glutathione disulfide in biological samples. Method. Enzymol. 113:548-554.

Asada K and Takahashi M (1987). Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CJ, Arntzen CJ(Eds), Photo inhibition: topics in Photosynthesis .Elsevier , Amsterdam p.227.

Bernal J, Ruvalacaba S (1995). Pharmacological prevention of acute lead poisoning in *Paramecium*. Toxicology. 108: 165-173.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principale of protein-dye binding. Anal. Biochem.72: 248-254.

Cao X, Ma LQ and Tu C (2004). Antioxydative reponses to arsenic in the arsenic hyperaccumulator Chinese brake fern. Environ. Pollut. 128: 317-325.

Chaffei C, Gouia H, Masciaux C, Ghorbel MH (2003). Réversibilité des effets du cadmium sur la croissance et l'assimilation de l'azote chez la tomate (*Lycopersicum esculentum*). C.R. Biologies. 26: 326-329.

Chaoui A, Mazoudi S, Ghorbal MH and Ferjani EEL (1997). Cadmium and Zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus ulgaris* L). Plant Science. 127:139-147.

De Vos CHR (1992). Glutathione depletion due to copper induced phytochelati, synthesis causes oxidative stress in Silene cucubalus. Pant Physiol. 98: 853-858.

Fellenberg G (2000). The chemistry of pollution.3rd Edition John Wiley and Sons Ltd. 14:83-88.

Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering I and Salt DE (2004). Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell. 16: 2176-2191.

Gallego SM, Benavides M, Tomaro M (2002). Involvement of anti-oxidant defense system in the adaptive response to heavy metal ions in *Helianthus annuus* L. Cells. Plant Growth Regul. 36: 267-273.

Grant CA, Bailey LD (1995). Cadmium accumulation in corps, Canadian Network of toxicology Centers National Workshop on Cadmium transport into Plants. Workshop Proceedings Ottawa. 55-71.

Habig L (1974). J. Biol. Chem. 249: 7130-7139.

Hagemeyer J, Breckle SW (1996). Growth under trace element stress, in : A. Eshel, U.Kafkafi (Eds.), Plant Roots: the Hidden Half. New York. 415-433.

Kauss H (1987). Some aspect of calcium-dependent regulation in plant metabolism. Annu. Rev. Plant Physiol. 38: 47-72.

Kinraide BT (1998). Three mechanisms for the calcium alleviation of mineral toxicities. Plant Physiol. 118: 513-520.

Kyunghee L, Junghoon U (2001). Protection of metal stress in *Saccharomyces cerevisae*: cadmium tolerance requires the presence of two ATP-Binding domains of HSP104 protein. Bull. Korean Chem. Soc. 22(5): 514-517.

Lagadic L, Caquet T, Amiard JC, Ramade F (1997). Biomarqueurs en écotoxicologie : Aspects fonda-mentaux. , Edition Masson. 33, 53, 97.

Loggini F (1999) in Youbi M (2005). Effets de deux fongicides Artea et Punch nouvellenment introduits en Algérie sur la physilogie et le métabolisme respiratoire du blé dur (*Triticum durum Desf).*Thèse de Magister de l'Université Badji Mokhtar de Annaba.

McBride MB (1995). Toxic metal accumulation from agricultural use of sludge. Are US EPA regulations protective, J. Environ. Qual. 25: 1025-1032.

Parameswaran A, Majeti NVP (2003). Zinc alleviates cadmium-induced oxidative stress in *Ceratop-hyllum demersum* L : a free floating freshwater macrophyte. Plant Physiol. Biochem. 41(4): 391-397.

Prasad KVSK, Saradhi PP, Sharmila P (1999). Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in Brassica juncea. Environ.Exp. Bot. 42: 1-10.

Ranieri A, Lencioni L, Schenone G, Soldatini GF (1993). Glutathion-ascorbic acid cycle in pumpkin plants grown under polluted air in open top chambers. J. Plant Physiol. 142:286-290.

Ravera O (2001). Monitoring of the aquatic environment by species accumulator of polluants. J. Limnol. 60(1): 63 – 78.

Romero-Puertas MC, Corpas FJ, Rodriguez-Serrano M, Gomez M, Del Rio LA, Sandalio LM (2007). Differential expression and regulation of antioxidative enzymes by cadmium in pea plants. J. Plant Physiol. 164(10): 1346-1357.

Rosen BP (2002). Biochemistry of arsenic detoxification. FEBS. Lett.

529: 86-92.
Rucinska R, Waplak S, Gwozoz EA (1999). Free radical formation and activity of antioxidant enzymes in lupin roots exposed to lead. Plant Physiol. Biochem. 37: 187-194.
Un-Haing C, Nam-Ho S (2004). Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Science.168(1): 113-120.
Vangronsveld J, Weckx J, Kubacka-Zebalska M, Clijsters H (1993). Heavy metal induction of ethylene production and stress enzymes: II. Is ethylene involved in the signal transduction from stress perception to stress responses? In: Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean *Phaseolus vulgaris* L. Plant Science, 127: 139-147.
Wagner GJ (1993). Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. 51: 173- 212.
Weckx J, Clijsters H (1996). Oxidative damage and defence mechanism in primary leaves of *Phaseolus vulgaris* L as result of root assimilation of toxic amounts of copper. Physiol. Plant. 96: 506-512.
Zhu YL, Pilon-Smits E, Tarun AS, Jouanin L, Terry N (1999). Overex pression of glutathione synthetase in indian mustard enhances cadmium accumulation and tolerance. Plant Physiol. 119: 73-80.
Zoghlami-Boulila L, Djebali W, Chaib W, Ghorbel MH (2006). Modification physiologiques et struc-turales induites par l'interaction cadmium-calcium chez la tomate (Lycopersicon esculentum). C.R Biologies. 329: 702-711.