RESEARCH ARTICLE

Effect of cadmium on growth, micronutrient concentration, and δ-aminolevulinic acid dehydratase and acid phosphatase activities in plants of *Pfaffia glomerata*

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Pfaffia glomerata (Spreng.) Pedersen plantlets were grown under different cadmium (Cd) concentrations (0, 20, 40, 60 and 80 μM) in a hydroponic system during 7 d. Plant growth, micronutrient, chlorophyll and carotenoid concentrations, as well as δ-aminolevulinic acid dehydratase (ALA-D; E.C.4.2.1.24) and acid phosphatase (AP; E.C.3.1.3.2) activities were analysed. Cadmium concentration in both shoots and roots increased with increasing external Cd levels. Metal concentration in roots were on average 12-fold higher than in shoot tissues. Root length was unaffected by Cd treatments. In contrast, dry weight of both shoot and roots increased significantly after exposure to 20 and 40 μM Cd. Additionally, shoot and total plant dry weight were only reduced in plants exposed to 80 μM Cd. In contrast, root dry weight decreased significantly after exposure with Cd concentrations above 40 μM. A micronutrient- and organ-dependent response to Cd toxicity was observed. Zinc and Cu concentrations in both, shoot and roots did not alter due to exposure to Cd. Cadmium stress reduced Mn uptake but not its translocation within the plant. A synergistic effect of Cd on Fe concentration in root at 20 μM and 80 μM Cd levels was observed. The activity of AP, and especially that of ALA-D, was reduced with increasing Cd levels. At these Cd levels, the chlorophyll concentration was also reduced. There was a positive correlation between concentrations of carotenoids and chlorophylls. Our results indicate that *P. glomerata* seems to have some degree of Cd tolerance.

Key Words: Brazilian ginseng, carotenoid, chlorophyll, heavy metal, micronutrient, phytoremediation

Efeito do cádmio no crescimento, concentração de micronutrientes e atividades da desidratase do ácido δ-aminolevulínico (ALA-D) e fosfatase ácida (AP) em plantas de *Pfaffia glomerata*: Plântulas de *Pfaffia glomerata* (Spreng.) Pedersen foram cultivadas em cinco níveis (0, 20, 40, 60 e 80 μM) de cádmio (Cd) em um sistema hidropônico durante 7 d, visando-se analisar o crescimento, as concentrações de micronutrientes, clorofilas e carotenóides, bem como as atividades da desidratase do ácido δ-aminolevulínico (ALA-D; E.C.4.2.1.24) e fosfatase ácida (AP; E.C.3.1.3.2) nas plantas. A concentração de Cd, na parte aérea e raízes, aumentou com o incremento dos níveis de Cd. A concentração de Cd nas raízes foi, em média, 12 vezes maior do que na

parte aérea. O comprimento das raízes não foi afetado pelos tratamentos de Cd. Em contraste, a biomassa seca da parte aérea e raízes aumentou significativamente pela adição de 20 e 40 μM Cd. Além disso, a biomassa seca total das plantas somente diminuiu no nível de 80 μM Cd em relação à das plantas-controle. Por outro lado, a biomassa radicular diminuiu significativamente pela adição de Cd a níveis superiores a 40 μM. Constatou-se resposta dependente do órgão e do micronutriente à toxicidade de Cd. As concentrações de Zn e Cu na parte aérea e raízes não foram alteradas pela presença de Cd. A absorção de Mn foi diminuída pelo estresse de Cd, porém sua translocação não foi alterada. Um efeito sinergístico do Cd na concentração de Fe nas raízes foi observado nos níveis de 20 e 80 μM Cd. As atividades da ALA-D e AP foram diminuídas com o incremento dos níveis de Cd, porém a ALA-D foi mais afetada. Naquelas concentrações de Cd, a concentração de clorofila também foi diminuída. Houve uma correlação positiva entre as concentrações de carotenóides e clorofila. Os resultados indicam que a *P. glomerata* parece ter algum grau de tolerância ao Cd.

Palavras-Chave: carotenóides, clorofilas, fitorremediação, ginseng brasileiro, micronutrientes, metal pesado.

INTRODUCTION

Heavy metal pollution is of considerable importance and relevant for the present situation of increasing levels of pollution and its obvious impact on human health through the food chain (Hadjiliadis, 1997; Almeida et al., 2007).

It is known that unfavourable effects of heavy metals on plants are manifested, e.g. by inhibiting the normal uptake and utilization of mineral nutrients (Jiang et al., 2004; Dong et al., 2006). Cadmium (Cd), for instance, can interfere with mineral nutrition by hampering the uptake and translocation of essential elements (Boussama et al., 1999; Jiang et al., 2004; Dong et al., 2006). Moreover, Cd can inhibit photosynthesis and plant growth (Gallego et al., 1996). Additionally, it affects the overall cell metabolism via alterations in (i) the behaviour of key enzymes of important pathways (Verma and Dubey, 2001), (ii) membrane composition and function (Fodor et al., 1995; Quariti et al., 1997) and (iii) by lowering the control of the cell redox state, that ultimately causes oxidative stress (Gratão et al., 2005). Acid phosphatases, AP (ortophosphoric-monoester phosphohydrolases; E.C.3.1.3.2) are widely distributed in plants and significantly differ in their susceptibility to be inhibited by various compounds (Penheiter et al., 1997). Acid phosphatases unspecifically catalyze the hydrolysis of a variety of phosphate esters in an acidic environment (Duff et al., 1994). These enzymes are proposed to act in the maintenance of the phosphorus status of the plant, particularly with respect to a role in accessing phosphorus from the soil (Duff et al., 1994). Several factors have been shown to influence the activity of AP (Duff et al., 1994), but the effects of heavy metals on AP are poorly understood (Tabaldi et al., 2007).

The enzyme δ-aminolevulinic acid dehydratase (ALA-D; E.C.4.2.1.24), which catalyzes the asymmetric condensation of two molecules of δ-aminolevulinic acid to porphobilinogen, is sensitive to metals due to its sulphydrylic nature (Pereira et al., 2006). The synthesis of porphobilinogen promotes the formation of porphyrins, hemes and chlorophylls, essential factors of an adequate aerobic metabolism and photosynthesis (Jaffe et al., 2000). Furthermore, altered ALA-D activity concomitant with reduced chlorophyll contents has been reported in many terrestrial plants exposed to various metals (Pereira et al., 2006).

The mechanism of action of heavy metals is due to their capability to form strong bonds with bases and phosphates of nucleic acids and with –SH groups of proteins, modifying both, their structure and function. They compete with other divalent cations such as Ca, Zn and Mg, replacing them, thus inhibiting their physiological roles (Pauza et al., 2005; Tabaldi et al., 2007).

The genus *Pfaffia* belongs to the Amaranthaceae family and has about 90 species distributed in Central and South America. In Brazil, 27 species have been described (Taniguchi et al., 1997). The roots of *Pfaffia glomerata* are of special interest due to their popular use as anti-tumoral, anti-diabetic and aphrodisiac tonic (Montanari et al., 1999). Due to the indications and the form of its roots, which are similar to the Asian ginseng, its common name is Brazilian ginseng (Montanari et al., 1999). In a recent study, Carneiro et al. (2002) showed that an undetermined species of the genus *Pfaffia* exhibited high tolerance to soil contamination, growing quite abundantly in a soil mix with 90 and 1,450 mg kg^{-1} of

Cd and Zn, respectively. In addition, this species showed a Cd content higher than 100 mg kg^{-1}, thus being considered a Cd hyperacumulator. Perhaps it contributes to phytoremediation in areas with high contamination of heavy metals.

Taking into account these characteristics and the high commercial value of *P. glomerata* to the pharmaceutical industry, it is important to determine whether this species accumulates and is tolerant to Cd. If there is a tolerance or an accumulation it is of interest, what mechanisms are involved. The present work was therefore designed to analyze the growth, micronutrient, chlorophyll and carotenoid concentrations, as well as ALA-D and acid phosphatase activities in *Pfaffia glomerata* (Spreng.) Pedersen plantlets during an extended 7-d period of exposure to different Cd concentrations.

MATERIAL AND METHODS

Experimental design: Tissue culture plantlets of *P. glomerata* (accession JB/UFSM) were obtained from the Ginseng Germplasm program, Universidade Federal de Santa Maria, State of Rio Grande do Sul, Brazil. Nodal segments (1.0 cm long) were micropropagated in Murashige and Skoog (1962) medium, supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar, according to the protocol established by Nicoloso et al. (2001).

Twenty-five-day-old plantlets from *in vitro* culture and after 3 d of acclimatization, following the protocol established by Skrebsky et al. (2006), were transferred into plastic boxes (10 L) filled with full nutrient solution of low ionic strength under aeration. The nutrient solution contained (mg L^{-1}): 85.31 of N; 7.54 of P; 11.54 of S; 97.64 of Ca; 23.68 of Mg; 104.75 of K; 176.76 of Cl; 0.27 of B; 0.05 of Mo; 0.01 of Ni; 0.13 of Zn; 0.03 of Cu; 0.11 of Mn and 2.68 of Fe. The pH of the solution was adjusted daily to 5.8 $\pm$ 0.1 by titration using either HCl or NaOH solutions (0.1 M). On the 14th day after transplanting, cadmium ($CdCl_2.H_2O$) was added to each container. In total, five final concentrations were generated: 0 (control), 20, 40, 60 or 80 μM. The nutrient solution in the growth containers was renewed once a week. Both *in vitro* and *ex vitro* cultured plants were grown in a growth chamber at 25 $\pm$ 1ºC during 16/8-h light/dark cycle with 35 μmol m^{-2} s^{-1} of irradiance. Cadmium-treated plantlets remained in each solution for 7 d. Three replicates with 54 plantlets were used for each treatment.

Growth analysis: After harvesting, plants were divided into shoots and roots. Roots were rinsed twice using fresh aliquots of distilled water. Subsequently, growth and biochemical parameters were determined. Length of roots was determined according to Tennant (1975), and the length of sprouts was measured with a ruler. To obtain dry weight, the plants were left at 65°C until reaching a constant weight.

Cadmium and micronutrient concentrations: Approximately 0.2 g of roots and shoots were digested with 4 mL HNO_3 using an exposure to high temperature: a) 50°C for 1 h; b) 80°C for 1 h; and c) 120°C for 1 h in a digester block (Velp, Italy). As a next step, the samples were diluted to 50 mL with deionized water. Concentrations of Cd, Zn, Mn, Fe, and Cu were measured by atomic absorption spectroscopy (Iyengar et al., 1997).

Acid phosphatase (AP; E.C. 3.1.3.2) activity: Fresh root and shoot extracts were centrifuged at 43200 *g* for 30 min at 4ºC and the supernatant used for enzyme assay. Acid phosphatase activity was determined according to Tabaldi et al. (2007) using a reaction medium consisting of 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffer, pH 5.5, in a final volume of 200 μL. An aliquot of 20μL of the enzyme preparation (10-20 μg protein) was added to the reaction mixture and preincubated for 10 min at 35°C. The reaction was started by the addition of substrate and stopped by the addition of 200 μL of 10% trichloroacetic acid (TCA) to a final concentration of 5%. Inorganic phosphate (Pi) was measured at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as the standard for the calibration curve. Controls were run to correct for nonenzymatic hydrolysis by adding enzyme preparation after TCA addition. Enzyme specific activities are referred to as nmol Pi released min^{-1} mg^{-1} protein. All assays were performed in triplicate using PPi as substrate at a final concentration of 3.0 mM.

δ-aminolevulinic acid dehydratase (ALA-D; E.C. 4.2.1.24) activity: Shoot tissue was homogenized in 10 mM Tris-HCl buffer, pH 9.0 (1:1, w/v). The homogenate was centrifuged at 12000 *g* at 4°C for 10 min to gain a supernatant (S1) to be used for the enzyme assay. The S1 was pre-treated with 0.1% Triton X-100 and 0.5 mM DTT. The ALA-D activity was assayed as described by Barbosa et al. (1998) by measuring the rate of porphobilinogen (PBG) formation. The incubation medium for the assays contained 100 mM Tris-HCl buffer, pH 9.0. For the enzyme assay,

the final concentration of ALA was 3.6 mM. Incubation was started by adding 100 μL of the tissue preparation to a final volume of 400 μL. 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. Activity of ALA-D was expressed as nmol PBG mg^{-1} protein h^{-1}.

Protein extraction: In all the enzyme preparations, protein was determined by the method of Bradford (1976) using BSA as standard and was expressed in mg mL^{-1}.

Chlorophyll and carotenoid concentrations: Chlorophyll and carotenoids were extracted using the method of Hiscox and Israelstam (1979) and estimated as described by Arnon (1949). Briefly, chopped fresh shoot sample (0.1g) was incubated at 65°C in dimethylsulfoxide (DMSO) until tissues were completely bleached. Absorbance of the solution was then measured at 470, 645, and 663 nm in order to determine the concentrations of carotenoids, chlorophyll a, and chlorophyll b, respectively. Chlorophyll and carotenoid concentrations were expressed as mg g^{-1} fresh weight.

Statistical analysis: The analyses of variance were computed for statistical significance based on *F*-tests. The results are mean ± standard deviation of at least three independent replicates. Significance was determined at $P < 0.05$. The mean differences were compared utilizing Duncan's multiple range tests.

RESULTS

Cadmium concentration under Cd exposure: Cd concentration in both, shoots (Figure 1A) and roots (Figure 1B) increased with increasing Cd levels. External Cd concentrations ranging from 20 to 60 μM generated the same enhancement of Cd concentration in both, shoots and roots (36 and 12.5-fold greater than controls, respectively). Cadmium concentration in roots was on average 12-fold greater than in the shoot. The maximum concentration of Cd in shoot and roots was 345 mg kg^{-1} DW and 3400 mg kg^{-1} DW, at the 80 μM Cd level, respectively.

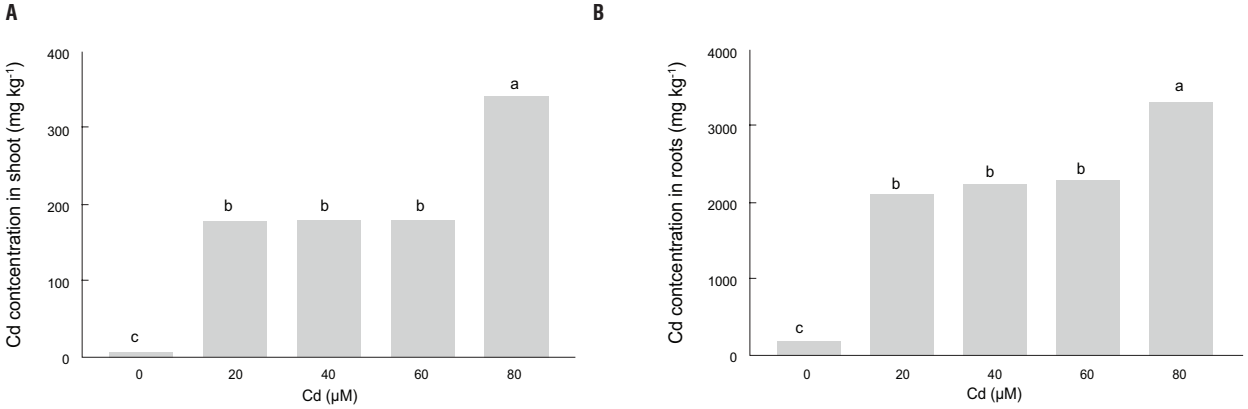

Figure 1. Cadmium concentration in shoot (**A**) and roots (**B**) of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Values are the mean of three individual replicates. Identical superscripts on bars indicate no significant difference ($P > 0.05$) between means according to Duncan's multiple range test.

Characterisation of Cd tolerance: There was no general pattern of plant growth responses to Cd stress. Number of leaves per plant was slightly, but not significantly, reduced by Cd concentrations up to 40 μM, whereas at the 80 μM Cd level it was reduced by 26% compared to control plants (Figure 2A). In addition, length of sprouts (Figure 2B) and length of the root system (Figure 2C) per plant were not affected by Cd. On the other hand, the root length/shoot length ratio increased significantly at the 20 μM Cd level and was slightly, but not significantly, increased after exposure to more than 20 μM Cd, as compared to control plants (Figure 2D).

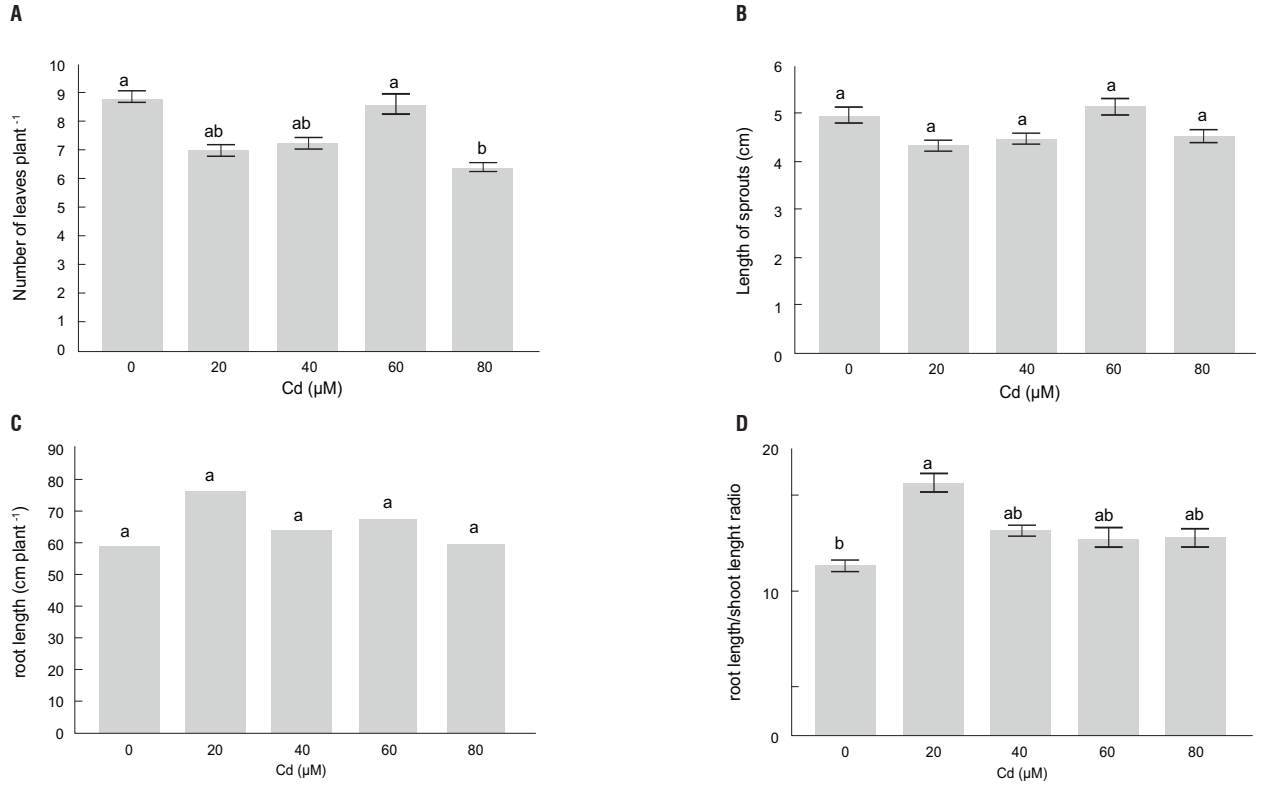

Figure 2. Number of leaves (**A**), length of sprouts (**B**), length of whole root system (**C**), root length/sprout length ratio (**D**) of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Statistics as in Figure 1.

Shoot fresh weight was reduced significantly only upon adding 80 μM of Cd (Figure 3A). In contrast, dry weight of both, shoot (Figure 3B) and roots (Figure 3C) increased significantly upon addition of 20 and 40 μM Cd. Moreover, shoot and total plant weight were reduced only at the 80 μM Cd level as compared with controls (Figure 3B and 3D, respectively). Conversely, root dry weight decreased significantly after exposure to Cd, when the amount exceeded 40 μM (Figure 3C).

Effect of Cd on micronutrient concentrations: A nutrient- and organ-dependent response to Cd toxicity was observed (Table 1). Zinc remained unaltered after applying Cd treatments. In roots however, Mn concentration was reduced significantly independent of the Cd levels added, Fe concentration increased significantly upon addition of 20 and 80 μM Cd, whereas Zn and Cu levels did not respond to the treatments.

Table 1. Concentrations of Zn, Mn, Fe and Cu in shoot and roots of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Values are the mean of three individual replicates. Means in columns for each plant tissue followed by the same letter did not differ significantly ($P > 0.05$) from each other according to Duncan's multiple range test.

Treatments Cd (μM)	Micronutrient concentration							
	Zn (mg kg-1 dw)		Mn (mg kg-1 dw)		Fe (mg kg-1 dw)		Cu (mg kg-1 dw)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	719a	128a	508a	140a	18209b	265a	79a	16a
20	573a	148a	168c	121a	24821a	395a	126a	14a
40	523a	91a	177c	118a	19539b	308a	93a	15a
60	571a	105a	244bc	99a	19320b	306a	90a	16a
80	650a	104a	322b	114a	21663a	411a	85a	14a
CV (%)	20.0	32.6	18.9	18.6	14.7	28.6	15.2	21.0

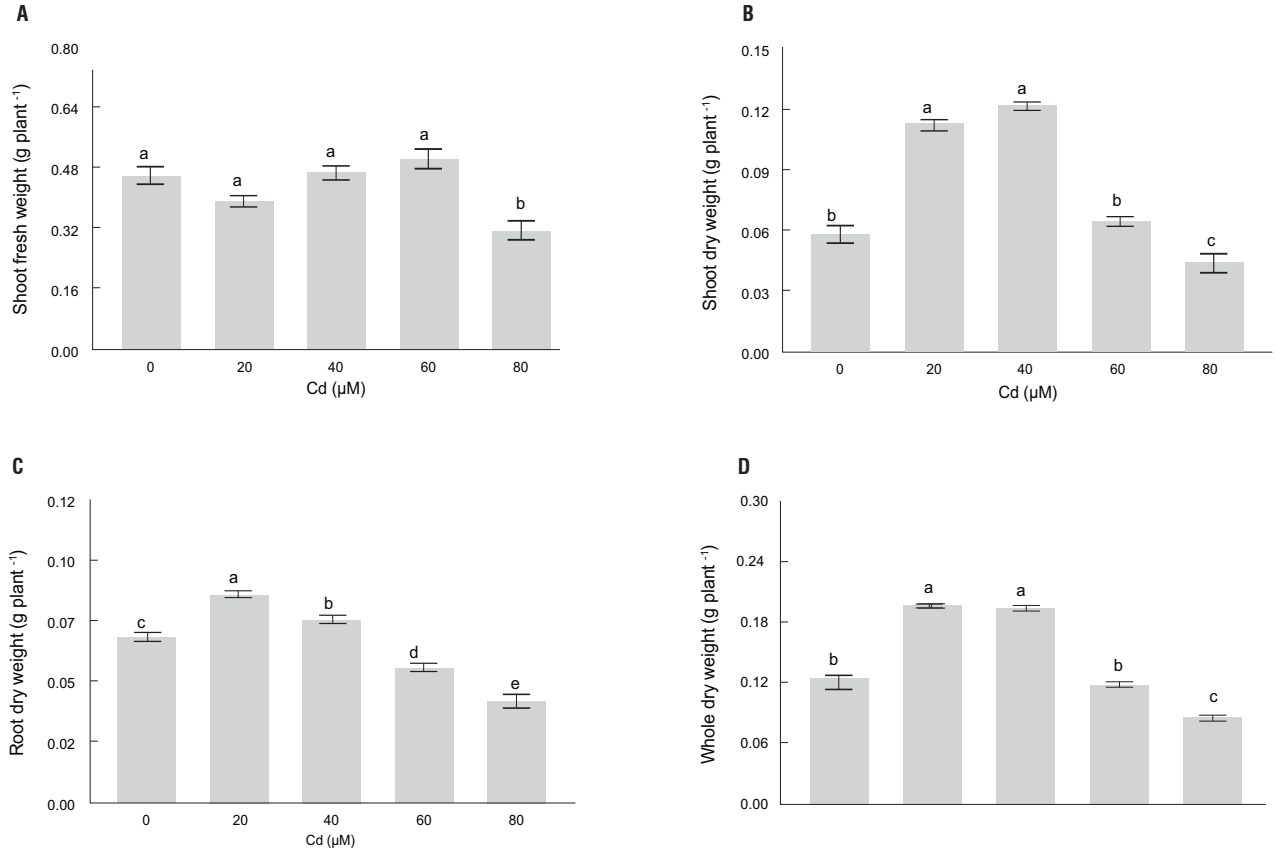

Figure 3. Shoot fresh weight (**A**), shoot dry weight (**B**), root dry weight (**C**), and whole plant dry weight (**D**) of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Statistics as in Figure 1.

Acid phosphatase activity (AP): Acid phosphatase activity was reduced in shoot (23%) and roots (30%) with increasing Cd levels. No difference in AP activity in both, shoot and roots was found for Cd treatments ranging from 20 to 80 μM (Figure 4).

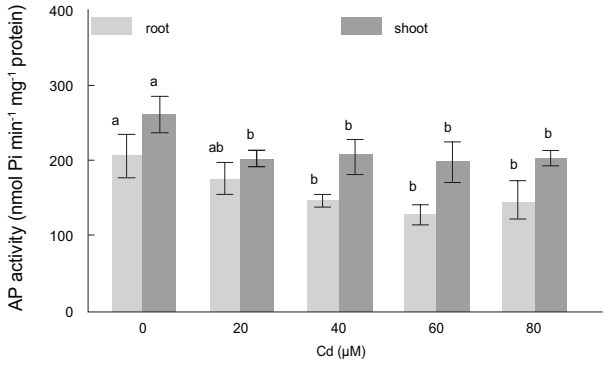

Figure 4. Acid phosphatase activities in shoot and roots of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Statistics as in Figure 1.

ALA-D activity and concentrations of chlorophyll and carotenoids: Shoot ALA-D activity decreased with increasing Cd levels in the nutrient solution (Figure 5A). A maximum of 89% depletion in ALA-D activity was found at 80 μM Cd. Total chlorophylls were also reduced but only at the 20 and 80 μM Cd levels (Figure 5B). On the other hand, carotenoid concentration was significantly reduced upon addition of 20, 60 and 80 μM Cd (Figure 5C).

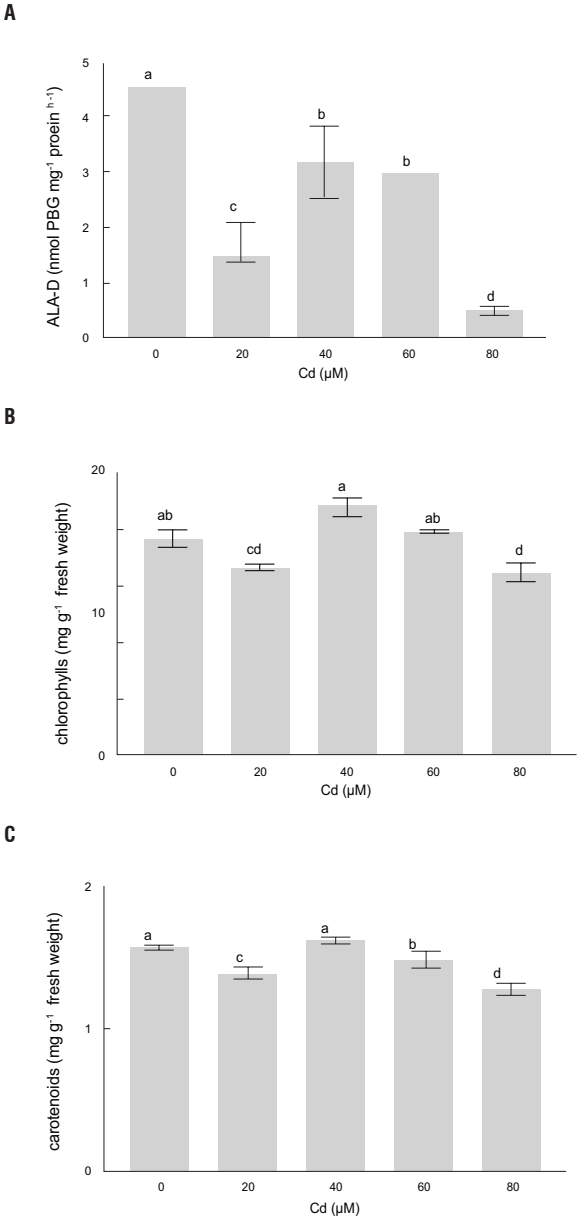

Figure 5. Activity of δ-aminolevulinic acid dehydratase (ALA-D) (**A**), chlorophyll (**B**) and carotenoid concentration (**C**) of *Pfaffia glomerata* plants grown under increasing concentrations of Cd for 7 d. Statistics as in Figure 1.

DISCUSSION

Cadmium concentration and plant tolerance to Cd: Our data (Figure 1) demonstrate that higher metal exposures led to remarkable Cd accumulation in both, root and shoot tissues, resulting in a high degree of toxicity as also by other authors (e.g., Carneiro et al., 2002; Lima et al., 2006; Mishra et al. 2006). Meanwhile, no significant difference in both shoot and root Cd concentrations was found between the 20 to 60 μM Cd treatments. We also checked Cd concentration in the nutrient solution at day 0 and 7 d after applying the treatments: external Cd was not significantly depleted during the experiment (data not shown). These data suggest that, up to a certain level of metal concentration, roots of *P. glomerata* have some mechanism to avoid excess of Cd uptake. Cadmium confinement in the root tissues may be due to an efficient binding and sequestration to the vacuoles by glutathione and phytochelatins, or by imobilization of Cd by the cell wall and extracellular carbohydrates (Lima et al., 2006; Mishra et al., 2006, Almeida et al., 2007).

Our data indicate, that the root length/shoot length ratio increased after treatment with all studied Cd concentrations (Figure 2D), which is not consistent with the results of Guo and Marschner (1995), who reported that usually the inhibition of root elongation of different plant species is the most sensitive parameter of Cd toxicity. In addition, our results also indicate that root elongation was much less affected than the decrease in biomass, which is not consistent with the earlier results of Lima et al. (2006) for *Pisum sativum* and of Meuwly and Rauser (1992) for *Zea mays*. According to Meuwly and Rauser (1992), since most of the root elongation is located in the first 10 mm of the root apex, the contribution of the biomass to this portion is probably too small to allow the detection of any toxic symptoms during the first days, but length inhibitions are enough to be detected. Therefore, since root biomass of *P. glomerata* was significantly reduced upon addition of Cd levels exceeding 40 μM, the data indicate, that *P. glomerata* has some degree of Cd tolerance. Meanwhile, there was an increase in both, root and shoot biomass (Figure 3) at lower Cd levels (20 and 40 μM). It should be emphasized that experiments of the present study were carried out three times (with some modifications), and results were almost the same for most parameters analyzed including the increased biomass we found (data not shown). The positive effects of low levels

of Cd on plant growth have been poorly discussed in the literature, and the mechanisms are not well understood. This phenomenon is normally related to a so-called hormetic effect that probably represents an "overcompensation" response to a disruption in the homeostasis of the organism (Aina et al., 2007). Khan et al. (2008) observed similar phenomena in sand culture where 10 μM Cd enhanced the activities of leaf superoxide dismutase, ascorbate peroxidase, glutathione reductase and carbonic anhydrase, net photosynthetic rate and plant dry mass of *Triticum aestivum* at low Zn level. These authors suggested that the synergies among the activities of antioxidative enzymes helped to maintain carbonic anhydrase and thus photosynthesis and plant biomass at low Cd levels under low Zn concentration. Taking into account the fact that the total dry biomass of *P. glomerata* was only significantly reduced at the 80 μM Cd level and such a concentration is within that observed in highly polluted soils, these results further indicate that *P. glomerata* seems to have some degree of Cd tolerance, as was found for an undetermined species belonging to the genus *Pfaffia*, reported by Carneiro et al. (2002). However, additional experiments should be performed in order to allow a better understanding of the mechanism of the effect of Cd toxicity on growth, photosynthesis and antioxidative mechanisms of *P. glomerata*.

Nutrients concentration under different degrees of Cd stress: It is well known that many toxic effects of Cd action result from interaction with micronutrients, in particular those with the same valence as Cd, such as Zn, Mn, Fe, and Cu. Our results showed that Zn and Cu concentrations in both shoot and roots remained unaltered upon Cd addition. Both synergistic and antagonistic effects of Cd on Zn and Cu were found in other studies (Jiang et al., 2004; Dong et al., 2006). Our data indicate that Mn uptake was affected by Cd stress but not the translocation of Mn within *P. glomerata* plants. An inhibition in Mn uptake and transport by Cd has been reported by Dong et al. (2006) for *Lycopersicon esculentum*. In contrast, an increase in Mn uptake and translocation to the shoots was observed in *Lactuca* sp. exposed to Cd stress and there was a higher Mn accumulation in chloroplasts when Cd was present in the growth medium (Ramos et al., 2002). Some studies showed that there is an antagonistic relationship between Cd and Fe (Sharma et al., 2004). Arabidopsis plants that overexpressed the *IRT1* gene, a major transporter responsible for high-affinity iron uptake from the soil, accumulated higher levels of Cd and Zn than the wild type, indicating that *IRT1* is responsible for the uptake of these metals (Connolly et al., 2002). Our data demonstrate that a synergistic effect of Cd on Fe concentration in root at the 20 and 80 μM Cd concentrations is likely to occur. Some of these conflicting results found in our study in relation to others might be presumably due to the differences in the culture methods, species, as well as growth conditions including Cd and micronutrient levels in medium, growth period, temperature and light.

Metabolic enzymes, and concentration of chlorophyll and carotenoid in different degrees of Cd stress: Our results showed that under Cd stress, AP activity in both shoot and roots was similarly reduced regardless of the amount of Cd added. Conversely, Tabaldi et al. (2007) found that Cd, Mn and Na did not significantly alter the AP activity in *Cucumis sativus*. Phosphatases are generally metalloenzymes depending on Ca^{2+} or Mg^{2+}. A possible mechanism explaining Cd-toxicity at high concentrations can be the replacement of Ca^{2+} and Mg^{2+} by Cd in the active site of enzyme, or the Cd can be interfering with the PO_4^{3-} binding sites. Other metals such as Hg and Zn also inhibited AP activity of cucumber, possibly through this mechanism (Tabaldi et al., 2007). Therefore, inhibition of AP activity in *P. glomerata* caused by Cd stress may impair phosphate mobilization, since this enzyme is involved in P metabolism, an essential element for plant growth and development (Duff et al., 1994).

Altered ALA-D activity concomitantly with reduced chlorophyll contents has been reported in many terrestrial plants exposed to various metals (Pereira et al., 2006). Our data also showed that total chlorophyll concentration was reduced significantly at 20 and 80 μM Cd (Figure 5B), at which greater reduction in ALA-D activity was observed. Moreover, there was a significant positive correlation (r^2= 0.73) between concentrations of carotenoids and chlorophylls. Carotenoids play a pivotal role in photoprotection of chlorophylls against photooxidative damage by quenching reactive oxygen species (ROS) such as singlet oxygen (Behera et al., 2002). Similarly, other authors have found a decrease in carotenoid content in Cd-treated plants, which might be interpreted as an overproduction of ROS (Mishra et al., 2006). In addition, ALA-D inhibition could have led to ALA accumulation that within the cell might contribute to enhance ROS production (Noriega et al., 2006). Therefore, in future studies it will be

necessary to analyze the effects of Cd on ROS formation and on both enzymatic and non-enzymatic antioxidant systems. These defence systems can remove, neutralise or scavenge oxy-radicals and their intermediates (Gratão et al., 2005).

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