Arsenic-Sensitivity in *Anadenanthera Peregrina* Due to Arsenic-Induced Lipid Peroxidation

Marcelo Pedrosa Gomes[1,2]; Marília Carvalho[2], Teresa Cristina Lara Lanza Sá e Melo Marques[3]; Daniela Moreira Duarte[4]; Claudia de Oliveira Gonçalves Nogueira[2]; Ângela Maria Soares[2]; Queila de Souza Garcia[4]

[1] Université du Québec à Montreal, Institut dês Sciences de l'environnement, Succ. Centre-Ville, C.P. 8888, Montreal, Québec, H3C 3P8, Canada.
[2] Universidade Federal de Lavras, Departamento de Biologia, Campus UFLA, 37200-000, Lavras, MG, Brazil
[3] Universidade Federal de Lavras, Departamento de Ciências do Solo, Campus UFLA, 37200-000, Lavras, MG, Brazil
[4] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Botânica, Avenida Antonio Carlos, 6627, Pampulha, C.P. 486, 31.270-901, Belo Horizonte, MG, Brazil

Abstract

Anadenanthera peregrina is legume that can grow on arsenic-contaminated sites when colonized by arbuscular mycorrhiza fungi (AMF). Previous studies demonstrated that these plants are subject to arsenic-toxicity in the absence of AMF. The present study investigated As-sensitivity in A. peregrina by submitting plants to increasing As concentrations (0, 10, 50 and 100 mg kg^{-1}) while monitoring alterations in their growth, root anatomical and morphology and associated biochemical features (oxidative damage and antioxidant enzymes). Our results demonstrated that arsenic affects early root growth, inducing root anatomical changes and altering plant antioxidant scavenging machinery by inactivating some antioxidant enzymes (CAT and APX). As-induced oxidative stress caused by lipid peroxidation (but not by H_2O_2 accumulation) may be the main cause of As-sensitivity. Since these plants become As-tolerant under AMF symbioses, the ability of AMF to minimize oxidative stress under these conditions needs to be more closely investigated.

Keywords: Environmental concern; growth; heavy metal; phytoremediation; soil contamination

1 Introduction

Arsenic (As) has been the focus of environmental concern in recent years because of its toxicity and potential risk to ecosystems as well as to human health (Duxbury and Zavala, 2008). Arsenic levels in soils have significantly increased through anthropogenic activities, with mining being one of the main sources of this environmental contaminant (Zhao-hui and Xu-feng, 2010). As-rich mining tailings have vertiginously increased soil heavy metal concentrations in Minas Gerais State (MG), Brazil, and arsenic concentrations higher than 27000 ppm have been reported in Santa Luzia in areas used to discard gold mining spoils (Gomes, 2011). The mobilization and migration of As from soils to other parts of the environment (especially to stream water) is of considerable concern because of its effects on human health, and this problem demands an effective solution.

Toxic heavy metals can provoke cellular disruption in plants through the induction of oxidative stress caused by increased generation of reactive oxygen species - ROS (Gratäo et al., 2005). Plants can usually increase antioxidant enzyme activities, however, reducing oxidative stress and increasing As tolerance (Singh et al., 2006). Antioxidative defense compounds fall into two general classes: 1) low molecular weight antioxidants, composed of lipid-soluble membrane-associated antioxidants (e.g., α-tocopherol and β-carotene) and water-soluble reductants (e.g., glutathione–GSH and ascorbate), and; 2) enzymatic antioxidants (e.g., superoxide dismutase-SOD, catalase–CAT, guaiacol peroxidase-GPX, and ascorbate peroxidase-APX) (Cao et al., 2004). The role of SOD in antioxidative defenses is to eliminate reactive oxygen species (ROS) that generate H_2O_2 (with the resulting H_2O_2 being removed by CAT, APX, and GPX enzymes) (Cao et al., 2004). High activity levels of these enzymes have been reported in plants exposed to heavy metals (Cao et al., 2004; Marques et al. 2011; Rossato et al. 2011).

Plants show great variations in their responses to As toxicity (Meharg, 2003). Generally, in hyperaccumulator species,

As exposure increases antioxidant mechanisms leading to its detoxification and its accumulation in tissues (Singh et al., 2006); in non-hyperaccumulator species, As induces oxidative stress resulting in cellular damage due to enhanced lipid peroxidation, H_2O_2 accumulation, and up-regulation of several scavenging enzymes (Mascher et al., 2002). Changes in plant anatomical and physiological characteristics have also been associated with arsenic exposure (Pereira et al., 2011; Singh et al., 2007), although the biochemical and anatomical responses of plants to this element are not well understood when compared to other metals (Srivatava et al., 2005).

Very few studies have examined As-induced oxidative damage and antioxidant responses in sensitive species and, since there are inter- and intra-specific variations in As-induced toxicity profiles, it is pertinent to evaluate arsenic toxicity in a wide variety of plant species (Singh et al., 2007). The present study investigated As-induced toxicity in *Anadenanthera peregrina* by studying oxidative damage in this As-sensitive plant and associated changes in biochemical and root anatomical attributes. We selected *A. peregrina* because it is a widespread Brazilian species that mainly grows in the Cerrado (Brazilian Savanna) biome, and seemingly healthy specimens have been recorded growing in soil with As levels ranging up to 540 ppm (Gomes, 2011). Previous studies indicated that As-tolerance in *A. peregrina* is correlated with root symbiosis with arbuscular mycorrhiza fungi (AMF) (Gomes, 2011), and plants without AMF colonization show heightened symptoms of As intoxication that has been correlated with As-induced nutritional disturbances (Gomes et al., 2012). The present investigation therefore examined how As (at concentrations of 10, 50 and 100 mg kg^{-1}) affected seedling growth, root anatomical features, oxidative stress markers (MDA and H_2O_2), and antioxidant enzyme systems in *A. peregrina*.

2 Materials and methods

2.1Greenhouse experiment

The cultivation substrate consisted of a sand/vermiculite mixture (1:1 v v^{-1}). Arsenic was added to the substrate as 0 (control), 10, 50 and 100 mg of Na_2HAsO_4 kg^{-1} substrate. As-doses were chosen based on soil As levels where *A. peregrina* plants have been found growing (Gomes 2011). The test concentrations of As with 2.5 $g.L^{-1}$ of extended time-release fertilizer (N-P-K ratios of 10-10-10) (Vida Verde Co., São Miguel) were mixed with the substrate, and the pH (1:1 soil water $ratio^{-1}$) was checked and adjusted to 7.1 $\pm$ 0.1. *A. peregrina* seeds were germinated in Styrofoam boxes containing vermiculite substrate without As and the seedlings grew under greenhouse conditions (temperature 15-31 °C; average photosynthetically active radiation 825 µmol m^{-2} s^{-1}) until the development of their first pair of full expanded leaves. These healthy plants were then transplanted to the As-contaminated substrate with daily watering; 20 ml of half-strength Hoagland solution was applied every two weeks. 25 days after transplanting (when symptoms of As toxicity were visible) the plants were harvested, washed thoroughly with tap water, and the lengths of their roots and shoots were measured.

2.2 Arsenic content

To determine their arsenic contents, five plants were separated into root and shoot portions, rinsed quickly with 0.1 mol L^{-1} HCl, followed by several rinses with deionized distilled water. The samples were then oven-dried for 3 days at 50-55 °C. Then samples were subsequently digested in a microwave oven (ETHOS 1, Milestone Italy) in 5 mL of concentrated HNO_3 (GR) at 80 °C for 10 min and then at 180 °C for 15 min. After digestion, the solutions were cooled and diluted to 50 mL using ultra-pure water. Arsenic concentrations were determined using a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 400, Norwalk, CT) following USEPA Method 7060A (Chen and Ma, 1998). The accuracies of the elemental analyses were checked by carrying a standard reference material (BCR-414 plankton) through digestion and analysis. Arsenic concentrations were expressed on a fresh weight basis in ppm (Mahmood et al., 2005) using the following formula: $ppm_{(fresh)} = (ppm_{(d)}$ x d.w.)/f.w, where d.w. = Dry weight, and f.w. = Fresh weight. Biological absorption (BAC) (Mahmood et al., 2005) was determined with the formula $BAC = ppm_{(fresh)}/ppm_{(sol)}$, where $ppm_{(sol)}$ = the heavy metal concentration in the original solution.

2.3 Morphological observations

For anatomical analyses, the lateral roots of five individuals from each treatment were collected. The material was fixed in FAA (formaldehyde + acetic acid + 70% ethyl alcohol, 5:5:90 L L^{-1} L^{-1}) for 72 hours and then stored in 70% ethyl alcohol. A table microtome was used to cut cross sections in the root maturation zone (4 ± 0.5 cm above the root apex). The cross sections were treated with 5% bleach (Kraus and Arduim,1997) and stained with an Astra Blue and safranin mixture (Kraus and Arduim, 1997).

All slides were examined and photographed using a Ken-a-Vision TT18 light microscope and a Canon Power Shot A620 digital camera. Lignin detection used fluorescence analyses: the sections were stained with 0.1% berberine hemisulfate (kg L^{-1}) for one hour (Brundrett et al., 1988), washed with distilled water, transferred to a solution of 0.5% (kg L^{-1}) aniline blue for 30 minutes, and again washed with distilled water. The sections were incubated in a 50% glycerin solution (L L^{-1}) with 0.1% $FeCl_3$ (kg L^{-1}) (Ederli et al., 2004), washed with distilled water for a few minutes, and then mounted on slides and examined under an Olympus BX60 UV light microscope (with a bandpass filter for wavelengths above 420 nm). The determinations of anatomical characteristics were performed using Sigma Scan software on three fields of five individuals per treatment. The items assessed in the root system included: thickness of epidermis, endodermis, and cortex, and the numbers and diameters of the tracheary elements. The Carlquist vulnerability index (CVI = diameter of tracheary elements/number of tracheary elements) was calculated following Carlquist (1975).

2.4 Oxidative damage and antioxidant enzymes

Oxidative responses (H_2O_2 production and lipid peroxidation) and antioxidant enzymes (SOD, CAT, APX, GPX and GR) were examined in five plants per treatment. After harvesting, samples of leaves and roots were stored at -40 °C and subsequently used for biochemical studies and assessments of oxidative damage.

For H_2O_2 content evaluation, samples (100 mg) were extracted with 5.0 mL of TCA (0.1%, w v^{-1}) in an ice bath, and the homogenate was centrifuged at 12.000 g for 15 min (Velikova et al., 2000). 0.5 ml of phosphate buffer (pH 7.0) and 1.0 ml of potassium iodide (1 M) were then added to 0.5 ml of the supernatant and the absorbance of the mixture was measured at 390 nm. H_2O_2 content was determined using an extinction coefficient (e) of 0.28 mM^{-1} cm^{-1} and was expressed as nmol g^{-1} FW.

Oxidative damage was estimated in terms of lipid peroxidation based on the production of 2-thiobarbituric acid-reactive metabolites, particularly malondialdehyde (MDA), following the methodologies of Heath and Packer (1968) and Buege and Aust (1978). Measurements of MDA were performed as in Hodges et al. (1999), which takes into account the possible influence of interfering compounds in the assay for 2-thiobarbituric acid (TBA)-reactive substances. Readings were made in a spectrophotometer at 535 and 600 nm, and the MDA concentrations were expressed as nmol g^{-1} of fresh tissue.

Antioxidant enzymes were extracted by macerating 200 mg of fresh leaves and roots in 800 µl of an extraction buffer containing 100 mmol L^{-1} potassium phosphate buffer (pH 7.8), 100 mmol L^{-1} EDTA, and 1 mmol L^{-1} L-ascorbic acid. The protein contents of all of the samples were determined using the Bradford method. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured photometrically at 560 nm, based on the inhibition of nitroblue tetrazolium (NBT) reduction by SOD. One unit of SOD was defined as the amount needed to bring about a 50% inhibition of the NBT reduction state (Beyer and Fridovich 1987). Catalase (CAT; EC1.11.1.6) activity was measured photometrically at 240 nm as described by Kraus et al. (1995) (with minor alterations as described by Azevedo et al. [1998]) by determining the rate of conversion of H_2O_2 to O_2. Total ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured as the decrease in absorbance at 290 nm resulting from ascorbate oxidation (Nakano and Asada 1981). Guaiacol peroxidase (GPX; E.C. 1.11.1.7) activity was measured photometrically at 470 nm (Souza and MacAdam, 1998) by following tetraguaiacol formation at 28 °C. Glutathione reductase (GR; E.C. 1.6.4.2) activity was determined as described by Cakmak and Horst (1991) by measuring the absorbance decreases at 340 nm due to NADPH oxidation.

2.3 Statistical analyses

The results were expressed as the averages of five replicates. The data were statistically analyzed using one-way analysis of variance run on the SAS software program (SAS Institute Ins., 1996).

3 Results

3.1 Growth and as contents

Visual symptoms of As intoxication were observed 25 days after initiating treatment, mainly in the highest As-treated plants. Chlorotic spots, leaf curling, reduced flexibility of the leaf blade, reduced growth, and reduced leaf emergences were observed in the aerial portions. Changes in the architecture of the roots after As exposure as root darkening and thickening was quite evident. Root length decreased as As levels increased above 10 mg As kg^{-1}, (Table 1) and decreases in shoot lengths were found with in As exposure plants (Table 1).

Regardless of the As substrate levels, fresh weight ($ppm_{(fresh)}$) As contents were always higher in the roots than in the shoots, although $ppm_{(fresh)}$ increased in both tissues as As levels increased (Table 1). The biological absorption coefficient (BAC) of both roots and shoots was highest in 10 mg As kg^{-1} treated plants, and was higher in roots than in shoots (Table 1).

3.2 As-induced morphological changes

Arsenic likewise induced anatomical changes in *A. peregrina* seedling roots (Fig. 1). The stele had a triarch form in the roots of control plant, but differentiation and pith formation was not complete with exposure to 10 and 50 mg As kg^{-1}, resulting in increases in the numbers of primary xylem poles to four and five respectively. The primary xylem poles did not increase in numbers in seedlings exposed to 100 mg As kg^{-1}, although the roots of these plants did show thick fibers alternating with the xylem poles, a situation that was not observed in the other treatments; fluorescence analysis also revealed lignin deposits in the endodermal cells of plants exposed to 100 mg As kg^{-1} that were not observed in any other treatments (Fig. 1). Changes in the sizes, shapes, and the arrangements of cortical parenchyma cell were also observed in the roots of As-treated plants, principally at 50 and 100 mg As kg^{-1}. Diameter of the tracheal element in cross section decreased in the presence of As, and was lowest in the highest As treatments (Table 2). Similarly, The Carlquist Vulnerability Index (CVI) was lower in plants treated with arsenic. The cortex and endodermis were thicker in the presence of As, although the epidermal thicknesses did not differ statistically ($P > 0.05$) among the treatments (Table 2).

3.3 As-induced oxidative damage and antioxidant enzyme activities

Arsenic increased the MDA contend in both the roots and leaves of *A. peregrina* seedlings, indicating enhanced lipid peroxidation (Fig. 2). However, while root MDA contents in plants exposed to 10 mg As kg^{-1} did not differ from the controls, an increase was observed in their leaves. With higher As doses, MDA contents increased from 25.8-28.4% and from 89.5-100.7% in roots and leaves respectively (Fig. 2). In contrast, arsenic exposure did not cause any H_2O_2 accumulation, with H_2O_2 content showing a significant reduction in the roots (at 50 and 100 mg As kg^{-1}), and in the leaves (in all As treated plants) (Fig. 2). Except for GPX and GR, antioxidant enzyme activity was higher in leaves than in roots. Arsenic significantly altered the activities of scavenging enzymes, and both SOD and GPX activities increased in the presence of As (Fig. 2).

Arsenic had differential effects on GR activity among the different plant tissues. GR activity in the roots did not statistically differ from control at 10 mg As kg^{-1}, but decreased more than 40% with exposure to 50 and 100mg As kg^{-1} (Fig. 2). GR activity increased in the leaves (from 185.0 to 271.7%) in As treated plants (Table Fig. 2). APX and CAT activities showed trends similar to GR. The highest CAT activity (79.7% over the control) was seen in roots exposed to 10 mg As kg^{-1}, but a sharp decrease (of 26.8%) was observed at 50 and 100 mg As kg^{-1}. CAT activity in the leaves decreased by more than 39.7% with 10 mg kg^{-1} arsenic doses and higher (Fig. 2). Likewise, root APX activity was highest at 10 mg As kg^{-1}, and decreases greater than 36.6% were seen with exposure to 50 and 100 mg As kg^{-1}. APX activity decreased in the leaves (4.5%) with exposure to 10 mg As kg^{-1}, but this was not significantly different from the control plants ($P > 0.05$). The lowest APX activities were seen in plants exposed to 50 and 100 mg As kg^{-1}, with decreases of up to 24.3% in relation to the control (Fig. 2).

4 Discussion

Arsenic-induced growth reductions and symptoms of intoxication have been described in numerous plant species (Hartley-Whitaker et al,. 2001; Singh et al., 2007; Wang et al., 2010). Our data was consistent with the observations of Gomes et al. (2012), who also reported decreased yields as well as symptoms of intoxication in *A. peregrina* plants grown in increasing As doses in the absence of AMF colonization. In absence of this symbiosis, *A. peregrina* is sensitive to the presence of As even at low concentrations, suggesting that AMF is responsible for the natural occurrence of this species in As-contaminated sites (as reported by Gomes [2011]). Arsenic-phytotoxicity has been related to As-induced oxidative stress due to the generation of free oxygen species that can damage proteins, amino acids, and nucleic acids (Dat et al., 2000). Grow reduction may also be related to heavy metal interference in photosynthetic processes such as chlorophyll synthesis and Calvin cycle reactions (Stoeva et al., 2004). Arsenic does not interfere with photosynthesis and yield in some species, however, due to unique morphological and biochemical features (Pereira et al., 2011), leading us to study these features to explain the As-sensitivity of *A. peregrina* seedlings. Different from hyperaccumulators (such as *Pteris vittata*, which accumulates As in its fronds [Ma et al. 2001]), many plant species show higher As accumulation rates in their roots than shoots (Shaibur et al., 2006) - especially As-sensitive species (Singh et al., 2007).

By avoiding As accumulation in their shoots, plants can reduce the deleterious effects of this heavy metal on photosynthetic sites. Additionally, As generally shows low mobility in terms of its translocation from roots to shoots (except among hyperaccumulator plants) (Zhao et al., 2008). The rapid reduction of arsenate to arsenite in the roots, followed by complexation with thiols (and possible sequestering in root vacuoles) may explain the observed reductions of As transport (Zhao et al., 2008). Nonetheless, both root and shoot ppm_{fresh} were high, and increased as As doses increased - indicating the capacity of *A. peregrina* to accumulates this heavy metal, as verified by Gomes et al. (2012). Root BAC was high even at the highest As doses, which demonstrated significant As uptake by the roots and relatively high transport rates to the shoots which may be linked to As-sensitivity of *A. peregrina*. According to Mahammod et al. (2005), heavy metal sensitivity in plants is correlated with their tendency and ability to accumulate heavy metals. According to Gomes et al. (2012) the ability of *A. peregrina* to absorb and accumulate As was related to As-induced nutritional disturbances and subsequent decreases in plant growth (Gomes et al., 2012).

Growth reductions and arsenic accumulation were accompanied by anatomical changes in *A. peregrina* roots. Heavy metal-induced root anatomical changes have previously been described in plants (Gomes et al., 2011; Lux et al., 2011; Marques et al., 2011; Pereira et al., 2011) and include decreases in root hair numbers, damage to epidermal and cortex cells in mung beans, and a lack of pith formation and differentiation in the stele (Singh et al., 2007). When viewed under a light microscope, the lateral roots of *A. peregrina* are usually triarch, and have a parenchymatous cortex that is delimited centripetally by the endodermis and centrifugally by an epidermal layer (Gross et al. 2003). In the stele, groups of phloem cells alternate with primary xylem poles and fibers (when present) that were probably formed by pericycle divisions (Gross et al., 2003).

Modifications of the vascular bundles in response to As exposure have also been reported in water hyacinth plants (Pereira et al., 2011). Changes in tracheary element size and number in *Brachiaria decumbens* were attributed to heavy metal effects on root maturation as a result of alterations in hormone balances (Gomes et al. 2011) that affected tissue morphogenesis and influenced cell division and the numbers of cells in these tissues (Sandalio et al. 2001). According Gomes et al. (2011), the reduction of tracheary element cross sectional diameter could represent a mechanism designed to maintain hydraulic functions. The Carlquist vulnerability index (IVC) did in fact decrease in the presence of As. This index is designed to infer the hydraulic vulnerability of xylem – with lower IVC values implying higher water conductivity (Carlquist, 1975) that may be influenced by environmental stress (Pereira et al., 2008). Reductions of IVCs have been reported in the roots of plants in the presence of As (Pereira et al., 2011).

In addition to affecting the vascular bundles, As induced other anatomical changes in *A. peregrina* roots, with both the cortex and the endodermis becoming thicker in the presence of As (and the latter structure became lignified at the highest As doses).

According to Castro et al. (2009), the epidermis is the first tissue to come into contact with soil contaminants and so one of the first to demonstrate symptoms of stress. The absence of changes in the epidermis in the presence of As as well as the thickening of the cortex seen in *A. peregrina* are probably due to increases in cortical cell diameters, which has been described as an As-tolerance mechanism (Pereira *et al.*, 2011). Arsenic was mainly found in the epidermis and endodermis (Smith *et al*., 2009), so endodermal thickening and lignification could be part of a plant strategy to minimize As translocation to the shoots, thus avoiding negative effects on photosynthetic sites (Gomes et al., 2011).

The As sensitivity of *A. peregrina* could be related to oxidative stress inducing lipid peroxidation but not by H_2O_2 accumulation. Earlier studies reports that As causes severe lipid peroxidation (Hartley-Whitaker et al. 2001; Stoeva et al. 2005; Srivastava et al. 2005; Singh et al. 2007), which in turn indicates membrane damage resulting from exposure to ROS (Montillet et al. 2005). Moreover, our results are in agreement with Gunes et al. (2009) which verified that As-inducing lipid peroxidation was not following by H_2O_2 accumulation. It may be related to the effective scavenging of H_2O_2 by antioxidant system. We confirmed the influence of As on antioxidant enzyme activity in *A. peregrina* (Fig. 2). Increased SOD activity in the presence of As was seen in roots and leaves of the arsenic-treated plants. CAT and APX activities in roots were greater in plants exposed to 10 mg As kg^{-1}, although their MDAs were similar to the controls and the roots of these plants did not show any growth inhibition. Unlike CAT and APX activity reductions, increased MDA contents and growth decreases were observed in the roots of plants exposed to 50 and 100 mg As kg^{-1}. This data suggests roles for these two enzymes in preventing MDA accumulation and lipid peroxidation.

This was also confirmed in leaves, with leaf CAT and APX activities decreasing, MDA content increasing, and growth decreasing even with small As doses. SODs are known to be major O_2^- scavengers and provide a first line of defense against cell injury by environmental stress factors (Gratäo et al., 2005). Increased SOD activity and decreased CAT and APX activities in plants exposed to As was reported by Singh et al. (2007). APX is mainly located in chloroplasts (Singh et al. 2007), and since As can cause chloroplast ultrastructural damage affecting their integrity and associated metabolism (Li et al. 2006), decreases in APX activity would be expected in the leaves. Arsenic also interferes with enzyme activity and with other proteins by binding to intracellular thiols (-SH) and thus inactivating them (Meharg and Hartley-Whitaker, 2002). The decreased activities of CAT and APX indicated their inactivation/degeneration due to As-induced oxidative stress (Singh et al., 2007).

The excess H_2O_2 from SOD activity could be detoxified by GPX, which, in fact, showed increased activity with As increased exposure. In contrast to leaves, which showed high GR activity, a significant decrease in GR activity was noted in roots exposed to 50 mg As kg^{-1}. GPX acts upon H_2O_2 and forms GSSH that is further reduced to GSH by GR (Singh et al., 2007). Decreasing root GR activity could also contribute to lipid peroxidation (and growth reduction) since GPX activity may be substrate-limited. However, Sigh *et al*. (2007) observed that GPX was not involved in providing protection against oxidative damage by H_2O_2 in roots – and the enhanced activity of GPX was correlated with higher lignification and stunted growth as a reaction to stress (as was seen in the roots exposed to 50 and 100 mg As kg^{-1}) (Gajewska et al., 2006).

In conclusion, our results demonstrated that arsenic affects early root growth, inducing root anatomical changes and altering plant antioxidant scavenging machinery by inactivating some antioxidant enzymes (CAT and APX). As-induced oxidative stress caused by lipid peroxidation (but not by H_2O_2 accumulation) may be the main cause of As-sensitivity and it appears to be linked to nutritional disturbances seen in *A. peregrina* without AMF colonization (Gomes et al., 2012). Since these plants become As-tolerant under AMF symbioses, the ability of AMF to minimize oxidative stress under these conditions needs to be more closely investigated.

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Table 1. Root and shoot growth and As-content of *Anadenanthera peregrina* seedlings grown in substrate amended with As-increased doses

	As (mg kg^{-1})			
	0	10	50	100
Root				
Length (cm)	9.3a	7.7a	5.2b	4.4b
As-content (fresh weight) ppm	-	90.41c	153.05b	304.47a
Biological absorption coefficient	-	9.04a	3.06b	3.04b
Shoot				
Length (cm)	16.0a	8.9b	5.6b	8.1b
As-content (fresh weight) ppm	-	35.86c	45.28b	104.64a
Biological absorption coefficient	-	3.58a	0.90b	1.04b

The results are presented as means of four replicates. Different letters within the same line indicate a significant difference (Scott-knott, $P < 0.05$)

Table 2. Root anatomical features of *Anadenanthera peregrina* seedlings grown in substrate amended with As-increased doses

	As (mg kg^{-1})			
	0	10	50	100
N° Tracheary elements	25.25 ± 6.13	32.75 ± 4.03	37.25 ± 5.12	30.00 ± 6.73^{NS}
Tracheary elements diameter (□ m)	68.25 ± 1.70a	59.75 ± 5.12b	60.50 ± 3.12b	51.50 ± 2.5c
CVI	2.83 ± 0.72a	1.83 ± 0.19b	1.65 ± 0.27b	1.81 ± 0.59 b
Cortex (□m)	114.82 ± 2.06c	123.04 ± 2.99b	135.42 ± 2.98a	138.35 ± 1.41a
Epidermis (□m)	26.13 ± 1.59	28.16 ± 2.16	25.46 ± 2.20	27.78 ± 1.84 ^{NS}
Endodermis (□m)	17.19 ± 0.94d	19.67 ± 1.04c	23.86 ± 1.84b	27.67 ± 1.13a

CVI, Carlquist Vulnerability Index

The results are presented as means ± SE of four replicates. Different letters within the same line indicate a significant difference (Scott-knott, *P*<0.05). NSnot significantly

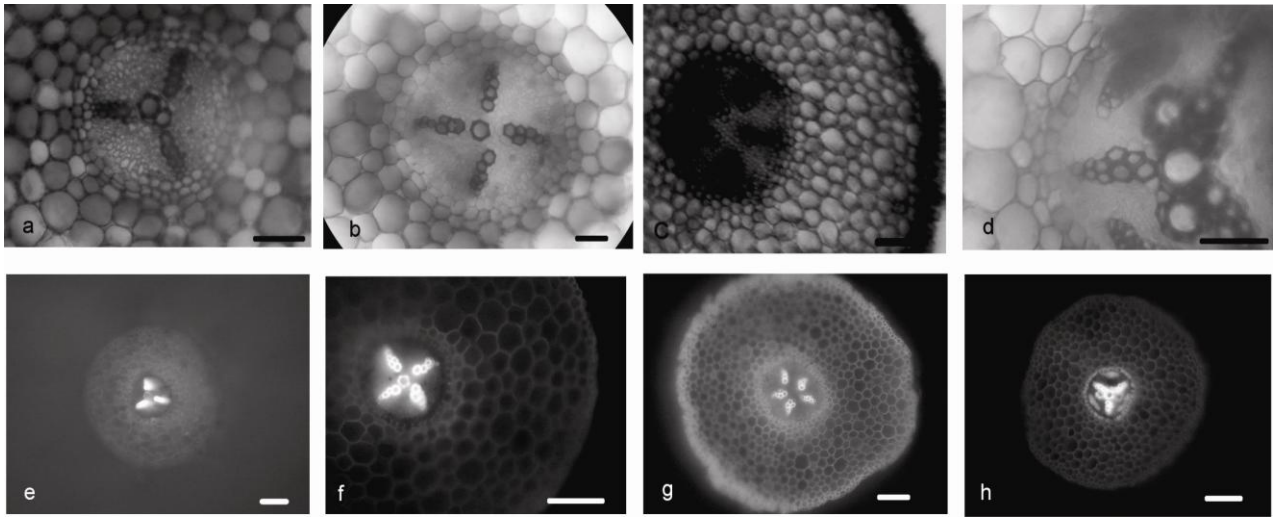

Fig. 1. Cross section (a – d) and Fluorescence photomicrography for lignin detection (e- f) of A. peregrina root showing anatomical changes upon exposure to 0 (a and e), 10 (b and f), 50 (c and g) and 100 (d and h) mg As kg^{-1}. Scale bars correspond to 50 □m.

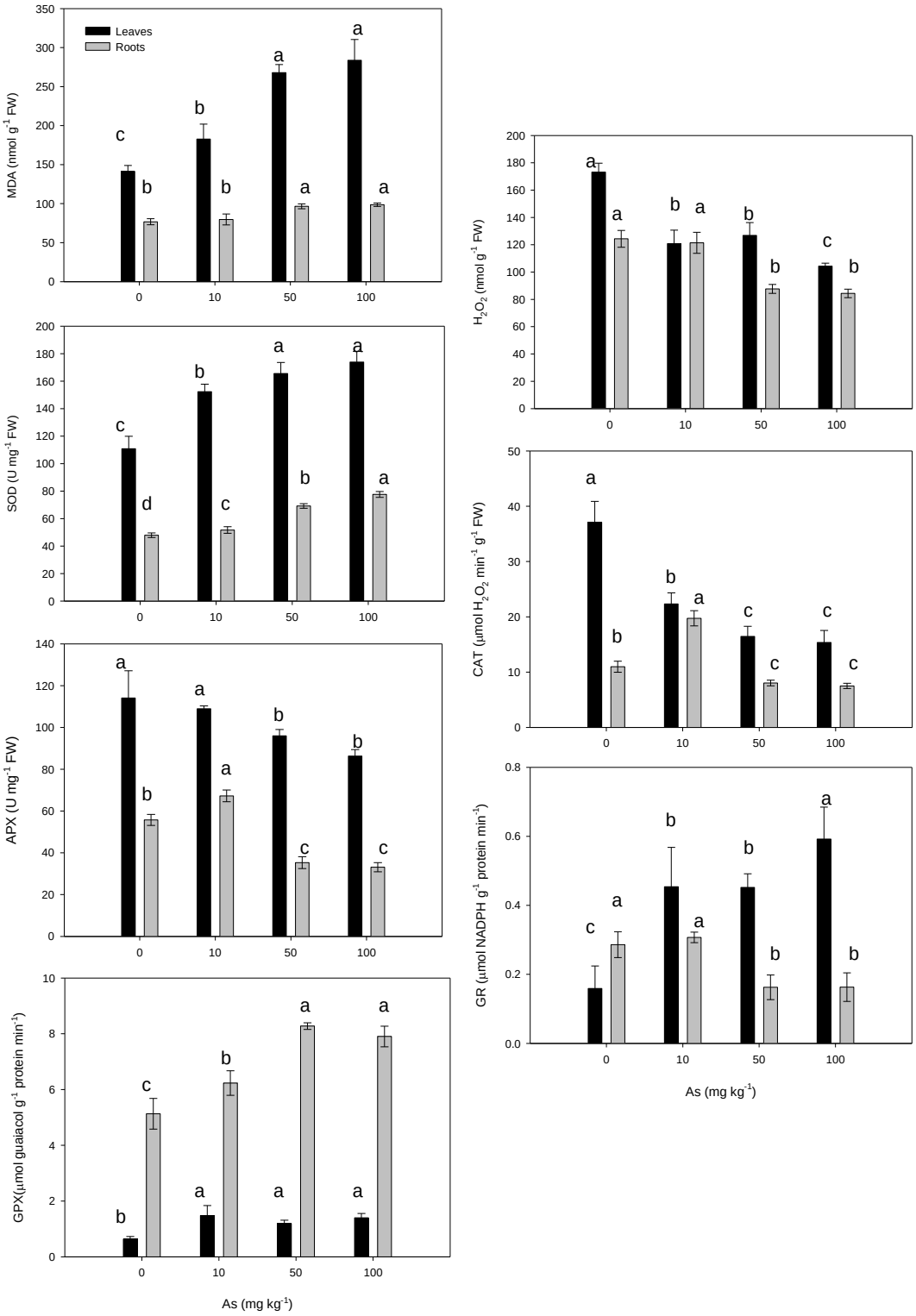

Fig. 2. Effects of arsenic (As) on root and leaves lipid peroxidation (MDA content), amounts of H_2O_2 and on antioxidant enzymes (SOD, CAT, APX, GPX and GR) of *Anadenanthera peregrina* seedlings. The data were recorded after 25 days' exposure to As, . Different letters within the same line indicate a significant difference (Scott-knott, $P<0.05$).