BIOLOGIA PLANTARUM 52 (2): 314-320, 2008

Glutathione and phytochelatin contents in tomato plants exposed to cadmium

W. BEN AMMAR*, C. MEDIOUNI, B. TRAY, M.H. GHORBEL and F. JEMAL

Faculté des Sciences de Tunis, Campus Universitaire, 1060 Tunis, Tunisia

Abstract

The effect of cadmium on growth and contents of glutathione (GSH) and phytochelatins (PCs) were investigated in roots and leaves of tomato plants (*Lycopersicon esculentum* Mill. cv. 63/5 F1). The accumulation of Cd increased with external Cd concentrations and was considerably higher in roots than in leaves. Dry mass production decreased under Cd treatment especially in leaves. In both roots and leaves, exposure to Cd caused an appreciable decline in GSH contents and increase in PCs synthesis proportional to Cd concentrations in the growth medium. At the same Cd concentration, PCs production was higher in roots than in leaves. The implication of glutathione in PC synthesis was strongly suggested by the use of buthionine sulfoximine (BSO). The major fraction of Cd accumulated by tomato roots was in the form of a Cd-PCs complex.

Additional key words: buthionine sulfoximine, complex Cd-PC, *Lycopersicon esculentum*.

Introduction

Cadmium is not essential for plant growth, but it is readily taken up by many plant species. Increased availability of Cd for root uptake may cause considerable alterations in mineral nutrition (Ben Ammar *et al.* 2005, Drazić *et al*. 2006), lipid biosynthesis (Nouairi *et al*. 2005), photosynthetic rate (Pietrini *et al*. 2003), and nitrogen metabolism (Gouia *et al*. 2003). Consequently, this led to a severe growth inhibition (Agrawal and Sharma 2006, Scebba *et al*. 2006). Higher plants, algae and some fungi react to excess Cd by stimulating sulphate absorption (Nocito *et al*. 2002) and production of phytochelatins (PCs) involved in Cd chelation and transport into vacuoles (Clemens 2001, Inouhe 2005). Phytochelatins or poly(γ-glutamyl-cysteinyl)glycines are small metal-binding peptides with the structure (γ -Glu-Cys)n-Gly, in which n varies from 2 to 11 (Rauser 1995). They are synthesized from reduced glutathione (GSH) (Ha *et al*. 1999). In fact, PCs induction in the presence of Cd coincided with a transient decrease in GSH content. Furthermore, the exposure of plants to an inhibitor of GSH biosynthesis, buthionine-S-sulfoximine (BSO), conferred depletion in PCs contents. Moreover, studies of transgenic *Brassica juncea* plants, in which the expression of the GSH biosynthetic pathway enzymes was increased, have shown that PCs biosynthesis and Cd tolerance have been correlated with overexpression of GSH (Zhu *et al*. 1999). The polymerization reaction of glutathione to form phytochelatins was catalyzed by phytochelatin synthase (Li *et al*. 2004, Clemens 2006). The PCs biosynthetic pathway may be regulated by a number of mechanisms, one of these was likely to be the regulation of GSH biosynthesis (Zhu *et al*. 1999). The crucial role of PCs in plant Cd^{2+} detoxification pathway was supported by the isolation of two mutants of *Arabidopsis*, cad1 and cad2, which are deficient in PC and GSH biosynthesis, respectively, and are consequently more sensitive to Cd (Cobett *et al.* 2000). Owing to their configuration and their high cysteine content, PCs are capable of chelating heavy metals, thereby reducing the concentration of cytotoxic free metal ions. PC-Cd complexes are sequestered in the vacuole. Some indications seem to attribute an important role to glutathione in metal detoxification. Like PCs, GSH forms sulphide complexes that could be transported in the vacuole (Rea *et al.* 1998).

Glutathione was considered as an abundant low molecular mass thiol in plants (Rennemberg 1982). Under normal conditions it was predominantly present in its reduced form (GSH), with only a small proportion of oxidized state (GSSG) (Noctor *et al*. 2002). The functions of GSH within the plant cell include *1*) a storage of reduced sulphur, *2*) a substrate for glutathione

Received 20 September 2006, *accepted* 9 March 2007.
Abbreviations: BSO - buthionine sulfoximine; GSH - glutathione; NPT - nonprotein thiol; PCs - phytochelatins; TBARS - thiobarbituric acid reactive substances.
* Corresponding author; fax: (+216) 71 885 480, e-mail: benammar.wided@voila.fr

S-transferases, which are involved in the removal of toxic compounds and in other key metabolic reactions, *3*) maintenance of the sulfhydryl groups of cysteine in a reduced form, and *4*) elimination of reactive oxygen species generated by heavy metals (Noctor et al. 2002). Likewise, glutathione was considered as an intermediary redox metabolite in the ascorbate-glutathione cycle of scavenging H_2O_2. The GSSG synthesized upon reduction of dehydroascorbate may be reduced by the glutathione reductase (GR; Noctor *et al*. 1998, Yu *et al*. 2000, Arbona *et al*. 2003).

In the present work, we have investigated how the presence of Cd, with or without BSO, in the growth medium of tomato plants interacted with glutathione and phytochelatins synthesis.

Materials and methods

Tomato (*Lycopersicon esculentum* Mill. cv. 63/5 F1) seeds were first sterilized in 10 % (v/v) H_2O_2 for 20 min, then, thoroughly washed with distilled water and germinated on moistened filter paper at 25 °C in the dark for 8-d. Individual plants were separated and hydroponically grown in the following medium: 0.5 mM KH_2PO_4, 1.25 mM Ca $(NO_3)_2$, 1.0 mM KNO_3, 0.5 mM $MgSO_4$, 100 µM Fe-SO_4-EDTA, 5 µM $MnSO_4 . 4\,H_2O$, 1 µM $ZnSO_4 . 7\,H_2O$, 1 µM $CuSO_4 . 5\,H_2O$, 30 µM H_3BO_3, 1 µM $(NH_4)_6Mo_7O_{24} . 4\,H_2O$. After an initial growth period of 10 d on control medium, some plants, used for studying growth and Cd accumulation, was directly treated with different concentrations (1, 5, 10, 25 and 50 µM) of $CdCl_2$. The other ones, used for non-protein thiols measurements, was performed in similar conditions except for Cd concentration which is kept at 10 µM with or without 100 µM buthionine sulfoximine (BSO; *Sigma*, St Louis, MO, USA) that was added to the growing medium 48 h before Cd treatment.

The plants were kept in a growing chamber at 20 to 25 °C and a 16-h photoperiod with irradiance of about 150 µmol(photon) m^{-2} s^{-1}. 7-d after Cd treatment, young leaves (leaves appeared after Cd treatment) and roots were harvested. Cd was removed from the root surface by placing the roots in a solution of 10 mM $CaCl_2$ for 30 min at 4 °C. Plant tissues was dried for 3 d in an oven at 80 °C for further determination of dry mass and mineral content or immediately frozen in liquid nitrogen, and stored at -80 °C.

To determine the Cd content, the various plant tissues were mineralized using a $HNO_3 : HClO_4$ (4:1, v/v) mixture. After mineralization the residues were solubilized in 7 ‰ HNO_3 and Cd concentrations in the tissue extracts were measured by atomic absorption spectrophotometry (*Perkin Elmer Analyst 300*, Norwalk, CT, USA).

Nonproteinthiols (NPT) were extracted by homogenizing frozen plant material in 10 % sulfosalicylic acid solution. The homogenate was centrifuged for 5 min at 13 000 *g* at 4 °C and the supernatants were immediately assayed for sulphydryl groups and GSH. The NPT content was measured spectrophotometrically with Ellman's reagent according to Ellman (1959): 0.25 cm^3 of acid soluble supernatant was neutralized with 0.5 cm^3 of Na-phosphate buffer (143 mM, pH 7.5). 0.02 cm^3 of 5,5'dithiobis[2-nitrobenzoic acid] (0.6 mM) was then added to that solution, followed by the measurement of A_{412}. Total glutathione and oxidized glutathione were assayed according to the method of Anderson (1985). The assay was based on sequential oxidation of glutathione by 5,5' dithiobis [2-nitrobenzoic acid] and reduced by NADPH in the presence of known amounts of GR. To quantify GSSG content, 2-vinylpyridine was added to the extract in order to eliminate the reduced form of glutathione. After 1 h of incubation in 25 °C, the 2-vinylpyridine was removed from the extract by addition of diethylether. Total phytochelatins concentrations were calculated by subtracting the amount of GSH from the total amount of NPT according to De Vos *et al*. (1992).

Frozen root material was powdered with in the presence of liquid nitrogen. The powder was then homogenized with extraction buffer Tris-HCl (20 mM, pH 8) mixed with 20 µM KCN, 0,5 mM ascorbate, 1 mM β-mercaptoethanol. After clearing by filtration through four layers of cheese cloth and subsequent centrifugation at 45 000 *g* for 30 min at 4 °C the extract was subjected to a gel filtration on *Sephadex G50*. Elution was performed using Tris-HCl (20 mM, pH 8) at a rate of 30 cm^3 h^{-1} and fractions of 5 cm^3 were collected to determine SH groups and cadmium concentrations. Cd content was measured as previously described. The concentration of SH groups in the fractions collected upon gel filtration was determined by spectrophotometry (*Perkin-Elmer Lambda I*) according to Ellman (1959).

Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reacting substances (TBARS), as described by Alia *et al.* (1995). The leaves and the roots were homogenized in 5 % (m/v) trichloroacetic acid (TCA). After centrifugation, a sample of the supernatant was added to 20 % TCA containing 0.5 % (m/v) thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 30 min. The absorbance was measured at 532 nm.

In all experiments three replicates were performed for each time of exposure to Cd and each tested concentration. Data presented here are the means ± SD of three independent experiments.

Results

Tomato plants exposed to $CdCl_2$ accumulated substantial amounts of Cd in the roots and leaves (Fig. 1*A*). There was a positive correlation between Cd concentrations in nutrient solutions and Cd content in roots and leaves. Moreover, Cd was mainly accumulated in roots. After 7-d exposure to 50 µM $CdCl_2$, the roots and the young leaves contained, respectively, 1846 and 20 µg g^{-1}(d.m.).

Increasing Cd concentrations in the nutrient solution induced a significant decrease in the biomass production of tomato plants (Fig. 1*B*). Young leaves seem to be more sensitive to Cd stress than roots and their dry mass decreased even in low Cd concentration (1 µM). In contrast, roots dry mass was slightly affected by Cd treatment. At 50 µM Cd, this reduction was about 80 % in the leaves and 31 % in the roots, as compared to controls. In spite of the high accumulation of Cd in roots, these organs seem to be more resistant. Growth inhibition was accompanied with visible symptoms of toxicity like chlorosis and necrosis.

GSH contents declined after the addition of Cd from 1600 nmol g^{-1}(f.m.) in leaves of untreated plants to 1000 nmol g^{-1}(f.m.) in leaves of plants treated with 1 µM $CdCl_2$ (Fig. 2*A*). The roots of untreated plants contained

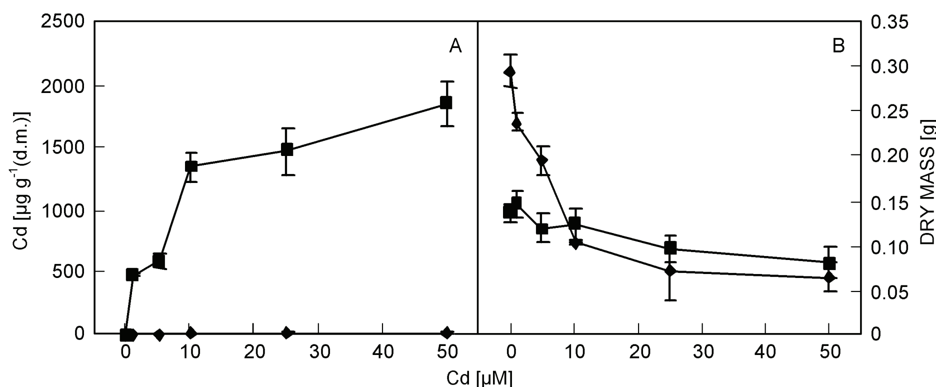

Fig. 1. Effect of cadmium supply on Cd accumulation (*A*) and dry mass production (*B*) of leaves (*diamonds*) and roots (*squares*) of tomato seedlings after 7-d exposure to various doses of cadmium. Means ± SE (*n* = 6).

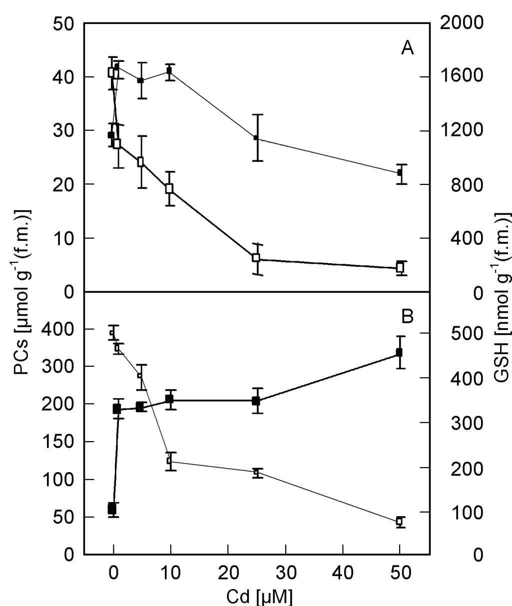

Fig. 2. Effect of Cd supply on the levels of total glutathione (GSH, *open squares*) and phytochelatins (PCs, *closed squares*) in leaves (*A*) and roots (*B*) of tomato seedlings after 7-d exposure to various doses of cadmium. Means ± SE (*n* = 3).

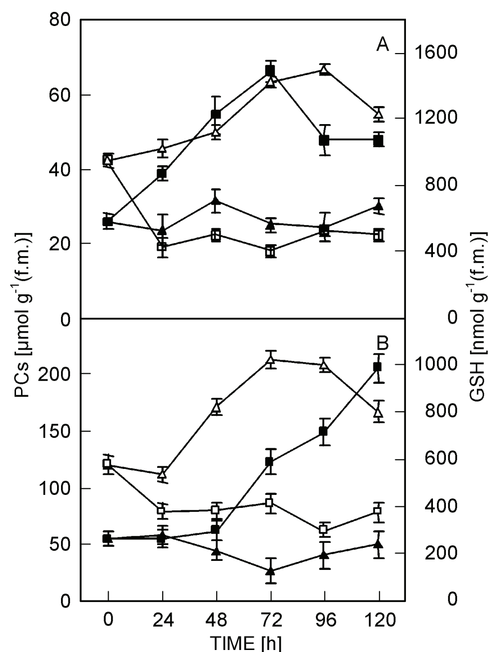

Fig. 3. Phytochelatins (PCs) accumulation and glutathione (GSH) depletion in leaves (*A*) and roots (*B*) of tomato exposed or not to 10 µM $CdCl_2$. Means ± SE (*n* = 3). *Open triangles* - GSH in control plants, *closed triangles* - PCs in control plants, *open squares* - GSH in Cd treated plants, *closed squares* - PCs in Cd treated plants.

500 nmol g^{-1}(f.m.) of GSH. Application of 50 µM $CdCl_2$ reduces the root GSH content to 100 nmol g^{-1}(f.m.) (Fig. 2*B*). The GSH reduction became more pronounced with increasing Cd treatment duration (Fig. 3).

In the leaves, treatment with 1, 5 and 10 µM Cd caused an increase in PCs content, but at higher concentrations PCs content decreased (Fig. 2*A*). PCs content increased from 60 µmol g^{-1}(f.m.), in the roots of control plants, to 193 µmol g^{-1}(f.m.), in 1 µM Cd treated ones and 266 µmol g^{-1}(f.m.) at 50 µM Cd (Fig. 2*B*). The PCs synthesis was associated with GSH decrease.

During the time course of experiment, the GSH content increased and a low PCs contents have been detected in leaves and roots of untreated plants (Fig. 3*A*,*B*). Addition of 10 µM $CdCl_2$ led to an immediate and significant decrease in the GSH content. At the same time, there was a marked increase in PCs contents, especially in the roots. However, in leaves, PCs concentrations decreased in leaves after treatment longer than 72 h.

Pre-treatment of plants with BSO, an inhibitor of γ-Glu-Cys synthetase, led to a significant depletion in the GSH contents both in leaves and roots (Fig. 4*A*,*B*). The decrease was more pronounced when plants were treated with BSO + 10 µM Cd. BSO treatment led, also, to a PCs synthesis inhibition.

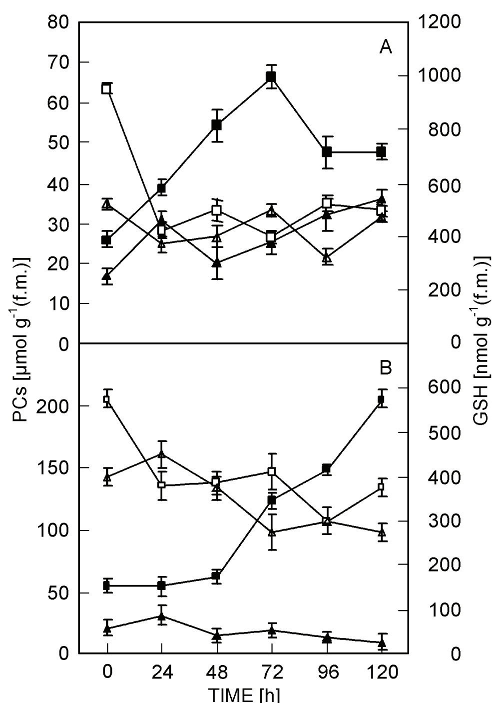

Fig. 4. Phytochelatins (PCs) accumulation and glutathione (GSH) depletion in leaves (*A*) and roots (*B*) of tomato exposed to 10 µM $CdCl_2$ with or without 100 µM BSO. Means ± SE (*n* = 3). *Open triangles* - GSH in plants treated with Cd + BSO, *closed triangles* - PCs in plants treated with Cd + BSO, *open squares* - GSH in Cd treated plants, *closed squares* - PCs in Cd + BSO treated plants.

In the root extract of untreated plants, a small quantity of Cd was associated with high molecular mass compounds (fractions 21 - 23) and the higher quantities were eluted in the low molecular mass fractions (fractions 40 - 55). In contrast, an additional intermediate UV absorbing peak appeared in the elution profile of the crude extract from Cd-treated plants. Superimposed to this UV peak, a Cd and SH groups were also detected (Fig. 5*A*,*B*). These results suggested that synthesis of Cd-binding peptides has been induced upon metal-treatment.

Cd induced oxidative damage as was demonstrated by a decrease of the GSH/GSSG ratio and by an increase of TBA content. In the non-treated leaves, GSH/GSSG ratio was at least 2 times higher than in the 50 µM $CdCl_2$ treated ones (Fig. 6).

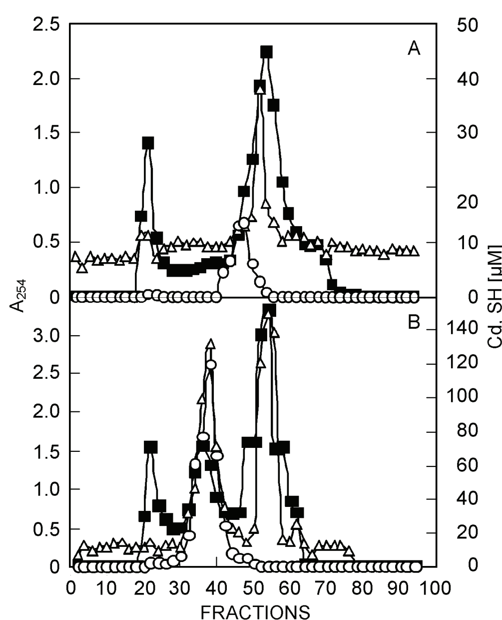

Fig. 5. Filtration chromatography on *Sephadex G50* of root extract of untreated (*A*) and treated tomato plants (*B*) with 10 µM $CdCl_2$. 60 µg of $CdCl_2$ was added to the control extract. *Squares* - absorbance at 254 nm, *triangles* - SH, *circles* - Cd.

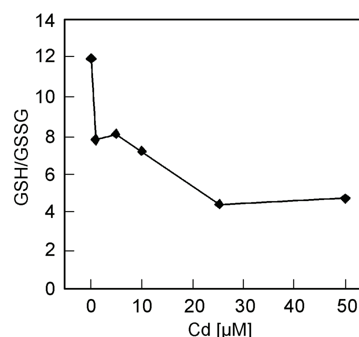

Fig. 6. Effect of Cd treatment in the GSH/GSSG ratio in tomato leaves. Means ± SE (*n* = 3).

Cd concentrations greater than 1 µM, caused a significant increase of lipid peroxidation products (Fig. 7). The content of thiobarbituric acid reactive substances (TBARS) increased from 26 nmol g^{-1}(f.m.) in

the control leaves to 67 nmol g^{-1}(f.m.) in leaves of plants treated with 50 µM $CdCl_2$, and from 10 nmol g^{-1}(f.m.) in the control roots to 32 nmol g^{-1}(f.m.) in roots of plants exposed to 50 µM $CdCl_2$.

The dry mass of leaves and roots of tomato plants treated by BSO, Cd and Cd+BSO was also determined (Fig. 8). The treatment of plants by BSO led to a small decrease in the leaves and the roots biomass production. The decrease of dry mass observed in the presence of Cd was accentuated by addition of BSO.

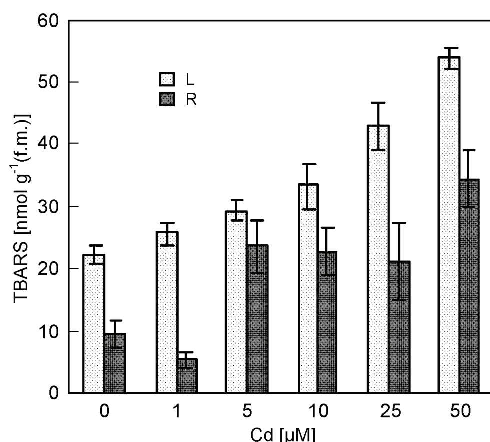

Fig. 7. Cadmium induced lipid peroxidation in tomato leaves (L) and roots (R) of treated plants. Means ± SE (n = 3).

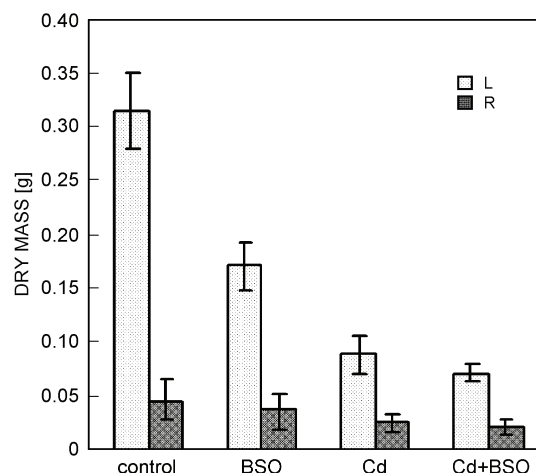

Fig. 8. Dry mass production of roots (R) and leaves (L) of tomato seedlings after Cd and BSO treatments. BSO was added to the culture medium 48 h before the Cd treatment. Means ± SE (n = 3).

Discussion

Tomato plants exposed to cadmium accumulated substantial amounts of Cd especially in roots. The higher accumulation of Cd in these organs may be due to their direct exposure to the metal. On the other hand, higher plant roots were considered as barriers of heavy metal translocation to shoots (Sandalio *et al*. 2001). This was an important factor to protect several physiological and metabolic processes active in shoots (Krupa *et al*. 1993). The presence of Cd, in the culture medium, resulted in an inhibition of leaf and root growth. The decrease in biomass production of leaves was mainly observed in spite of the low metal concentrations. However, root growth decreased only at the highest Cd concentrations. The higher sensitivity exhibited by tomato leaves compared to roots can be related to the different mechanisms of tolerance implicated by the two plant organs to alleviate the deleterious effects imposed by Cd like PCs synthesis.

In the untreated tomato plants, the content of PCs was rather low and this low content can be explained by the presence of Cu and Zn in the culture medium (Inouhe 2005). Cd-exposure led to an increase in the PCs amount in the different plant organs. PCs production, that has a crucial role in the intracellular metal chelation, has been considered as a general response of plants to heavy metal exposure. Their production has been reported in plant seedlings of several Gymnosperms as well as in Angiosperms (Inouhe *et al*. 2005), in fungi (Yurekli *et al*. 2004) and in algae (Scarano and Morelli 2002).

The accumulation of PCs was higher in roots than in leaves and was proportional to Cd-concentrations used. This can be related to the higher accumulation of Cd in roots (Grill *et al*. 1985, De Vos *et al*. 1992). The significant difference in PCs production between leaves and roots can explain, in part, the variability in Cd-sensitivity already announced between the two plant organs. In addition, production of PCs was coupled to a decrease in the GSH content. In spite of the high synthesis of PCs in roots, the GSH decrease was more pronounced in leaves. Similar results have been reported in tobacco plants (Rennenberg *et al*. 1982). It seems that GSH was transported from leaves to roots in order to participate to PCs biosynthesis (Meneguzzo *et al*. 1999). The inhibition of PCs synthesis following the application of BSO, an inhibitor of GSH synthesis, was an agreement with the hypothesis demonstrating that GSH constitute the precursor of PCs synthesis.

Experiment carried out by the gel filtration chromatography showed the presence of an additional peaks of UV absorption and of SH content in root extract from Cd-stressed plants, compared with untreated ones. This can be considered as a supplementary evidence for cadmium induces a PCs synthesis in tomato plants, estimated by SH-containing compounds. Moreover, the high amount of Cd in the root extract (95 %) was found in the intermediate peak that superimposed to the SH peak. In agreement with results of Jemal *et al*. (1998), above mentioned results proved that the PCs were implicated in the binding of absorbed Cd. Thus phytochelatins might be involved in the Cd detoxification

and plant tolerance to heavy metal stress (Hall 2002). This can diminish the availability of intracellular Cd and therefore reduce its toxicity. In fact, like other heavy metals, available Cd in the intracellular compartment can induce reactive oxygen species (ROS) generation and thus oxidative stress. ROS accumulation was reported to cause a breakdown of cell membrane lipids, evidenced by the accumulation of lipid peroxidation products such as malonyldialdehyde (MDA), a major product of poly-unsaturated fatty acid oxidation (Buege and Aust 1972). Our TBARS determination showed that Cd led to in increase in the lipid peroxidation both in leaves and in roots of tomato plants. Decreases in the GSH/GSSG ratio constitute a second evidence for Cd-induced an oxidative stress. On the other hand, decline in the GSH/GSSG ratio may explain the reduction in PCs content observed in leaves of plants treated with $CdCl_2$ concentrations greater than 10 μM and confirm the stimulation of GSH oxidation. The increase in the GSSG content suggested that glutathione was implicated in the elimination of ROS induced by Cd. Indeed, GSH was reported to be strongly implicated in the ascorbate/GSH cycle and in the protein thiol-disulphide redox state regulation (De Pinto *et al.* 2000).

Moreover, our results suggested that PCs have a very important role in tomato plants tolerance to Cd. In fact, the reduction in the leaves biomass production observed after BSO treatment becomes more pronounced when plants are treated by Cd+BSO. Roots growth, which seems to be unaffected by BSO treatment alone, showed a clear decrease after addition of Cd+BSO to the culture medium. Similar results have been presented by Xiang *et al.* (2001) who reported that *Arabidopsis* plants with low GSH content were unable to produce PCs and were hypersensitive to Cd.

Summarizing our results, we can conclude that, in tomato plants, Cd exposure lead to a great inhibition of biomass production especially in leaves and to an oxidative stress. The glutathione detected, in leaves as well as in roots, seems to be implicated both in ROS scavenging and in PCs synthesis. In roots, the increase in PCs production might be an efficient mechanism implicated in Cd chelation and can explain the difference in sensitivity between the two plant organs.

References

Agrawal, V., Sharma, K.: Phytotoxic effects of Cu, Zn, Cd and Pb on in vitro regeneration and concomitant protein changes in *Holarrhena antidysenterica*. - Biol. Plant. **50**: 307-310, 2006.

Alia, K.V.S.K., Prasad, P., Pardha Saradhi, P.: Effect of zinc on free radicals and proline in *Brassica* and *Cajanus*. - Phytochemistry **42**: 45-47, 1995.

Anderson, M.E.: Determination of glutathione and glutathione disulfide in biological samples. - Methods Enzymol. **113**: 548-555, 1985.

Arbona, V., Flors, V., Garcia-Agustin, P., Gomez-Cadenas, A.: Enzymatic and non-enzymatic antioxidant responses of *Carrizo citrange*, salt-sensitive citrus rootstock, to different levels of salinity. - Plant Cell Physiol. **44**: 388-394, 2003.

Ben Ammar, W., Nouairi, I., Tray, B., Zarrouk, M., Jemal, F., Ghorbal, M.H.: Effets du cadmium sur l'accumulation ionique et les teneurs en lipides dans les feuilles de tomate (*Lycopersicon esculentum*). - J. Soc. Biol. **199**: 157-163, 2005.

Buege, A.J., Aust, S.D.: Microsomal lipid peroxidation, - Method Enzymol. **52**: 302-310, 1972.

Clemens, S.: Molecular mechanisms of plant metal tolerance and homeostasis. - Plant **212**: 475-486, 2001.

Clemens, S.: Evolution and function of phytochelatin synthase. - J. Plant Physiol. **163**: 319-332, 2006.

Cobbett, C.S.: Phytochelatin biosynthesis and function in heavy-metal detoxification. - Curr. Opin. Plant Biol. **3**: 211-216, 2000.

De Pinto, M.C., Tommasi, F., De Gara, L.: Enzymes of the ascorbate biosynthesis and ascorbate-glutathione cycle in cultured cells of tobacco bright yellow (BY-2 line). - Plant Physiol. Biochem. **38**: 541-550, 2000.

De Vos, C.H., Marjolein, R.V.J., Vooijs, R., Schat, H.: Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. - Plant Physiol. **98**: 853-858, 1992.

Drazić, G., Mihailović, N., Lojić, M.: Cadmium accumulation in *Medicago sativa* seedlings treated with salicylic acid. - Biol. Plant. **50**: 239-244, 2006.

Ellman, G.L.: Tissue sulfhydryl groups. - Arch. Biochem. Biophys. **82**: 70-77, 1959.

Gouia, H., Suzuki, A., Brultfert, J., Ghorbal, M.H.: Effects of cadmium on the co-ordination of nitrogen and carbon metabolism in bean seedlings. - J. Plant Physiol. **160**: 367-376, 2003.

Grill, E., Winnacker, E., Zenk, M.H.: Phytochelatins the principal heavy-metal complexing peptides of higher plants. -Science **230**: 674-676, 1985.

Ha, S.B., Smith, A.P., Howden, R., Dietrich, W.H., Bugg, S., O'Connell, M.J., Goldsbrough, P.B., Cobbet, C.S.: Phytochelatin synthase genes from *Arabidopsis* and yeast *Schizosaccharomyces pombe*. - Plant Cell. **11**: 1153-1164, 1999.

Hall J.L.: Cellular mechanisms for heavy metal detoxification and tolerance. - J. exp. Bot. **53**: 1-11. 2002.

Inouhe, M.: Phytochelatins. - Braz. J. Plant Physiol. **17**: 65-78, 2005.

Jemal, F., Didierjean, L., Ghrir, R., Ghorbal, M.H., Burkard, G.: Characterization of cadmium binding peptides from pepper (C*apsicum annuum*). - Plant Sci. **137**: 143-154, 1998.

Krupa, Z.., Öquist, G., Huner, N.P.A.: The effects of cadmium on photosynthesis of *Phaseolus vulgaris*. A fluorescence analysis. - Physiol. Plant. **88**: 626-630, 1993.

Li, Y., Parkash, O.D., Laura, C., David, L., Alice, C., Julian, I.S., Rebecca, S.B., Richard, B.M.: Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. - Plant Cell Physiol. **45**: 1787-1797, 2004.

Meneguzzo, S., Navari-Izzo, F., Izzo, R.: Antioxidant responses of shoots and roots of wheat to increasing NaCl concentrations. - J. Plant. Physiol. **155**: 274-280, 1999.

Nocito, F., Pirovano, L., Cocucci, M., Sacchi, A.: Cadmium-

induced sulfate uptake in maize roots. - Plant Physiol. **129**: 1872-1879, 2002.

Noctor, G., Foyer, C.H.: Ascorbate and glutathione: keeping active oxygen under control. - Annu Rev. Plant Physiol. Plant mol. Biol. **49**: 249-279, 1998.

Noctor, G., Gomez, L., Vanacker, H., Foyer, C.H.: Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. - J. exp. Bot. **53**: 1283-1304, 2002.

Nouairi, I., Ben Ammar, W., Ben Youssef, N., Ben Miled Daoud, D., Ghorbal, M.H., Zarrouk, M.: Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. - Plant Sci. **170:** 511-519, 2005.

Pietrini, F., Iannelli, M.A., Pasqualini, S., Massacci, A.: Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis*. - Plant Physiol. **133**: 829-837, 2003.

Rauser, W.E.: Phytochelatins and related peptides. Structure, biosynthesis and function. - Plant Physiol. **109**: 1141-1149, 1995.

Rea, P.A., Li, Z.S., Lu, Y.P., Drozodowicz, Y.M., Martinoia, E.: From vacuolar GS-X pumps to multispecific ABC transporters. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 727-760, 1998.

Rennenberg, H.: Glutathione metabolism and possible biological roles in higher plants. - Phytochemistry **21**: 2771-2781, 1982.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C., Del Rio, L.A.: Cadmium induced changes in the growth and oxidative metabolism of pea plants. - J. exp. Bot. **152**: 2115-2126, 2001.

Scarano, G., Morelli, E.: Characterization of cadmium and lead-phytochelatins complexes formed in marine microalgae in response to metal exposure. - BioMetals **15**: 145-151, 2002.

Scebba, F., Arduini, I., Ercoli, L., Sebastiani, L.: Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. - Biol. Plant. **50**: 688-692, 2006.

Xiang, C., Werner, B.L., Christensen, E.M., Oliver, D.J.: The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. - Plant Physiol. **126**: 564-574, 2001.

Yu, C..W., Murphy, T.M., Sung, W.W., Lin, C.H.: H_2O_2 treatment induces glutathione accumulation and chilling tolerance in mung bean. - Funct. Plant Biol. **29**: 1081-1087, 2002.

Yurekli, F., Unyayar, A., Porgali, Z.B., Mazmanci, M.A.: Effects of cadmium exposure on phytochelatin and the synthesis of abscisic acid in *Funalia trogii*. - Eng. Life Sci. **4**: 478-380, 2004.

Zhu, O.Y.L., Pilon-Smits, E.A.H., Jouanin, l., Terry, N.: Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. - Plant Physiol. **119**: 73-79, 1999.