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Cadmium-induced oxidative stress in two potato cultivars

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Abstract A hydroponic experiment was carried out to characterize the oxidative stress responses of two potato cultivars (*Solanum tuberosum* L. cvs. Asterix and Macaca) to cadmium (Cd). Plantlets were exposed to four Cd levels (0, 50, 100, 150 and 200 μM) for 7 days. Cd concentration was increased in both roots and shoot. Number of sprouts and roots was not decreased, whereas Cd treatment affected the number of nodal segments. Chlorophyll content and ALA-D activity were decreased in both cultivars, whereas carotenoids content was decreased only in Macaca. Cd caused lipid peroxidation in roots and shoot of both cultivars. Protein oxidation was only verified at the highest Cd level. H_2O_2 content was increased in roots and shoot of Asterix, and apparently, a compensatory response between roots and shoot of Macaca was observed. SOD activity was inhibited in roots of Asterix at all Cd treatments, whereas in Macaca it was only increased at two highest Cd levels. Shoot SOD activity increased in Asterix and decreased in Macaca. Root CAT activity in Asterix decreased at 100 and 150 μM, whereas in Macaca it decreased only at 50 μM. Shoot CAT activity was decreased in Macaca. Root AsA content in Macaca was not affected, whereas in shoot it was reduced at 100 μM and increased at 200 μM. Cd caused increase in NPSH content in roots and shoot. Our results suggest that Cd induces oxidative stress in both potato cultivars and that of the two cultivars, Asterix showed greater sensitivity to Cd levels.

Keywords Antioxidant system · ALA-D · Cd toxicity · Growth · *Solanum tuberosum* · Oxidative stress

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Introduction

Contamination of heavy metals such as cadmium (Cd) through human activities has been increased since the last century. In urban areas or agricultural land with a long history of crop production, the concentrations of trace elements, especially heavy metals, in soil can be higher than those found in parent materials (Senesi et al. 1999). Heavy metal inputs include those from commercial fertilizers, liming materials, and agrochemicals, sewage sludges and others wastes used as soil amendments, irrigation waters and atmospheric deposition (Senesi et al. 1999). Highly polluted soils containing over 100 mg kg^{-1} Cd were reported in China, France and some other countries (Kabata-Pendias and Pendias 2001).

At very high concentrations in soils, Cd can adversely affect plant growth and also human health after introduction to the food chain (Tiryakioglu et al. 2006). In plants, stomatal opening, transpiration, water balance and nutrient uptake have been reported to be affected by Cd (Barceló and Poschenrieder 1990). Cd also results in negative effects on photosynthetic enzymes, particularly the enzymes involved in the Calvin cycle and chlorophyll biosynthesis such as the enzyme δ-aminolevulinic acid dehydratase (ALA-D) (Vitória et al. 2001). ALA-D is a metalloenzyme that catalyses the asymmetric condensation of two molecules of ALA (δ-aminolevulinic acid) to form porphobilinogen (PBG) (Noriega et al. 2007).

One possible mechanism which elevated concentrations of Cd may damage plant tissues is the stimulation of reactive oxygen species (ROS) production, by imposing oxidative stress (Benavides et al. 2005). ROS include superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) which are necessary for the correct functioning of plants, but in elevated concentrations they react with lipids, proteins, pigments, and nucleic acids and cause membrane damage, inactivation of enzymes, thus affecting cell viability (Gratão et al. 2005).

Plants possess several antioxidative defense systems to scavenge ROS in order to protect themselves from the oxidant stress including that caused by heavy metals (Benavides et al. 2005). The antioxidative system falls into two general classes: (1) low molecular weight antioxidants, which consist of lipid-soluble membrane-associated antioxidants such as α-tocopherol and β-carotene, and water-soluble reductants such as glutathione and ascorbate and (2) antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Gratão et al. 2005).

Two main research lines have been followed in studying plants exposed to Cd pollution, namely (1) identification and characterization of hyperaccumulating species that are potentially useful for phytoremediation and (2) studies on Cd accumulation and effects in crops of significant economic and alimentary interest (Rascio et al. 2008). Among the latter, potato occupies the fourth place in volume of world production of food crops, being only overcome by the wheat, maize and rice (FAO 2007). Moreover, of particular concern is the concentration of Cd in the tubers of potatoes which may contribute more than 50% of total human Cd consumption (Stenhouse 1992).

Genotypic variations in uptake and translocation of Cd in food crops have been observed not only among plant species but also among cultivars within the same species including potato (Dunbar et al. 2003; Liu et al. 2007). Based on these genetic differences, the manipulation of Cd uptake and translocation to plant tissues will provide a long-term effective and economical means in reducing Cd contamination in crops (Liu et al. 2007). However, the differences among potato cultivars to Cd toxicity are poorly understood. The potato cultivars have been shown to differ in their ability to accumulate Cd, although the differences between cultivars are not consistent (Dunbar et al. 2003).

In view of this, the objective of the present study was to contribute to a better understanding of the toxicology of cadmium in potato plantlets. In order to obtain these results, two potato cultivars were used to evaluate the effect of this metal on the enzymatic and non-enzymatic antioxidant system and its relation to pigments content, ALA-D activity, lipid peroxidation and protein oxidation.

Materials and methods

Plant materials and growth conditions

Tissue culture potato (*Solanum tuberosum* L.) plantlets were from the Potato Breeding and Genetics Germplasm Program, Universidade Federal de Santa

Maria, RS, Brazil. Two potato cultivars, Asterix and Macaca, widely planted in southern Brazil, were used in this study. Explants of 1.0 cm nodal segments without leaves were micropropagated in MS medium (Murashige and Skoog 1962), supplemented with 30 g l^{-1} of sucrose, 0.1 g l^{-1} of myo-inositol and 6 g l^{-1} of agar.

Fifteen-day-old plantlets from in vitro culture were transferred into plastic boxes (10 l) with polystyrene plates with holes that were used as a physical support for the plants; roots were submerged in aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition (mg l^{-1}): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. Evaporate and transpired water was continuously replaced with distilled water and the nutrient solution was completely renewed every week. After 2 weeks of plantlets acclimatization, Cd was added to nutrient solution as cadmium chloride ($CdCl_2 \cdot H_2O$) to final concentrations of 0 (control), 50, 100, 150 and 200 μM.

After 7 days of Cd exposure, 24 plantlets per replicate (each treatment consisted of three replicates) were randomly harvested from hydroponic recipients and potato plantlets were carefully washed with distilled water and then divided into roots and shoot for growth and biochemical analysis.

The levels of Cd utilized in the present study were above the toxic thresholds of many plants in the environment. However, the pure hydroponic system was designed to study basic phytotoxic effects, and not to mimic situations found in agricultural or natural ecosystems (Hernández et al. 1998).

Metal determination

To metal determination plantlets were oven-dried at 65°C to constant mass to determine Cd concentration. Dried plant tissues (0.01–0.1 g) were ground and digested only in 2.5 ml HNO_3. Samples decomposition was made in an open system utilizing a block digestor *Velp Scientifica* (Milano, Italy), heated at 130°C, during 2 h. Cd concentration was estimated by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) PerkinElmer *Optima 4300 DV* (Whaltam, USA) equipped with a cyclonic spray chamber and a concentric nebulizer.

Growth analysis

Growth of potato plantlets was determined by measuring the number of nodal segments, sprouts, roots, and leaves and shoot and root (Tennant 1975) length.

Carotenoids and chlorophyll contents

Carotenoids and chlorophyll contents were determined following the method of Hiscox and Israelstam (1979) and estimated with the help of Lichtenthaler's formula (Lichtenthaler 1987). Briefly, 0.1 g chopped fresh leaves sample was incubated at 65°C in dimethylsulfoxide (DMSO) until the tissues were completely bleached. Absorbance of the solution was then measured at 470, 645 and 663 nm in order to determine the contents of carotenoids, chlorophyll *a*, and chlorophyll *b*, respectively.

Delta-aminolevulinic acid dehydratase (ALA-D; E.C. 4.2.1.24) activity

Potato shoots were homogenized in 10 mM Tris–HCl buffer, pH 9.0, at a proportion of 1:1 (w/v). The homogenate was centrifuged at 12,000*g* at 4°C for 10 min. The supernatant was pre-treated with 0.1% Triton X-100 and 0.5 mM dithiotreithol (DTT). ALA-D activity was assayed as described by Morsch et al. (2002) by measuring the rate of porphobilinogen (PBG) formation. The incubation medium for the assays contained 100 mM Tris–HCl buffer, pH 9.0 and 3.6 mM ALA. Incubation was started by adding 100 μl of the tissue preparation to a final volume of 400 μl and stopped by adding 350 μl of the mixture containing 10% trichloroacetic acid (TCA) and 10 mM $HgCl_2$. The product of the reaction was determined with the Ehrlich reagent at 555 nm using a molar absorption coefficient of 6.1×10^4 l mol^{-1} cm^{-1} (Sassa 1982) for the Ehrlich-porphobilinogen salt.

Estimation of lipid peroxidation

The level of lipid peroxidation products was estimated following the method El-Moshaty et al. (1993) by measuring the concentration of malondialdehyde (MDA) as an end product of lipid peroxidation by reaction with thiobarbituric acid (TBA). Both roots

and shoot of potato plantlets were homogenized at 4°C in 20 ml of 0.2 M citrate-phosphate buffer, pH 6.5, containing 0.5% Triton X-100 at a proportion of 1:20 (w/v). The homogenate was filtered through two layers of paper and centrifuged for 15 min at 20,000*g*. One milliliter of the supernatant fraction was added an equal volume of 20% (w/v) TCA containing 0.5% (w/v) TBA. The mixture was heated at 95°C for 40 min and then quickly cooled in an ice bath for 15 min. After centrifugation at 10,000*g* for 15 min, the absorbance of the supernatant was measured at 532 nm. A correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm.

Protein oxidation

The reaction of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) was used to determine the amount of protein oxidation, as described in Levine et al. (1990). Both root and shoot of potato plantlets were homogenized with 25 mM K_2HPO_4 containing 10 ml l^{-1} Triton X-100, pH 7.0, at a proportion of 1:5 (w/v). The homogenate was centrifuged at 15,000*g* for 30 min at 4°C, the supernatant was used for immediate determination of protein oxidation. After DNPH-reaction, the carbonyl content was calculated by absorbance at 370 nm, using the extinction coefficient for aliphatic hydrazones (22 l $mmol^{-1}$ cm^{-1}).

Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 content of potato plantlets were determined according to Loreto and Velikova (2001). Approximately 0.1 g of both root and shoot were homogenized at 4°C in 2 ml of 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000*g* for 15 min and 0.5 ml of 10 mM potassium phosphate buffer pH 7.0 and 1 ml of 1 M KI. The H_2O_2 content of the supernatant was evaluated by comparing its absorbance at 390 nm with a standard calibration curve.

Superoxide dismutase (SOD; E.C. 1.15.1.1) activity

The activity of superoxide dismutase was assayed according to Misra and Fridovich (1972). About 0.2 g of both root and shoot of potato plantlets were homogenized in 5 ml of 100 mM K-phosphate buffer (pH 7.8) containing 0.1 mM ethylenediaminetetracetic acid (EDTA), 0.1% (v/v) Triton X-100 and 2% polyvinylpyrrolidone (PVP) (w/v). The extract was filtered and centrifuged at 22,000*g* for 10 min at 4°C, and the supernatant was utilized for assay. The assay mixture consisted of a total volume of 1 ml, containing glycine buffer (pH 10.5), 1 mM epinephrine and enzyme material. Epinephrine was the last component to be added. The adrenochrome formation in the next 4 min was recorded at 480 nm in UV-Vis spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation in the experimental conditions. This method is based on the ability of SOD to inhibit the autoxidation of epinephrine at an alkaline pH. Since the oxidation of epinephrine leads to the production of a pink adrenochrome, the rate of increase of absorbance at 480 nm, which represents the rate of autoxidation of epinephrine, can be conveniently followed. SOD has been found to inhibit this radical-mediated process.

Catalase (CAT; 1.11.1.6) activity

Catalase activity were determined from both root and shoot of potato plantlets homogenized in a solution containing 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 g l^{-1} PVP, 0.2 mM EDTA and 10 ml l^{-1} Triton X-100, at a proportion of 1:5 (w/v). The homogenate was centrifuged at 12,000*g* for 20 min at 4°C. The supernatant was used for determination of catalase activity according to the modified method of Aebi (1984) by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm in a reaction mixture with a final volume of 2 ml containing 15 mM H_2O_2 in 50 mM KPO_4 buffer (pH 7.0) and 30 µl of the extract.

Ascorbic acid and non-protein thiol groups contents

Both root and shoot of potato plantlets were homogenized in a solution containing 50 mM Tris–HCl and 10 ml l^{-1} Triton X-100 (pH 7.5), centrifuged at 6,800*g* for 10 min. To the supernatant obtained was

added 10% TCA at proportion 1:1 (v/v) followed by centrifugation (6,800*g* for 10 min) to remove protein. The supernatant was used to determine Ascorbic acid (AsA) and Non-protein thiol groups contents (NPSH) contents.

AsA determination was performed as described by Jacques-Silva et al. (2001). An aliquot of the sample (300 µl) was incubated at 37°C in a medium containing 100 µl TCA 13.3%, 100 µl deionized water and 75 µl DNPH. After 3 h, 500 µl of 65% H_2SO_4 was added and samples were read at 520 nm. A standard curve was constructed using L(+) ascorbic acid.

NPSH content in potato plantlets was measured spectrophotometrically with Ellman's reagent (Ellman 1959). An aliquot of the sample (400 µl) was added in a medium containing 550 µl 1 M Tris–HCl (pH 7.4). Reaction was read at 412 nm after the addition of 10 mM 5-5-dithio-bis (2-nitrobenzoic acid) (DTNB) (0.05 ml). A standard curve using cysteine was used to calculate the content of thiol groups in samples.

Protein determination

In all the enzyme preparations, protein was measured by the Coomassie Blue method according to Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

The experiments were done as randomized design. The analyses of variance were computed on statistically significant differences determined based on the appropriate *F*-tests. The results are the means ± SD of at least three independent replicates containing 24 plantlets of each cultivar. The mean differences were compared utilizing Duncan's multiple range test ($P < 0.05$).

Results

Cd concentration, visible symptoms, and plantlets growth

Cd concentration in both roots and shoot increased with Cd treatments. However, most of the Cd taken up by the plantlets was accumulated in roots (Fig. 1a, b). Roots of Macaca had significantly greater Cd concentration than Asterix. In shoot, Asterix had greater Cd concentration at 200 µM Cd level compared with Macaca.

As visible symptoms, older leaves of both potato cultivars showed necrosis at two highest Cd levels (150 and 200 µM). In time, some of leaves collapsed down.

Number of sprouts in both potato cultivars was not affected at all Cd treatments, and did not have any difference between the cultivars at the same Cd level (Fig. 1c). Number of nodal segments in Asterix was reduced at 200 µM Cd, but it was not affected by increasing Cd level in Macaca. Asterix had lower number of nodal segments at two highest Cd levels compared with Macaca (Fig. 1d). Number of leaves in Asterix was reduced upon addition of Cd level exceeding 50 µM. By contrast, number of leaves in Macaca was reduced only at two highest Cd levels. Asterix had significantly greater number of leaves at all Cd treatments than Macaca (Fig. 1e). Shoot length in Macaca was not affected by increasing Cd level, but it showed a decrease at two highest Cd levels in Asterix. Asterix showed lower shoot length at two highest Cd levels compared with Macaca (Fig. 1f). Number of roots in both potato cultivars was not affected at all Cd treatments, however, Macaca showed lower number of roots than Asterix at 0, 50 and 200 µM Cd (Fig. 1g). Root length in Macaca was not affected by increasing Cd level, whereas in Asterix it showed a Cd-dependent decrease. Asterix had significantly greater root length at all Cd treatments compared with Macaca (Fig. 1h).

Photosynthetic pigments and ALA-D activity

Carotenoids content in Asterix was not affected by increasing Cd level, but in Macaca it showed a decrease at two highest Cd levels. Moreover, Macaca had greater carotenoids content at 0 and 50 µM Cd compared with Asterix (Fig. 2a). Total chlorophyll content of both cultivars was significantly decreased at two highest Cd levels, but in Macaca chlorophyll content was greater than in Asterix at 50 µM Cd (Fig. 2b).

ALA-D activity in Asterix showed a continuous inhibition with increasing Cd level, whereas in Macaca it was reduced only upon addition of Cd level exceeding 50 µM. Moreover, Asterix showed

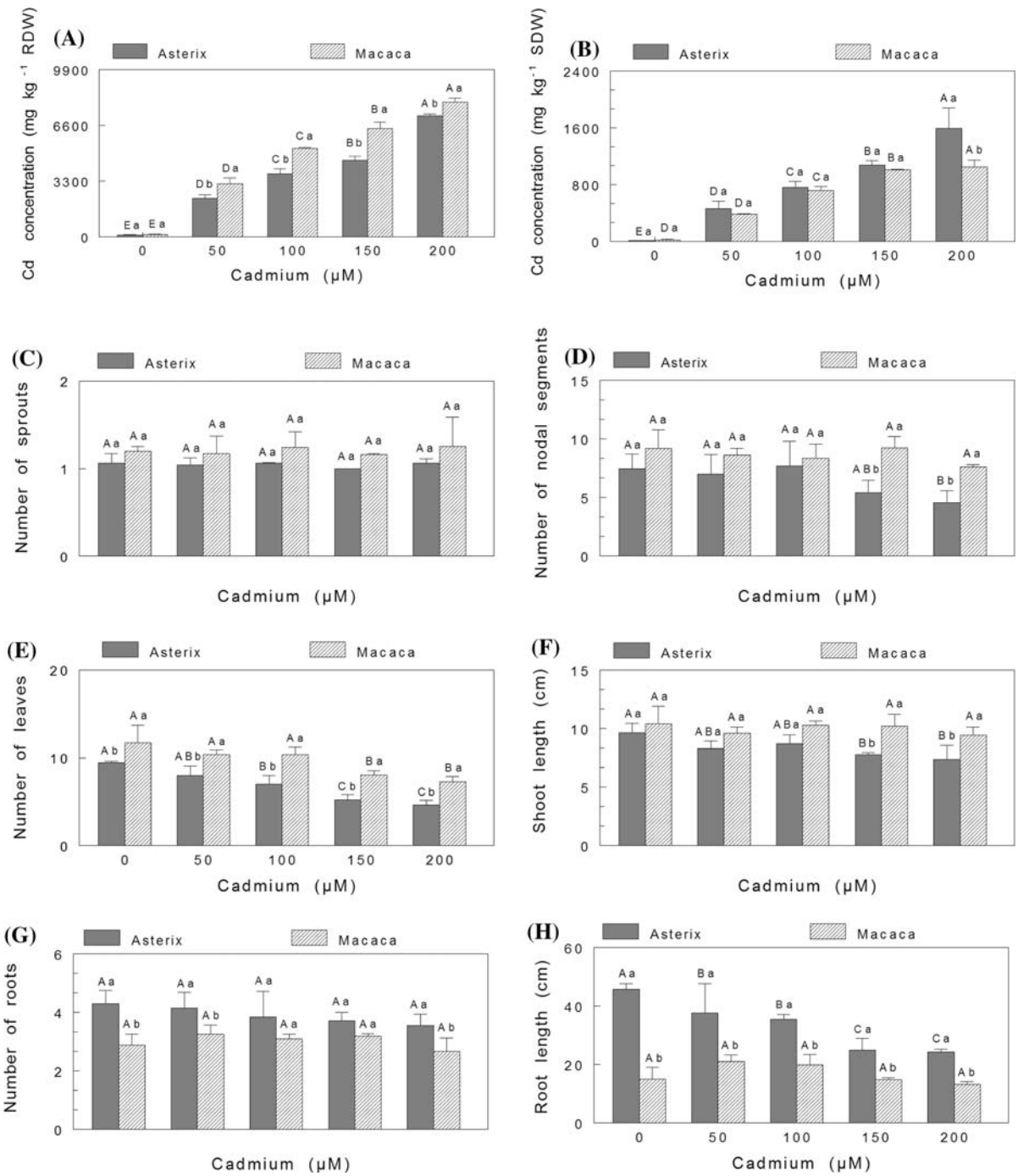

Fig. 1 Effect of increasing Cd concentration on the Cd concentration in roots (**a**) and in shoot (**b**), number of sprouts (**c**), nodal segments (**d**), roots (**e**) and leaves (**f**), and shoot (**g**) and root (**h**) length of two potato cultivars. *RDW* root dry weight; *SDW* shoot dry weight. Data represent the mean ± SD of three different experiments. Different *capital letters* indicate significant differences among the Cd concentrations in the same potato cultivar ($P < 0.05$). Different *lowercase letters* indicate significant differences between potato cultivars in the same Cd concentration ($P < 0.05$)

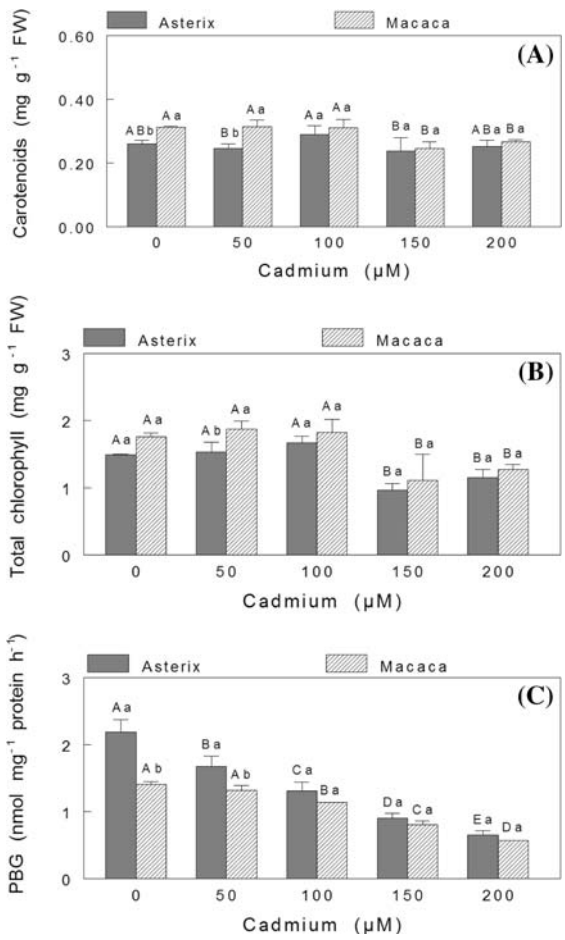

Fig. 2 Effect of increasing Cd concentration on the carotenoids (**a**) and chlorophyll (**b**) contents, and δ-aminolevulinic acid dehydratase activity (**c**) in leaves of two potato cultivars. *FW* fresh weight. Statistics as in Fig. 1

greater ALA-D activity than Macaca at 0 and 50 μM Cd (Fig. 2c).

Lipid peroxidation and protein oxidation

The level of lipid peroxidation was measured in terms of MDA accumulation. As shown in Fig. 3a, roots MDA content was significantly increased at all Cd treatments in Asterix, whereas in Macaca it increased only at two highest Cd levels. Roots of Asterix had significantly higher MDA accumulation compared with Macaca. Shoot MDA content was significantly increased in Asterix at all Cd treatments, whereas Macaca showed an increase only at three highest Cd levels. Shoot of Macaca had significantly higher MDA accumulation upon addition of Cd level exceeding 50 μM compared with Asterix (Fig. 3b).

The level of protein oxidation was measured in terms of carbonyl accumulation only in shoot. Carbonyl content was significantly increased in shoot of both cultivars only at 200 μM Cd, and at this level Asterix had significantly lower carbonyl accumulation compared with Macaca (Fig. 3c).

H_2O_2 content and SOD and CAT activities

As shown in Fig. 4a, roots H_2O_2 content in Asterix was significantly increased at all Cd treatments. Conversely, roots H_2O_2 content in Macaca increased at 50 μM Cd, whereas upon addition of Cd exceeding this level it was reduced compared with control. H_2O_2 content of roots was greater in Macaca than in Asterix at 0 and 50 μM Cd, whereas at 100 and 200 μM Cd Asterix showed greater H_2O_2 content than Macaca. As shown in Fig. 4b, shoot H_2O_2 content in both potato cultivars was increased at three highest Cd levels compared with control, whereas in Macaca it was reduced at 50 μM Cd. Moreover, Macaca had greater shoot H_2O_2 content than in Asterix at 0, 150 and 200 μM Cd, whereas at 100 μM Cd Asterix showed greater H_2O_2 content than Macaca.

SOD activity was significantly inhibited in roots of Asterix at all Cd treatments. By contrast, SOD activity in Macaca increased significantly upon addition of Cd levels exceeding 100 μM. The roots of Macaca had significantly lower SOD activity at 0, 50 and 100 μM Cd compared with Asterix (Fig. 4c). Shoot SOD activity was significantly activated in Asterix at three highest Cd levels. On the other hand, in Cd treatment ranging from 100 to 200 μM Cd shoot SOD activity in Macaca was reduced. At such Cd levels, Asterix had higher SOD activity than Macaca (Fig. 4d).

CAT activity was significantly inhibited in roots of Asterix at 100 and 150 μM Cd, whereas in Macaca it showed an inhibition only at 50 μM Cd. Roots of Asterix had greater CAT activity at 50 μM Cd than Macaca, whereas Macaca showed greater CAT activity at 150 μM Cd compared with Asterix (Fig. 4e). Shoot CAT activity in Asterix was slight, but not significantly, reduced at 200 μM Cd. On the other hand, shoot CAT activity in Macaca was significantly inhibited at all Cd treatments. Asterix

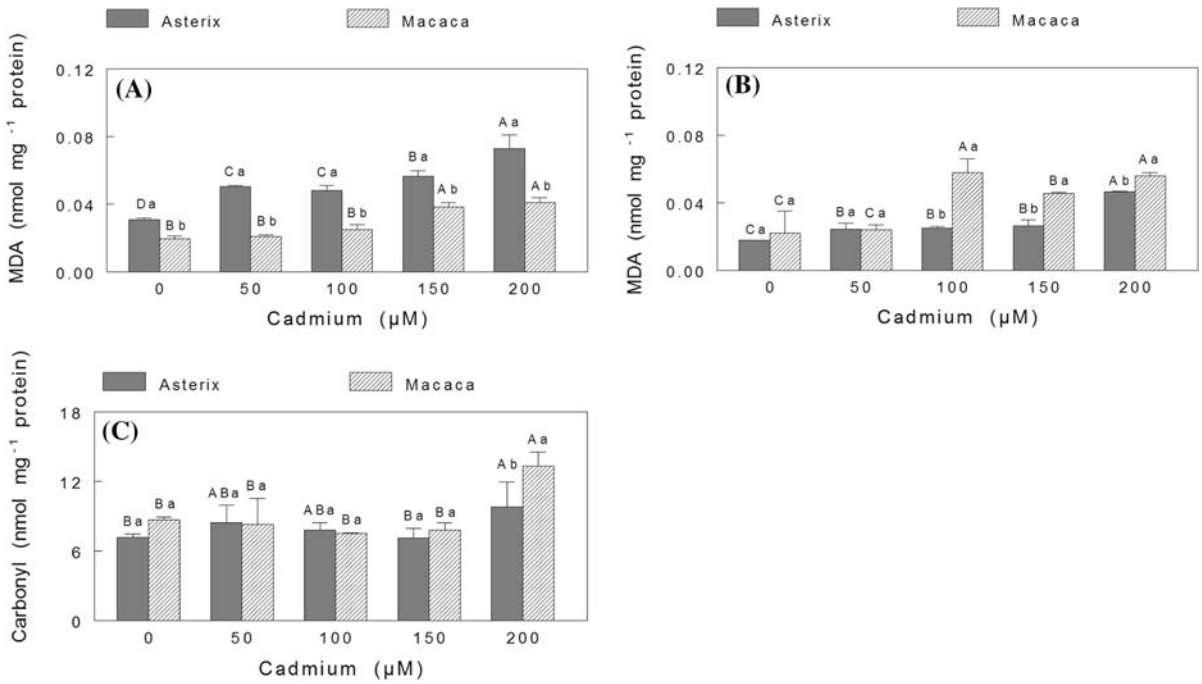

Fig. 3 Effect of increasing Cd concentration on lipid peroxidation in roots (**a**) and in shoot (**b**) and protein oxidation in shoot (**c**) of two potato cultivars. Statistics as in Fig. 1

had greater shoot CAT activity at 50, 100 and 150 μM Cd compared with Macaca, whereas at 0 μM Cd CAT activity was greater in Macaca than Asterix (Fig. 4f).

AsA and NPSH contents

Roots AsA content in Asterix was significantly increased at 150 μM Cd, whereas at 200 μM Cd it was decreased. By contrast, roots AsA content in Macaca was not affected by increasing Cd treatments. Roots of Macaca had lower AsA content at 150 μM Cd compared with Asterix (Fig. 5a). Shoot AsA content in Asterix was significantly decreased at 150 μM Cd, whereas at 200 μM Cd it was increased. Conversely, shoot AsA content in Macaca was significantly decreased at 100 μM Cd, but it was increased at 200 μM Cd. Shoot of Macaca had lower AsA content at 100 μM Cd compared with Asterix, whereas at 150 μM Cd AsA content was greater in Macaca than Asterix (Fig. 5b).

Roots NPSH content of both cultivars was significantly increased at all Cd treatments. Excepting at 100 μM Cd in the substrate, NPSH content was higher in roots of Macaca than Asterix (Fig. 5c). Shoot NPSH content increased at two highest Cd levels in Asterix, but in Macaca it was increased at three highest Cd levels. Shoot of Macaca had greater NPSH content at 100 and 200 μM Cd compared with Asterix, whereas Asterix had greater NPSH content at 150 μM Cd than Macaca (Fig. 5d).

Discussion

Cd concentration, visible symptoms, and plantlets growth

In the present study, both potato cultivars showed a continuous increase in the concentration of Cd in roots and shoot with increasing Cd external concentration and accumulated significantly higher Cd concentration in roots. This finding has been reported by other researches working with distinct plant species exposed to varied Cd concentrations (Mishra et al. 2006; Tiryakioglu et al. 2006; Rascio et al. 2008) and was expected since that *Solanum* is a plant with a "normal = typical" Cd tolerance level and

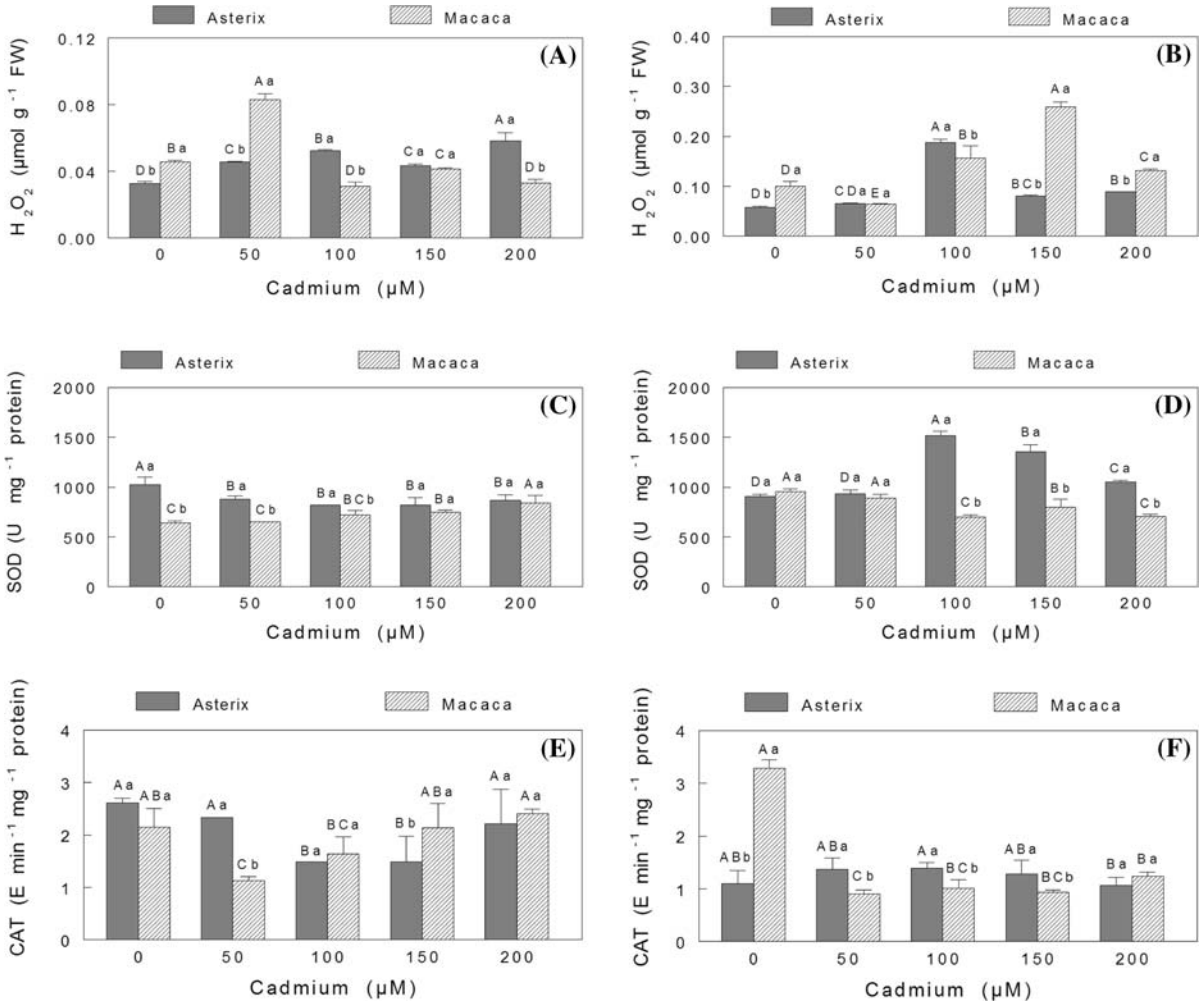

Fig. 4 Effect of increasing Cd concentration on the hydrogen peroxide content and superoxide dismutase and catalase activities in roots (**a**, **c**, and **e**, respectively) and, in shoot (**b**, **d**, and **f**, respectively) of two potato cultivars. *FW* fresh weight. Statistics as in Fig. 1

accumulates this metal according to shoot-excluder strategy. Such metal confinement in the root tissues may be due to an efficient binding and sequestration to the vacuoles by glutathione and phytochelatins, or by immobilization of Cd by cell wall and extracellular carbohydrates (Mishra et al. 2006 and references herein). This Cd accumulation in the root system can indicate that roots serve as a partial barrier to Cd transport to the shoots. Moreover, Macaca accumulated more Cd in the roots than Asterix. On the other hand, in shoot, Asterix had higher Cd concentration at 200 μM Cd compared with Macaca. This data suggests a genotypic difference between potato cultivars concerning the partitioning of Cd. In fact, differences in metal accumulation among cultivars of the same species have been observed in different plants (Dunbar et al. 2003; Liu et al. 2007).

According to Rascio et al. (2008) inhibition of seedling growth is a common effect of many heavy metals and is used as a parameter to characterize phytotoxicity. The growth analyses of potato plantlets showed that number of sprouts and roots was not affected by Cd in both cultivars, whereas Cd treatment affected the number of nodal segments, and shoot and roots length only in Asterix. Reduction in shoot and roots length have been reported by other researches for distinct species exposed to Cd (Tiryakioglu et al. 2006; Rascio et al. 2008). In addition, it was observed a reduced number of leaves as well as leaves abscission and necrosis for both cultivars at

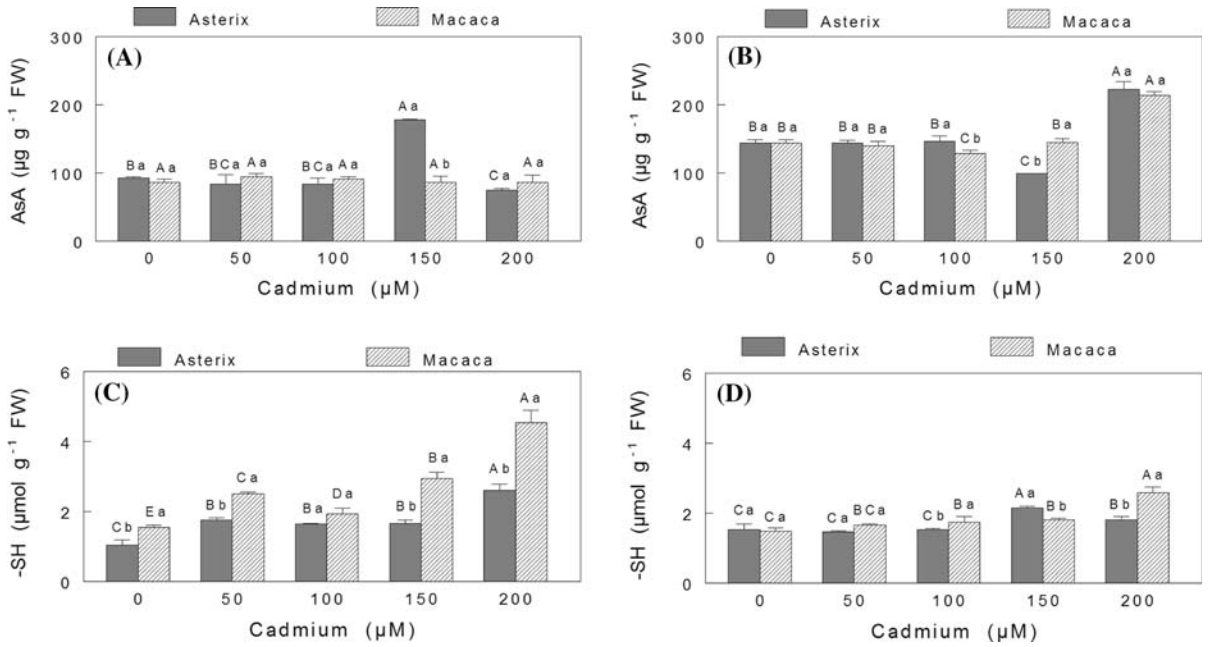

Fig. 5 Effect of increasing Cd concentration on ascorbic acid and non-protein thiol groups contents in root (**a** and **c**, respectively) and in shoot (**b** and **d**, respectively) of two potato cultivars. *FW* fresh weight. Statistics as in Fig. 1

high Cd levels. Soares et al. (2005) and Tiryakioglu et al. (2006) also reported necrosis and shedding of leaves in eucalyptus and barley, respectively, exposed to Cd. Therefore, in the present study, regarding the growth analysis it seems that Asterix was more sensible to Cd toxicity compared with Macaca.

Photosynthetic pigments and ALA-D activity

The level of chlorophyll and total carotenoids was postulated as a simple and reliable indicator of heavy metal toxicity for higher plants (Gratão et al. 2005). Carotenoids are essential for photosynthesis where they serve as accessory light-harvesting pigments and as photoprotectants that quench tissue-damaging free radicals such as singlet oxygen species (Frank and Cogdell 1996; Behera et al. 2002 and references herein). Results of the present study showed a decrease of carotenoids content only in Macaca at two highest Cd levels. This genotypic variations has been reported for other species (Mishra et al. 2006; Singh et al. 2006; Drazkiewicz and Baszynski 2005) and, in the present study, it suggests that might be occurred an overproduction of ROS in leaves of Macaca. Interestingly, Macaca had higher H_2O_2 content at two highest Cd levels than Asterix which can be correlated with the decreased content of carotenoids.

Lower amounts of chlorophyll were found in leaves of both potato cultivars exposed at both 150 and 200 μM Cd. A bleaching effect of Cd on leaf tissues has been reported, although the mechanism by which the metal brings about chlorosis is still unclear (Rascio et al. 2008). This event has been attributed to a number of effects including inhibition of chlorophyll biosynthesis, chlorophyll degradation, hastened senescence and disorganization of chloroplasts, decreased number of photosynthetic membranes, and oxidative stress (Mishra et al. 2006; Rascio et al. 2008 and references herein).

Cd-exposure caused a severe inhibition of ALA-D activity in both potato cultivars. The reaction catalyzed by ALA-D is common to tetrapyrrol biosynthesis, including chlorophyll molecules, and is essential for cellular life (Noriega et al. 2007). So, the reduced chlorophyll content verified in the present study may be attributed to reduced chlorophyll synthesis because Cd interferes with heme biosynthesis and chlorophyll formation by interacting with functional –SH groups of sulfhydryl-requiring

enzymes like ALA-D (Morsch et al. 2002; Noriega et al. 2007). Indeed, the substrate of reaction catalyzed by ALA-D, the ALA, undergoes enolization and further metal-catalyzed aerobic oxidation at physiological pH to yield superoxide radical, hydrogen peroxide and hydroxyl radical. So, ALA-D inhibition could have led to an ALA accumulation that in cell might endogenously contribute to enhanced level of ROS (Noriega et al. 2007 and references therein).

Lipid peroxidation and protein oxidation

In the present study, there was a gradual increase in MDA content in roots and shoot of both potato cultivars, which was positively correlated with the Cd concentration in the tissues (root: $r = 0.93$ and $r = 0.87$ to Asterix and Macaca, respectively; shoot: $r = 0.87$ and $r = 0.82$ to Asterix and Macaca, respectively). The significant increase in MDA concentration observed at highest Cd levels suggests that Cd caused oxidative damage to the plant (Singh et al. 2006). Interestingly, Asterix had higher lipid peroxidation in roots than Macaca which can be correlated with the reduction observed in root length only in Asterix. Corroborating with our results, others researches have showed an increased MDA content in Cd-exposed plants (Mishra et al. 2006; Singh et al. 2006; Rellán-Álvarez et al. 2006). Mishra et al. (2006) reported that ROS induce severe lipid peroxidation due to removal of hydrogen from unsaturated fatty acids leading to formation of lipid radicals and reactive aldehydes. This results in cyclic cascade of reactions causing distortion of lipid bilayer and membrane proteins (Reinheckel et al. 1998).

In addition, the present work showed an increased carbonyl content in both cultivars at highest Cd treatments, indicating that Cd toxicity caused protein oxidation in potato plantlets. This result corroborates with reports of Arvind and Prasad (2005), Ortega-Villasante et al. (2005) and Rellán-álvarez et al. (2006), who noticed carbonyl accumulation in *Ceratophyllum demersum*, *Medicago sativa* and *Zea mays* plants, respectively, exposed to Cd. Oxidatively modified proteins can undergo chemical fragmentation or form aggregates because of covalent cross-linking reactions and increase surface hydrophobicity, leading to a loss of function (Berlett and Stadman 1997). In fact, protein carbonylation is an irreversible oxidative process and it appears to contribute to inhibition or the impairment of multiple enzymes (Sohal et al. 2002).

H_2O_2 content and SOD and CAT activities

H_2O_2 serves as a signaling molecule to activate a rescue/defense system for restoring the redox homeostasis in plant cells (Gratão et al. 2005). Conversely, during oxidative stress, H_2O_2 is a strong toxic oxidant causing cell damage or even cell death and also can contribute to the carbonylation of proteins (Bienert et al. 2006) which was observed in the present paper. The present investigation indicates that Cd-exposure resulted in increased H_2O_2 content in roots and shoot of Asterix, and apparently, a compensatory response to Cd level exceeding 50 μM between roots and shoot of Macaca. According to Aebi (1984) increased H_2O_2 levels may be related with low levels of SOD because H_2O_2 may inactivate enzymes by oxidizing their thiol groups. In fact, in the present study, the content of H_2O_2 was negatively correlated with SOD activity in roots of both potato cultivars ($r = 0.62$ and $r = 0.55$ to Asterix and Macaca, respectively). However, it was observed a positive correlation between these parameters in shoot of Asterix ($r = 0.82$). Therefore, SOD activity had an organ- and cultivar-dependent response to Cd treatments. Increase in SOD activity may be due to increase in superoxide radical concentration and due to de novo synthesis of enzymatic proteins (Verma and Dubey 2003), which is associated with an induction of genes of SOD by superoxide mediated signal transduction (Fatima and Ahmad 2004). On the other hand, the reduction of the SOD activity is probably due to enhanced level of H_2O_2 as explained above.

Root CAT activity in Asterix decreased at 100 and 150 μM Cd, whereas in Macaca it was inhibited only at 50 μM. In shoot, CAT activity in Asterix was not affected by Cd, but in Macaca it was decreased at all Cd treatments. According to Ogawa et al. (1997) the decline observed in CAT activity may be due to inhibition of enzyme synthesis or change in assembly of enzyme subunits.

Previous reports have demonstrated erratic and contradictory responses of antioxidant enzymes to Cd treatment depending on the species and the growing experimental conditions (Gomes-Júnior et al. 2006; Tiryakioglu et al. 2006).

Stroinski and Kozlowska (1997) reported CAT activity and verified that Cd induced oxidative stress in potato tuber, but although the point of view of its utility only potato tubers are important it is interesting to note that the study of the whole plant physiology is relevant for the understanding of Cd accumulation and detoxification.

In future studies it will be necessary to analyze the effects of Cd on antioxidant enzymes activities in native polyacrylamide gel electrophoresis to verify possible differences in activity among specific isoenzymes as showed by some authors (Vitória et al. 2001; Gomes-Júnior et al. 2006).

AsA and NPSH contents

Ascorbic acid (AsA) is a key antioxidant for elimination of ROS especially H_2O_2 (Noctor and Foyer 1998). The reaction of AsA with H_2O_2 can occur directly or it can be catalyzed by ascorbate peroxidase (APX) (Chen and Gallie 2004). Root AsA content in Macaca was not affected by Cd, whereas in shoot it was reduced at 100 μM Cd and increased at 200 μM Cd. On the other hand, a compensatory response to Cd (150 and 200 μM) between roots and shoot of Asterix was observed on AsA content. The increase of AsA content for both potato cultivars indicates that AsA is involved in antioxidant response to Cd toxicity (Tiryakioglu et al. 2006). Moreover, ROS are assumed to be involved in the oxidation of ascorbic acid to dehydroascorbic acid, leading to reduction in the ascorbic acid content of the plant (Singh et al. 2006).

NPSH are known to be affected by the presence of several metals (Xiang and Oliver 1998). In the present study, there was a gradual increase in NPSH content in roots and shoot of both potato cultivars, which was positively correlated with the Cd concentration in the tissues (root: $r = 0.91$ and $r = 0.81$ to Asterix and Macaca, respectively; shoot: $r = 0.57$ and $r = 0.71$ to Asterix and Macaca, respectively). Other investigations have showed an increased NPSH in Cd-treated plants (Mishra et al. 2006; Singh et al. 2006; Tiryakioglu et al. 2006; Rascio et al. 2008). Noctor and Foyer (1998) reported that such increase may be due to stimulation of enzymes of sulfate reduction pathway. In addition, increased synthesis of phytochelatins that play an important role in Cd detoxification could be related to NPSH enhancement (Romero-Puertas et al. 2007).

Conclusion

In conclusion, the growth reduction of potato plantlets may be related to decreased chlorophyll content with consequent reduction on rate of photosynthesis and an increase in tissue damage, which is evidenced by high levels of lipid peroxidation and protein oxidation. In general, Cd availability above of 100 μM caused great oxidative stress and the antioxidant system of the plantlets was not sufficiently efficient to reverse the stress burst. In addition, there is a significant difference in partitioning of Cd between two cultivars, where Macaca accumulated higher levels of Cd in root tissues than Asterix. Such characteristic was accompanied by some detoxification mechanisms. Under Cd treatment, roots of Macaca had increased SOD activity and higher NPSH content, which might be important to detoxify ROS. In parallel, root lipid peroxidation was not alter upon addition of Cd for up to 150 μM, whereas ALA-D activity decreased only upon addition of Cd exceeding 50 μM. These data suggest that Asterix seems to be more sensitive to Cd toxicity compared with Macaca. However, future studies at metabolic and molecular levels with these potato cultivars are necessary for a better understanding of their responses to Cd.

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