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Effects of short-term arsenic exposure in *Arabidopsis thaliana*: tolerance *versus* toxicity responses

A. PITA-BARBOSA[1]*, T.C.R. WILLIAMS[2], and M.E. LOUREIRO[1,3]

Centre for Coastal, Limnological and Marine Studies, Federal University of Rio Grande do Sul, Litoral Norte Campus, Imbé, RS, 95625-000 *Brazil*[1]
Department of Botany, University of Brasilia (UNB), Darcy Ribeiro Campus, Brasilia, DF, 70297-400 *Brazil*[2]
Arts, Humanities and Sciences Institute, Federal University of Southern Bahia (UFSB), Paulo Freire Campus, Teixeira de Freitas, BA, 45988-058 *Brazil*[3]
Plant Biology Department, Federal University of Viçosa (UFV), Viçosa Campus, Viçosa, MG, 36570-900 *Brazil*[4]

Abstract

The metalloid arsenic (As) is highly phytotoxic, in part due to the similarity of the arsenates to phosphates, but also due to its ability to induce reactive oxygen species (ROS) formation, and in the form of arsenite directly interact with certain enzymes. Here we aimed to determine the effects of a short period of As exposure on *Arabidopsis thaliana*. Particular focus was given to shoot responses, which have received less attention in previous studies. *A. thaliana* (ecotype Col-0) plants (28-d-old) were cultivated hydroponically in the presence of 0, 27, 108, and 216 μM arsenic in the form of sodium arsenate for five days. Translocation of As from root to shoot increased with increasing As concentration in the medium and caused a reduction in growth. Photosynthesis was severely affected due to stomatal closure, increased ROS accumulation, and alterations in expression of genes involved in oxidative stress responses and As detoxification. Primary metabolism was also perturbed, suggesting both the direct inhibition of certain enzymes as well as active defensive responses. Overall the effects of As toxicity depended greatly on the degree of translocation from root to shoot and involved both direct effects on biological processes and secondary effects caused by the accumulation of ROS.

Additional key words: arsenate, gene expression, leaf anatomy, metabolic profile, oxidative stress, photosynthesis.

Introduction

Arsenic is an extremely toxic metalloid that is found in nature as the anions AsO_4^{-3} (arsenate) and AsO_3^{-3} (arsenite) (Tripathi *et al.* 2007), and its presence in over two hundred minerals means that it is continuously released into the environment by weathering. The ingestion of As-contaminated water represents a serious health risk worldwide, leading to both cancerous and non-cancerous pathologies (Martinez *et al.* 2013, Rodríguez-Lado *et al.* 2013). Environmental contamination with As is also a serious problem in Brazil (Figueiredo *et al.* 2007) where the main source of As is mining; gold-bearing ore can contain up to 11 % As that may be released during gold extraction (Borba *et al.* 2003).

The high toxicity of arsenate is due to its chemical similarity to phosphate. In plants, the phosphate and arsenate anions compete for the same transporter present in root epidermal cells. Arsenate may therefore cause phosphorus deficiency (Clarck *et al.* 2000) and is also known to downregulate the high affinity phosphate transport system as part of a mechanism to avoid As uptake

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Abbreviations: AOX1D - alternative oxidase 1D; CA - carbonic anhydrase; c_i/c_a - internal to atmospheric CO_2 concentration ratio; E - transpiration rate; EL - electrolyte leakage; F_v/F_m - variable to maximum fluorescence ratio (maximum potential yield of photosystem II); g_s - stomatal conductance; GSH - reduced glutathione; GSSH - glutathione (oxidized form); GSTF8 - glutathione S-transferase phi 8; MDA - malondialdehyde; PAR - photosynthetic active radiation; P_N - net photosynthetic rate; PC - phytochelatin; PCD - programmed cell death; ROS - reactive oxygen species; RWC - relative water content; TF - translocation factor.
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* Corresponding author: pitabarbosa@yahoo.com.br

in conditions of low soil Pi availability (Catarecha *et al.* 2007, Castrillo *et al.* 2013). Phosphate may also be replaced by arsenate in metabolic processes. During mitochondrial oxidative phosphorylation ADP-As may be formed, which will hydrolyse spontaneously, ultimately reducing ATP production, whereas in glycolysis arsenate may be used by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to produce 1-arseno-3-phosphoglycerate, that is hydrolysed to 3-phosphoglycerate without formation of ATP (Finnegan and Chen 2012).

Immobilization in root vacuoles represents an important mechanism for defence against As toxicity, a process preceded by the conversion of arsenate to arsenite by the enzyme arsenate reductase (Chao *et al.* 2014, Sanchez-Bermejo *et al.* 2014) and complexation with reduced glutathione (GSH) and phytochelatins (PC) (Schulz *et al.* 2008). Non-complexed arsenite is even more toxic than arsenate, due to its ability to inactivate a range of thiol-rich proteins (Bleeker *et al.* 2006) and react with the dihydrolipoamide cofactor encountered in the pyruvate dehydrogenase complex, glycine decarboxylase, and 2-oxoglutarate dehydrogenase (Finnegan and Chen 2012). Plant tolerance to As is strongly influenced by the extent of its translocation from root to shoot (Verbruggen *et al.* 2009), as As in leaves may lead to severe inhibition of photosynthesis (Li *et al.* 2006), increased accumulation of reactive oxygen species (ROS), and damage to cellular components (Li *et al.* 2007, Singh *et al.* 2007).

The physiological and genetic basis of As stress in the model plant *Arabidopsis thaliana* has been studied (Catarecha *et al.* 2007, Chao *et al.* 2014, Sanchéz-Bermejo *et al.* 2014), but little attention has been given to the shoot. For this reason, the aim of this study was to identify the tolerance and toxicity responses to As in this non-hyper-accumulator species in order to subsidize future studies with species of ecological and agronomic importance.

Materials and methods

Plants, cultivation and exposure to arsenic: *Arabidopsis thaliana* L. ecotype Col-0 plants were cultivated hydroponically (Norén *et al.* 2004) in half strength Clark nutrient solution (Clark 1982), pH 5.8, which was renewed on alternate days. When plants were 28-d-old, sodium arsenate (Na_2HAsO_4) was added to the nutrient solution to a final concentration of 0, 27, 108, and 216 μM and maintained at this As concentration for five days. The experiments were performed at the Federal University of Viçosa, in a growth room with a temperature of 20 °C, a 16-h photoperiod, a photosynthetically active radiation (PAR) of 100 μmol m^{-2} s^{-1}, and a relative humidity around 73 %.

Growth and mineral composition: Plants with homogeneous size and appearance were examined for As toxicity symptoms during the second and fifth day of exposure. The roots and shoots were collected and weighed to obtain their fresh mass (f.m.). The samples were then dried for 72 h in an oven to obtain the dry mass (d.m.). Relative water content (RWC) was evaluated using the leaf disc method (Downey and Miller 1971) and calculated using the formula RWC = [(f.m. - d.m.)/(water saturated mass - d.m.)] × 100.

Shoots (0.3 g) and roots (0.1 g) were washed with distilled water, dried in an oven for 72 h and ground to powder prior to digestion with nitric-perchloric solution (3:1, v/v) (Tedesco *et al.* 1995). The content of As, Ca, Cu, Fe, K, Mg, Mn, Mo, P, S, and Zn in the plant extracts were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES; *Optima 8300 DV*, *Perkin Elmer*, Waltham, USA) using a standard curve with known concentrations of all analyzed elements. The As translocation factor (TF) was calculated according to the formula: TF = As content in shoots/As content in roots.

Leaf anatomy: Visually healthy leaf samples from control plants and those exposed to 108 μM were fixed according to Karnovsky (1965), dehydrated using an ethanol series, and embedded in methacrylate (*HistoResin*, *Leica Biosystems*, Wetzlar, Germany). For each treatment, we prepared four historesin blocks, each one containing three leaf fragments from distinct leaves of one plant. Therefore, each treatment had four biological replicates and 12 replicates each. Cross sections of 4 μm (with 100 μm between two adjacent cuts) were cut using an automatic microtome (*RM 2155, Leica Biosystems*) and stained in toluidine blue at pH 4.0. The slides were mounted in synthetic resin (*Permount*, *Thermo Fisher Scientific*, Hampton, USA). Images were captured using a light microscope with a coupled digital camera (*AX70TRF*, *Olympus*, Tokyo, Japan) for measurement of the thickness of leaf regions and tissues. The analyses were performed on the middle portion of the leaves between the margin and midrib covering an area of 600 μm^2 using the software *Image Pro Plus 5.1*.

Oxidative stress analysis: Malondialdehyde (MDA) content was assessed spectrophotometrically in leaves and roots using a thiobarbituric acid (TBA) assay (Hodges *et al.* 1999) and membrane damage in leaves was evaluated through electrolyte leakage quantification (Garraway *et al.* 1989). Hydrogen peroxide and the superoxide radical were detected as proposed by Ramel *et al.* (2009) in entire roots, young leaves, and fully expanded non-senescent leaves. Quantification of soluble phenolics (antioxidants) was done in the same extract as for MDA analysis using the Folin-Ciocalteau method (Sánchez-Viveros *et al.* 2011).

Metacaspase-3 activity was measured using a caspase-3 assay kit (*DEVDR110*, *Biotium*, Fremont, USA) according to the manufacturer's instructions. Fluorescence was measured using a fluorometer (*Victor X5*, *Perkin Elmer*, Waltham, USA). The results were expressed as relative fluorescence units (RFU) mg^{-1}(protein).

Photosynthesis: Gas exchange parameters (net photosynthetic rate, P_N, stomatal conductance, g_s, and internal/external CO_2 concentration, c_i/c_a) were determined using an infrared gas analyser coupled with fluorometer (*LI-6400XT, Li-Cor*, Lincoln, USA) in fully expanded non-senescent leaves, on the fifth day of As exposure. The PAR was supplied by a halogen lamp (600 µmol m^{-2} s^{-1}). Possible photoinhibition was accessed by "light curves" using PAR 0 - 1000 µmol m^{-2} s^{-1} in descending order, with intervals of 2 - 3 min between each reading. Light saturation point (LSP), light compensation point (LCP), and dark respiration rate were obtained from this curve using regression analysis. The following chlorophyll *a* fluorescence-related parameters were measured on the same leaves used for gas exchange analysis (which were dark adapted prior to analysis): initial fluorescence, F_0, potential quantum efficiency of photosystem II, F_v/F_m, electron transport rate, ETR, and the non-photochemical quenching, NPQ.

The anthocyanin content: Leaf discs were placed in -20 °C methanol-HCl (0.1 % HCI, v/v) and kept at 2 °C for 24 h. The extracts obtained were used for spectrophotometric determination of anthocyanins, as described by Murray and Hackett (1991).

Shoot temperature: Thermal images of shoots were obtained using an infrared camera (*T420, Flir*, Wilsonville, USA). We measured the area corresponding to three temperature intervals (14.7 - 16.2, 16.2 - 17.0, and 17.0 - 18.5 ºC) using the software *Image Pro Plus v. 5.1.*

Carbonic anhydrase activity: Fresh leaf samples were ground in liquid nitrogen and homogenized with 0.1 M sodium phosphate buffer (pH 8.3), supplemented with 5 mM cysteine, 1 mM EDTA, and 125 µg of sucrose and centrifuged (10 000 *g,* 4 ºC, 20 min). To 0.05 cm^3 of supernatant we added 1.5 cm^3 of 0.05 M sodium phosphate buffer (pH 8.3) and 1.5 cm^3 of cold water saturated with CO_2. After addition of this last component, the time necessary for the pH change from 8.3 to 7.3 (T_{Sample}) was determined. The blank consisted of 0.05 M sodium phosphate buffer (pH 8.3) and cold water saturated with CO_2 and without the extract (T_{Blank}). The CA activity was given by the formula: AC = 10 (T_{Blank}/T_{Sample}) - 1, and expressed as Wilbur-Anderson units (WA) (adapted from Lazova *et al.* 2004).

Metabolic profile: Metabolites were extracted using around 0.1 g of fresh root or leaf tissue. For gas chromatography coupled mass spectrometry (GC-MS) analysis samples were derivatized using N-methyl-N-(trimethylsilyl) trifluoroacetamide and methoxyamine hydrochloride (Lisec *et al.* (2006). GC-MS analysis was carried out using an *Agilent 7890-5795* instrument equipped with a 30-m column (*HP-5ms*, *Agilent Technologies*, Santa Clara, USA). Metabolites were identified using the National Institute of Standards and Technology (NIST) spectral libraries and by comparison with standards. Chromatograms were aligned, and relative metabolite abundance determined using the software *MetAlign* (Lommen 2009). Data were expressed on a dry mass basis. The content of sucrose, fructose, glucose, and starch was determined spectrophotometrically using NADH coupled enzymatic assays according to Trethewey *et al.* (1998).

Real-time quantitative PCR: Samples from plants submitted to 0, 27, and 108 µM As for leaves and 0 and 27 µM As for roots were analysed (good quality RNA could not be obtained from treatments with higher As concentrations). Total RNA was extracted (Wang *et al.* 2008) for synthesis of the first-strand cDNA (*ImProm-II, Promega*, Madison, USA) according to the manufacturer's instructions. The real-time qPCR assays were performed using the *SYBR Green* detection system (*Thermo Fischer Scientific*) using primers for the amplification of the candidate genes (Table 1 Suppl.). The specificity of the reaction was checked by a final melting curve and the Ct values were corrected based on the efficiency of single reactions using the Miner algorithm (Zhao and Fernald 2005) and results expressed by the comparative Ct method ($2^{-\Delta\Delta Ct}$) (Livak and Schmittgen 2001). The values were normalized based on *EF1-a* gene expression. Samples for this analysis were run in four biological replicates and three technical replicates.

Statistical analysis: The randomized block design was used for the photosynthesis, shoot temperature, and gene expression analyses. In the others analyses, we used the completely randomized design. The "light curves" were analyzed by non-linear regression (rectangular hyperbola) with the software *Sigma Plot 10.0*. The other data were submitted to analysis of variance (*ANOVA*) and compared by Tukey's test at $P \leq 0.05$ with the software *Assistat 11.0.*

Results

Arabidopsis thaliana plants exposed to arsenate in the hydroponic nutrient solution accumulated substantial amounts of As in their roots (Table 1), which were saturated by the 27 µM As treatment, as levels remained

almost constant with further increases in As concentration. In the shoot, treatment with 108 and 216 μM As led to significant increases in As content as a result of increased As translocation to shoot (Table 1), which reached 61 % in higher As doses. The symptoms of As toxicity in both shoots and roots intensified with increasing time of exposure and As concentration. Plants exposed to 108 and 216 μM As exhibited a purple colour mainly on the lower side of the leaf, resulting from anthocyanin accumulation, which was greater in the expanded leaves. Compared to control, the increase in anthocyanin content was 146 and 286 % in young leaves, and 307 and 443 % in mature leaves, respectively. At the highest As concentration most of the leaves (including young ones) exhibited signs of early senescence. Roots exposed to 108 μM As showed a higher density of hairs and at 216 μM As acquired a brownish colouring and gelatinous appearance (Fig. 1 Suppl.).

Exposure to As led to greater reductions in f.m. than in d.m. (Table 1). Shoot f.m. was reduced by ~40 and 70 % in treatments with 108 and 216 μM As and the RWC decreased by 29 and 49 % compared to the control under these treatments (Table 1). Leaf blade thickness was reduced in both young (-8.2 %) and fully expanded (-10.8 %) leaves (Fig. 1, Table 2 Suppl.). In the former, the spongy parenchyma was the most affected tissue, with a reduction of ~20 % in thickness. A reduction in intracellular spaces was noticeable even without measurement, but no other changes in young leaves were observed. In mature leaves all other tissues exhibited reduced thickness. The spongy parenchyma was also the most affected tissue, followed by the palisade parenchyma (-10.8 %), without significant differences in the number of cells in each tissue in either young or mature leaves.

Exposure to As led to changes of several gas exchange parameters (Table 1). There was a gradual decrease in P_N with increasing As concentration. Stomatal conductance (g_s) also decreased under 27 and 108 μM As, when compared to the control, but at 216 μM As g_s was similar to that detected for 27 μM As. The c_i/c_a ratio followed a similar pattern, being reduced in treatments with 27 and 108 μM As. Interestingly, the c_i/c_a ratio was higher in plants treated with 216 μM As than in control.

CA activity and electron transport rate (ETR) gradually decreased with increasing As concentration (Table 1), showing that the observed reductions in P_N were not only due to stomatal closure. At 216 μM minimum fluorescence (F_0) was increased by almost 50 % compared to the control, showing the occurrence of biochemical damage and explaining why CO_2 assimilation rates were close to zero at such a rather high stomatal aperture (Table 1). High As doses also promoted photoinhibition under higher

Table 1. Characteristics of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0, 27, 108, and 216 μM of arsenic for five days. Means ± SEs, n = 5. Different letters indicate significant differences at $P < 0.05$ (Tukey's test). PS II = photosystem II, WA = Wilbur-Anderson units, Rh = rhodamine.

Physiological parameters	Organs	As concentration [μM] 0	27	108	216
Arsenic accumulation [g kg^{-1}(d.m.)]	shoot	$2e^{-4}$ d	0.12 c	0.64 b	0.89 a
	root	$7e^{-3}$ d	1.90 a	1.83 a	1.46 b
Arsenic translocation factor, TF [%]	shoot/root	0.02	0.06 c	0.35 b	0.61 a
Fresh mass [g]	whole plant	1.80 a	1.56 a	1.09 b	0.56 c
Dry mass [g]	whole plant	0.59 ab	0.74 a	0.54 b	0.34 c
Relative water content [%]	leaf	90.9 a	87.5 a	72.1 b	59.3 b
Shoot temperature [%]	17.0-18.5 °C	35.7 b	33.3 b	33.3 b	48.0 a
	16.2-16.9 °C	21.2 c	25.2 c	54.6 a	34.0 b
	14.7-16.1 °C	43.1 a	41.5 a	12.1 b	18.0 b
Net photosynthetic rate [μmol m^{-2} s^{-1}]	leaf	12.91 a	7.80 b	2.84 c	0.05 d
Stomatal conductance [mmol m^{-2} s^{-1}]	leaf	0.19 a	0.05 b	0.02 c	0.04 b
Internal/external CO_2 concentration ratio	leaf	0.81 b	0.60 c	0.55 c	1.16 a
Electron transport rate, ETR [μmol m^{-2} s^{-1}]	leaf	45.2 a	29.1 b	18.8 bc	11.1 c
Initial fluorescence, F_0	leaf	553.7 ab	503.1 ab	436.5 b	652.4 a
Potential quantum efficiency of PS II, F_v/F_m	leaf	0.82 a	0.83 a	0.79 a	0.69 b
Non-photochemical quenching, NPQ	leaf	0.29 c	0.42 bc	0.49 ab	0.58 a
Carbonic anhydrase activity [WA units]	shoot	5042 a	2468 b	604 c	76 d
Malondialdehyde content [nmol g^{-1} (d.m.)]	shoot	122 b	152 ab	260 a	247 a
	root	129 c	515 b	1679 a	1692 a
Eletrolytes leakage [%]	leaf	13.9 b	14.1 b	21.5 a	26.3 a
Soluble phenols [mg g^{-1} (d.m.)]	shoot	11.6 b	21.4 a	25.4 a	23.4 a
	root	6.5 b	12.3 ab	18.9 a	8.1 a
Caspase-3 activity [RFU g^{-1}(protein)]	shoot	96.8 a	86.0 a	66.7 a	56.4 a
	root	341.0 a	148.0 b	141.6 b	84.4 c

irradiances, as indicated by a dramatic reduction in the light saturation point, the low F_v/F_m ratio (~0.70) and increased non-photochemical quenching (NPQ) observed at 108 and 216 μM As (Table 1, Fig. 2 Suppl).

With respect to shoot temperature, three temperature levels were determined: low (14.7 - 16.2 ºC), moderate (16.2 - 17 ºC) and high (17.0 - 18.5 ºC). Plants exposed to the lowest As dose showed the same temperature as control plants, with the lower temperature range being predominant while the moderate and high temperature ranges were predominant in plants submitted to 108 and 216 μM As, respectively (Table 1, Fig. 3 Suppl.).

Roots of plants exposed to 27 μM showed a 4-fold increase in MDA content in relation to the control, reaching increases of around 10-fold at higher As concentrations. Oxidative stress was also detected in the shoot (though to a lesser extent than in roots), since increased MDA content and electrolyte leakage were detected in the treatments with 108 and 216 μM As (Table 1).

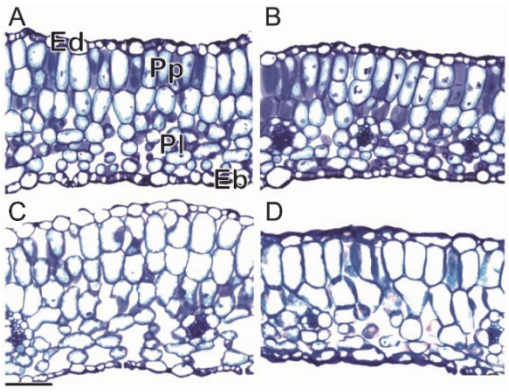

Fig. 1. Transversal sections of young (*A*, *B*) and fully expanded (*C*, *D*) leaves (light microscopy) of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0 (*A*, *C*) and 108 μM arsenic (*B*, *D*) for five days. Eb - abaxial epidermis, Ed - adaxial epidermi, Pl - spongy parenchyma, Pp - palisade parenchyma. *Bar* = 100 μm.

The production of formazan was used to visualize $O_2^{\bullet-}$ accumulation (Fig. 4 Suppl.). In young and expanded leaves, blue staining was detected in an apparently random pattern in different regions of the leaf blade, and both the number of spots and colour intensity increased with increasing As concentration. In roots, the greatest content of $O_2^{\bullet-}$ were found in the treatment with 108 μM As. At the lower As dose almost no stain was detected, with the exception of the region closest to the root-stem transition zone. The 216 μM As treatment apparently led to severe root cell death, which is likely to have compromised the detection of $O_2^{\bullet-}$. DAB was used to detect H_2O_2 production after As exposure (Fig. 5 Suppl.). In leaves, brown coloured regions corresponding to areas of H_2O_2 accumulation were found at the leaf margins, and principally close to the shoot apex. Increasing As concentrations led to a gradual increase in H_2O_2 in leaves, with a greater effect in expanded leaves. The presence of H_2O_2 in roots was also detected at the highest concentration of As. We also detected a reduction in metacaspase-3 activity, an apoptotic signalling enzyme, in roots of *A. thaliana*; the lowest concentration of As (27 μM As) led to a 57 % reduction in its activity (Table 1). In the shoot, no significant changes in the enzyme activity were detected under all As treatments.

Exposure to As strongly affected sugar metabolism in *A. thaliana*. Content of glucose increased in young leaves under all As treatments, and in mature leaves under 108 μM As. Starch decreased in both mature and young leaves under 216 μM As and in young leaves also under 108 μM As. Roots exhibited significant decreases in fructose under 216 μM As, whereas content of starch, glucose, and sucrose was not affected (Table 3 Suppl.).

GC-MS based metabolite profiling also revealed extensive changes in primary metabolism (Table 3 Suppl.). In mature leaves, 216 μM As led to increases in the content of amino acids glycine, alanine, proline, valine, and β-alanine. The content of valine and alanine was increased more than 100-fold and the content of proline more than 2000-fold under the 216 μM As in comparison with the control. Amongst the intermediates of the TCA cycle, citrate, fumarate, and succinate decreased with increasing As concentration, while the content of malate and 2-oxoglutarate increased. The amount of glycolate (photorespiratory intermediate) peaked at 108 μM As. Results in young leaves were similar to those for mature leaves, though the content of alanine and glycolate was not affected by As, and the content of raffinose increased with increasing As concentration. In roots, we detected significant increases in the content of maltose and pinitol/ononitol. The content of glucose-6-phosphate was higher at 27 μM As than at 216 μM As. The content of succinate and pyruvate increased specifically under 108 μM As. The content of glycerate firstly increased but then decreased under 216 μM As, whereas the content of glycolate increased gradually with increasing As concentration. Threonic and erythreonic acids and the amino acids lysine, leucine, valine, phenylalanine, tyrosine and β-alanine also increased with increasing As (Fig. 3 Suppl.).

Alterations in the content of micro and macronutrients were detected in shoots and roots in response to As (Table 4 Suppl.). Alterations in macronutrients were more evident in the root system, while the content of micronutrients varied with As treatment in both roots and shoots. I shoots, P was the only macronutrient that suffered a reduction of 33 and 36 % in the treatments with 108 and 216 μM As, respectively. In roots, we detected alterations in all the elements analysed. Only the Ca content increased under As treatments. The content of K was reduced by 36, 59,

and 80 % under 27, 108, and 216 μM As, respectively. An average reduction of 27 % in the content of P and Mg was detected in plants exposed to 108 and 216 μM As. The content of micronutrients Fe and Mn was similar in the root and shoot: in the case of Fe an increase of 9 and 34 % was observed in roots treated with 108 and 216 μM As respectively, while an increase of 32 % was detected in the shoot under the highest As concentration. In the 108 μM As treatment, an increase of 234 and 14 % in Mn content was detected in the roots and shoot, respectively. The content of both Mo and Zn were decreased by all treatments more severely in roots than in shoots.

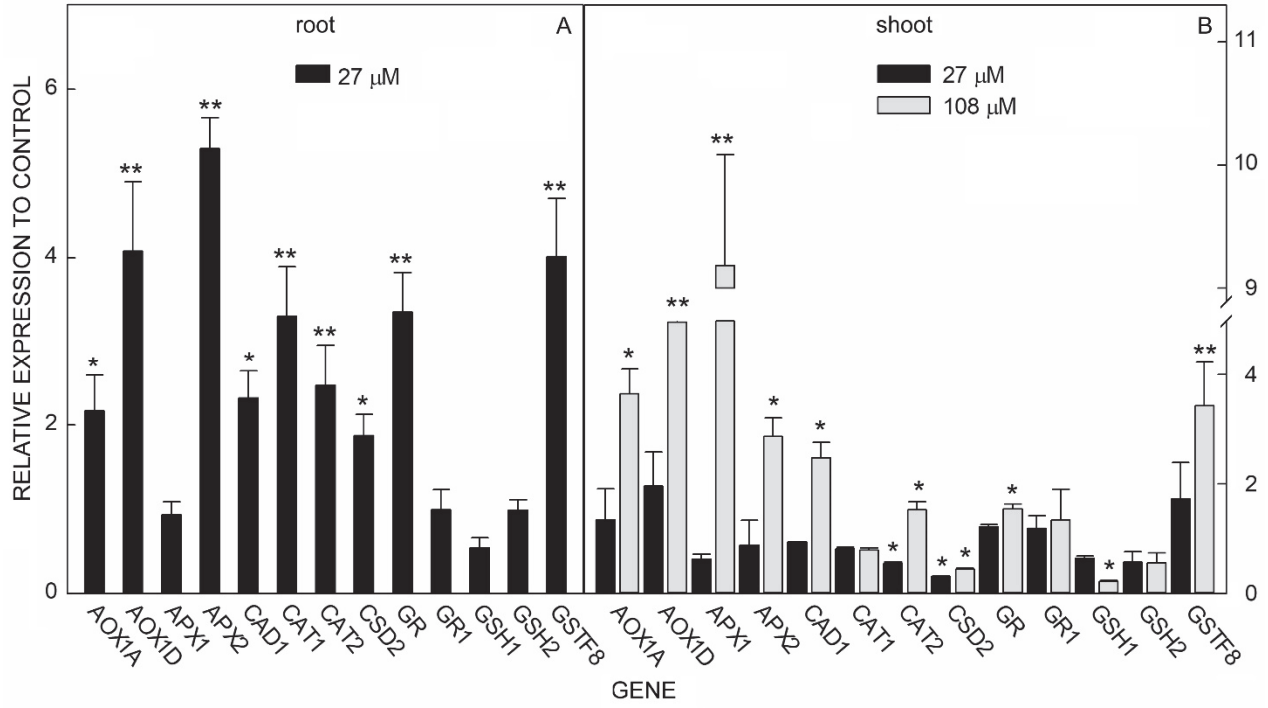

Fig. 2. Gene expression analysis in root (*A*) and shoot (*B*) of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0, 27, and 108 μM arsenic for five days. The values are relative to control (fixed at 1). Black bars refer to 27 μM As and grey bars refer to 108 μM As. Means ± SEs, $n = 4$, * and ** - differences between control and arsenate treatments at $P < 0.05$ and $P < 0.01$, respectively (Student *t*-test).

Alterations in expression of several genes related to As detoxification and antioxidant defence were observed in both root and shoot after As exposure (Fig. 2). In roots treated with 27 μM As, most genes were significantly upregulated, with *AOX1D* and *APX2* showing a 3-fold increase in expression compared to control plants. In leaves, only peroxisomal catalase (*CAT2*) and superoxide dismutase 2 (*CSD2*) decreased under this treatment. In the 108 μM treatment, expression of most genes was increased. Expressions of an ascorbate peroxidase (*APX1*) and an alternative oxidase (*AOX1D*) were greater by 718 and 299 %, respectively, than in the control. Decreased expressions of *CSD2* and glutamate-cysteine ligase (*GSH1*) were also detected in this treatment.

The content of soluble phenolics increased in both roots and shoots under As treatments (Table 1). In shoots, we observed differences between the three As concentrations tested while in the roots a significant increase was only detected in the 108 μM As treatment.

Discussion

In non-hyperaccumulating plants such as *A. thaliana* tolerance to As is achieved through restriction of absorption by the roots or through limitation of translocation to the shoot, avoiding the spread of the contamination (Catarecha *et al.* 2007, Wang *et al.* 2018). Here we highlight the importance of the prevention of high degrees of shoot translocation in plant-As tolerance. Under the lowest As dose this element already reached maximum accumulation in roots, even with the plants not showing any visible symptoms of toxicity. This tolerance was achieved by the low root-to-shoot TF observed in this treatment, possibly through As complexation by glutathione/phytochelatins in roots, followed by storage in vacuoles, as suggested by the increased expression of

glutathione-related genes in roots exposed to 27 μM As. Under higher As concentrations, leaves showed symptoms of toxicity, including darkening, wilting, senescence, and disruption of physiological processes. The symptoms observed allowed us to classify the 27, 108, and 216 As concentrations as being slightly, moderately, and extremely toxic to the plants after short-term exposure.

In the treatment with 108 μM As, the content of As in roots was the same as that detected at 27 μM As, but the TF almost doubled, and many symptoms of damage were observed. This suggests the saturation of the detoxification system for As transport into root vacuoles, and that As toxicity symptoms are mainly due to effects in the shoots. At the highest As dose, we observed severe damage at the whole plant level, despite the fact that accumulated As was not severely affected, but more than 60 % of total As was located in the shoot. We suggest that under extremely high As concentrations, the capacity of the plant to detoxify As becomes even more limited due to the inhibitory effects of As on respiration and photosynthesis. This may occur as energy available for reduction of arsenate to arsenite, a step necessary for arsenic storage in vacuoles, is reduced together with the availability of substrates for the production of chelating agents, such as oxidized glutathione (GSSH) and phytochelatin (PC). This reinforces the great importance of studying the effects of As in shoots instead of focusing only in roots, in order to understand As toxicity at the whole plant level. In roots of plants exposed to the lowest As concentration we detected increased expression of two genes involved in vacuolar As storage: *GR1* (glutathione-disulfide reductase) and *GSTF8* (glutathione S-transferase phi 8). *GR1* helps maintain the pool of GSH, while *GSTF8* binds GSH to arsenite prior to transport into the vacuole. However, for an optimal detoxification the upregulation of *GR1* and *GSTF8* must be accompanied by increases in glutathione content, otherwise chelating agents are unlikely to be sufficient to bind arsenite, leaving it free in the cytosol and ultimately leading to its translocation to the shoot (Bleeker *et al.* 2006). Despite the upregulation of *CAD1* (glutathione γ-glutamylcysteinyltransferase 1), which codes for an enzyme that catalyses PC synthesis from GSSH, no upregulation of *GSH1* (glutamate-cysteine ligase) or *GSH2* (glutathione synthetase 2) expression was found in this study. This suggests that there may be a lack of available GSSH for PC biosynthesis, explaining the high TF of As in *A. thaliana* plants. Similarly, in *Holcus lanatus*, exposure to high concentrations of As led to rapid uptake of this element, meaning that content of GSH and PC available in root cells were insufficient for the detoxification of all As absorbed and ultimately resulting in toxicity due to the presence of significant quantities of arsenite in the cytosol of root cells (Bleeker *et al.* 2006).

In the leaves of plants exposed to 27 μM As no alterations in gene expression were found, as might be expected given the low TF in this treatment. However, leaves of plants submitted to 108 μM As exhibited a similar gene expression response to that observed in roots at 27 μM As, suggesting that a failure in the detoxification process through vacuolar compartmentalization also occurred in leaf cells, probably resulting in high free arsenite, which explains the symptoms in the shoot. This is reinforced by the absence of alterations in GSSH-related genes in leaves under the 108 μM As treatment. It is likely that, both in root and shoot cells, the detoxification process would be improved with increased GSSH biosynthesis, since this process (from GSSH reduction to PC production) responds to the presence of As. Indeed, expression of a rice glutathione S-transferase in *Arabidopsis* lead to improved seedling growth in the presence of arsenate (Kumar *et al.* 2013).

Even the lowest As concentration led to severe effects on photosynthesis, due to a reduction in stomatal conductance. At the highest As concentration we detected high c_i/c_a ratio and the stomata almost totally closed, indicating a problem with CO_2 consumption *via* the Calvin cycle, which is also explained by the reduced CA activity upon As treatment. The decreased CA activity may be explained by the reduced Zn content in leaves, since Zn acts as a cofactor for this enzyme and its deficiency is already known to lead to decreased CA activity (Sasaki *et al.* 1998). The "light curve" shows the significant limitation of the photosynthetic reactions in response to increases in irradiance, and this is accompanied by the reduced ETR and F_v/F_m ratio detected in the presence of As. The decrease in g_s promoted a reduction in transpiration under all As treatments (data not shown), and this excludes the possibility that the reductions in relative water content that we detected result from excessive water loss *via* transpiration. Instead, the reduction in RWC may result from decreases in transpiration as a result of stomatal closure, which in turn decreases water uptake by roots (O'Toole and Cruz 1980).

Stomatal closure affected heat energy dissipation through transpiration, as shown by high NPQ and the increased leaf surface temperature at 108 and 216 μM As (Costa *et al.* 2013). Increases in temperature may also result from increases in the activity of alternative mitochondrial electron transport chain components, given that we observed increased expression of alternative oxidases in the shoot. These routes of respiration are often activated under stress (Vishwakarma *et al.* 2015) and we observed symptoms of oxidative stress caused by As.

Exposure of *Cajanus cajan* seedlings to 20 μM arsenate led to an increase in density of root cortex cells as well as a reduction in the percentage of intercellular spaces (Pita-Barbosa *et al.* 2015). In leaves of *A. thaliana* exposed to As, the spongy parenchyma was the most severely affected, which in turn is likely to interfere in respiration and photosynthesis *via* reductions in mesophyll conductance. The reduction observed in leaf tissues/ regions may result from the reduced RWC, given that plant

cell growth requires the efficient absorption of water to produce the pressure potential necessary for cell wall extension (Stoeva *et al.* 2004). Absorption of water by the root depends upon the presence of a water potential gradient between soil and root and upon the accumulation of solutes in the stele, a process likely to be complicated by the effects of arsenate on ATP production. Indeed, we detected a drastic reduction in content of K in the root that may contribute to impaired water absorption.

The leaves of plants exposed to the highest As concentrations exhibited a darker colour than those of control plants due to an increase in anthocyanin content, which increased by 90 and 97 % in young leaves and 212 and 172 % in expanded leaves in treatments with 108 and 216 μM As, respectively. Anthocyanin production in *A. thaliana* plants treated with As is thought to be related to P deficiency (Catarecha *et al.* 2007). Arsenate is transported by phosphate transporters in the root and thus competes with this nutrient (Irtelli and Navari-Izzo 2008), and we also detected decreased content of P in this study, which in turn explains the increased density of root hairs, a typical response to phosphorus starvation (Ma *et al.* 2001). In general, the fully expanded leaves were more affected by As as explained by the higher accumulation of As in older leaves, as previously reported in *Sorghum bicolor* (Shaibur *et al.* 2008), *Camellia sinensis* (Shi *et al.* 2008), and *Pteris vittata* (Cao *et al.* 2004), in which expanded leaves exhibited symptoms of As toxicity when exposed to high content of this element while young leaves remained apparently healthy (Li *et al.* 2006).

A common response to toxic metals/metalloids is the occurrence of oxidative stress (Catarecha *et al.* 2007, Singh *et al.* 2007, Finnegan and Chen 2012, Castrillo *et al.* 2013). The histochemical tests for H_2O_2 and $O_2^{\bullet-}$ detection, together with the increased MDA content and EL are strong evidence of oxidative stress occurring in the treatments with 108 and 216 μM As. It is highly likely that the damage to photosynthesis resulting from thylakoid electron transport failure is the main source of ROS, which resulted in the photoinhibition. Moreover, it is thought that ROS may interact with the PS II reaction centre and cause the cleavage of specific fragments of the D1 protein or inhibit the mechanisms used to repair PS II photodamage (Murata *et al.* 2007). It is worth noting that we did not detect macroscopic symptoms of toxicity at 27 μM As nor alterations in MDA content and EL, suggesting that at this concentration the antioxidative mechanisms were sufficient to remove excess ROS. Previous reports have indicated an important role for antioxidant responses in As tolerance, in which enzymatic antioxidants appear more relevant at low As concentrations while at higher concentrations non-enzymatic antioxidants become more important (Cao *et al.* 2004). Here we observed increases in the content of soluble phenolic compounds, which are known to act as antioxidants (Campos *et al.* 2014).

H_2O_2 represents a potentially dangerous ROS in biological systems, as it may be converted into the hydroxyl radical ($OH^{\bullet-}$) through interaction with transition metals (Fenton 1984). Long distance transport of H_2O_2 occurs *via* the xylem (Slesak *et al.* 2007), what explains its marginal and apical location within leaves. Gene expression analysis showed upregulation of catalases and peroxidases, enzymes involved in the detoxification of H_2O_2, suggesting that this molecule may make the most important contribution to the generation of oxidative stress in plant tissues in response to As (Gunes *et al.* 2009). Since this molecule is not charged, it can move between different compartments crossing membranes through aquaporins (Bienert *et al.* 2006) and ultimately reach the nucleus where it promotes DNA breaks. In this case, the activation of programmed cell death (PCD) may be a protective response, and one that also participates in nutrient recycling. One characteristic of PCD is the activation of the enzyme caspase-3, which occurs towards the beginning of the signalling pathway (Del Pozo and Lam 1998). In animal cells apoptosis can be activated by reductions in rates of glycolysis and ATP synthesis (Plas and Thompson 2002), both of which are typical effects of As exposure (Hughes 2002). However, little is known regarding the participation of caspases in plant cells (where these enzymes are called metacaspases). Here we showed that As exposure promoted partial inhibition of metacaspase-3 activity in roots (though not in leaves), and that this inhibition depends on the As concentration. The presence of cysteine as a principal component of the active site of caspases indicates that substances with great affinity for thiols, such as arsenite, could interact with this amino acid and result in the oxidation and inactivation of the enzyme (Nicholson *et al.* 1995), explaining these results. We suggest that the reduction in metacaspase-3 activity is due to a combination of inhibitory effects generated directly through arsenite and indirectly through the generation of ROS.

The pronounced effects of As on glycine and glycolate in mature leaves suggests that As affected photo-respiration; indeed increased leaf temperature and decreased stomatal conductance are both likely to favour photorespiration. Whilst glycine content was elevated at 216 μM, glycolate content was only increased at 108 μM As possibly due to the partial recovery of g_s and elevated c_i/c_a at 216 μM As. These effects may also result from inhibition of glycine decarboxylase (GDC) by arsenite (Finnegan and Chen 2012) as we observed much larger increases in content of glycine than in serine with increasing As. Similar effects on glycine content was previously reported for mutants of *A. thaliana* that are unable to produce the lipoamide dehydrogenase 2 enzyme component of GDC (Chen *et al.* 2014).

Arsenite can also inhibit other enzymes containing dihydrolipoamide groups including 2-oxoglutarate dehydrogenase, pyruvate dehydrogenase, and branched-chain α-keto acid dehydrogenase, and these enzymes may also be targets of ROS (Finnegan and Chen 2012, Chen

et al. 2014). Increased content of 2-oxoglutarate together with decreased content of succinate and fumarate may well reflect the inhibitory effect of As on 2-oxoglutarate dehydrogenase (Chen *et al.* 2014) whilst an increase in malate and decrease in citrate may result from the inhibition of the mitochondrial pyruvate dehydrogenase complex. Arsenic is also known to inhibit activity of branched-chain α-keto acid dehydrogenase, explaining the accumulation of valine in both young and mature leaves and in roots, and the accumulation of leucine in roots; senescence caused by As may result in the release of amino acids that cannot then be degraded *via* their normal route (Araujo *et al.* 2010).

The presence of As also appears to produce certain effects that mirror those encountered under drought stress, including stomatal closure and decrease in RWC. The significant accumulation of proline and raffinose may therefore reflect osmotic adjustment and proline is also known to act in the removal of ROS (Rejeb *et al.* 2014). Increases in γ-aminobutyric acid, lysine, glycine, and alanine were also observed previously in *A. thaliana* roots treated with arsenate (Chen *et al.* 2014), though in this case, proline and β-alanine only increase in mutant plants defective in lipoamide synthesis which exhibit increased sensitivity to As suggesting that pyruvate metabolism is a target of As. In roots we also found increased content of threonate in the presence of As; threonate is a product of ascorbate breakdown and its presence, together with increased ascorbate peroxidase, reinforces the importance of antioxidant defences in the presence of As.

Finally, different defence mechanisms may be activated under different experimental conditions (Zabludowska *et al.* 2009) and studies using hydroponics with *A. thaliana* are scarce. The high sensitivity observed in the treatments with 108 and 216 μM As is related to the use of hydroponic system, where As in the medium is entirely bioavailable for absorption by the root system. This type of culture system has the advantage of allowing the study of the effects of As without the interference of any other toxic elements or additional factors that promotes toxicity or avoids As availability. Our study shows that As had a range of effects on gas exchange, metabolism, gene expression, and antioxidant defence in *Arabidopsis thaliana*. Crucially, toxicity was dependent on translocation from root to shoot, since plants with the same As content in roots, but different translocation factors showed different tolerance. Our results also indicate that As may act both directly on a number of physiological processes and also indirectly *via* induction of ROS production, reinforcing the importance of antioxidant defences in tolerance to As.

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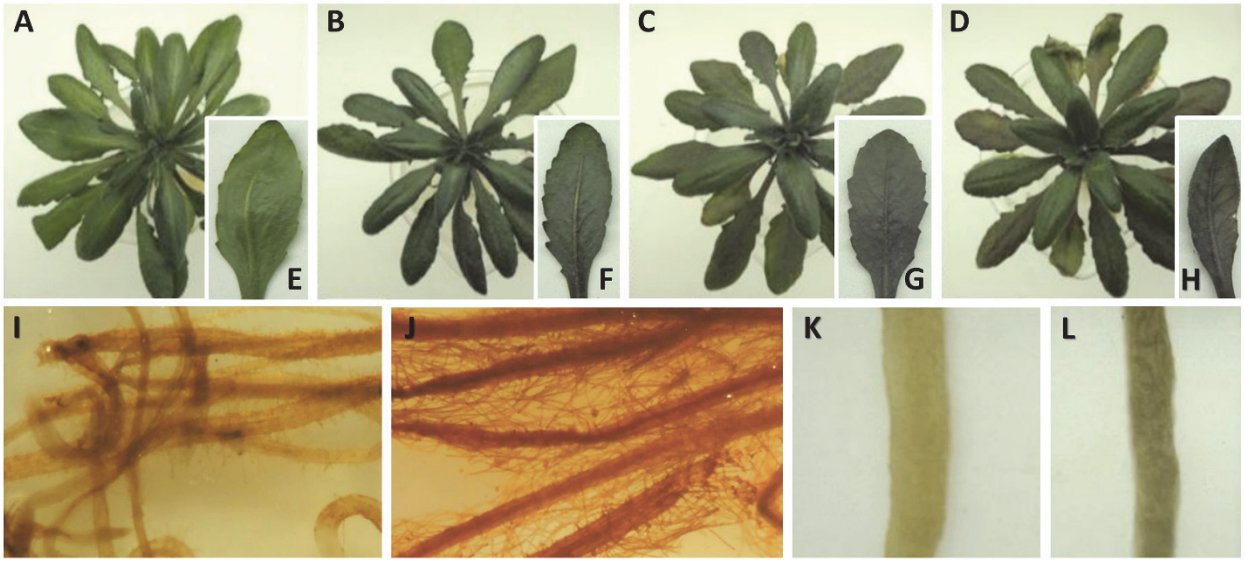

Fig. 1 Suppl. Arsenic toxicity symptoms of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0 (*A*, *E*, *I*, *K*), 27 (*B*, *F*), 108 (*C*, *G*, *J*) and 216 (*D*, *H*, *L*) µM arsenic in the form of sodium arsenate for five days. Leaves showing a purple colour detectable mainly on the abaxial side (*E* - *H*) and roots showing increased hair density (*J*), brownish colouring and gelatinous appearance (*L*).

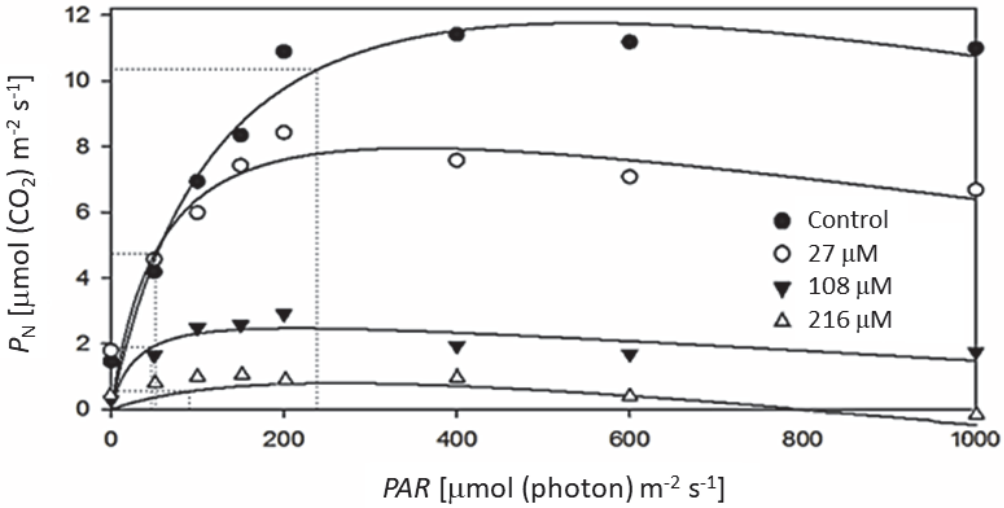

Fig. 2 Suppl. Photosynthetic light response curve and light saturation point (dashed lines, 80 % of the maximum P_N value) of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0, 27, 108, and 216 µM arsenic for five days.

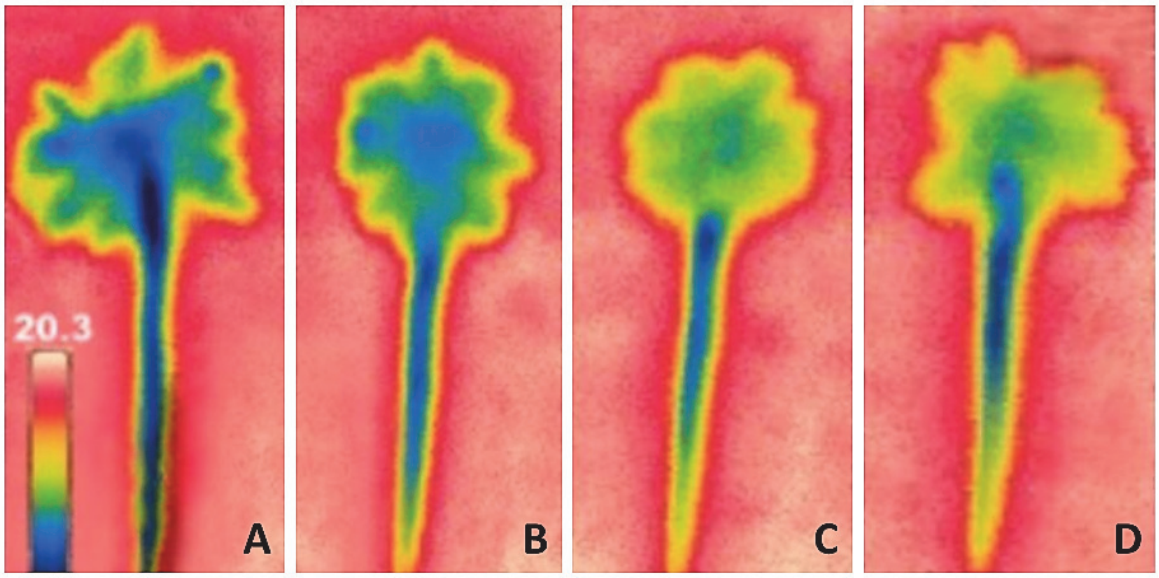

Fig. 3 Suppl. Images of *Arabidopsis thaliana* plants cultivated in nutrient solution with 0 (*A*), 27 (*B*), 108 (*C*), and 216 μM (*D*) arsenic obtained using an infra-red photographic camera.

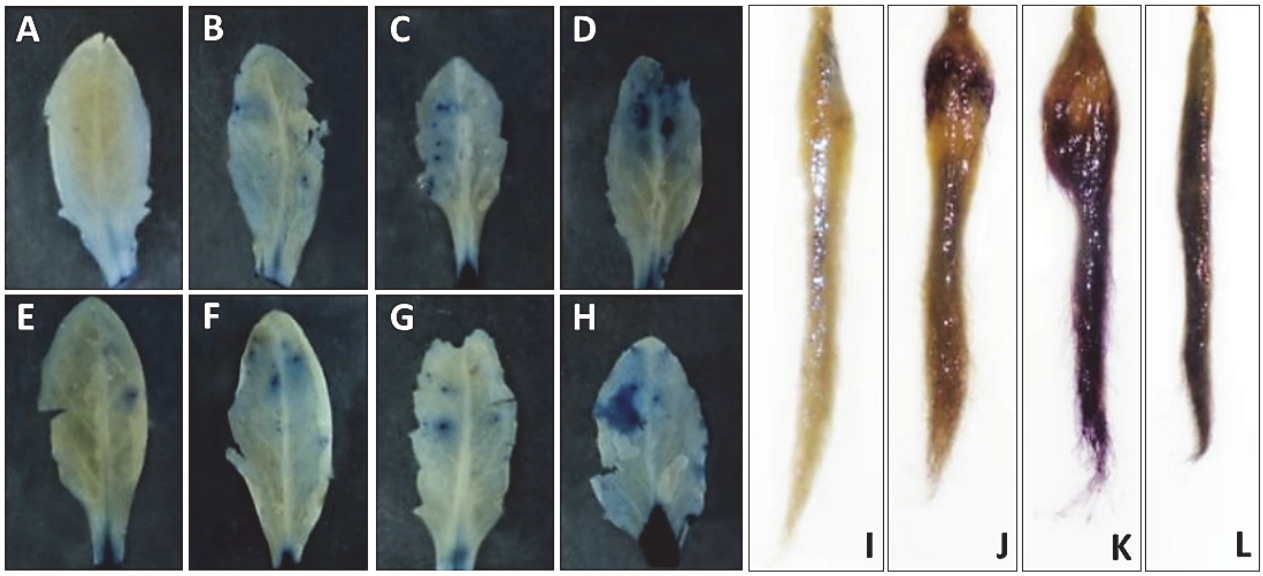

Fig. 4 Suppl. Superoxide radical detection using the NBT reagent in leaves (*A* - *H*) and roots (*I* - *L*) of *Arabidopsis thaliana* plants cultivated in nutrient solution containing 0 (*A*, *E*, *I*), 27 (*B*, *F*, *J*), 108 (*C*, *G*, *K*), and 216 (*D*, *H*, *L*) μM arsenic for five days.

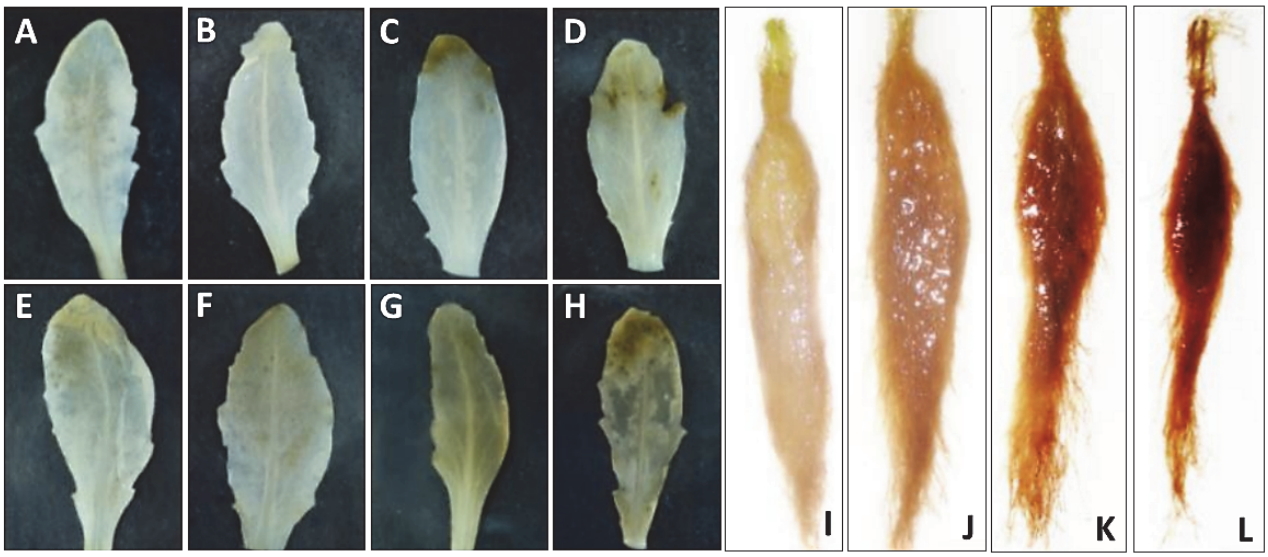

Fig. 5 Suppl. Hydrogen peroxide detection using the DAB reagent in leaves (*A* - *H*) and roots (*I* - *L*) of *Arabidopsis thaliana* plants cultivated in nutrient solution containing 0 (*A*, *E*, *I*), 27 (*B*, *F*, *J*), 108 (*C*, *G*, *K*) and 216 (*D*, *H*, *L*) μM arsenic for five days.

Table 1 Suppl. Primer pairs used for quantitative gene expression analysis. Accession numbers are based on the National Center for Biotechnology Information (NCBI); F and R indicate forward and reverse primers.

Gene	Accession	Primer pairs (5′ → 3′)
ACT2 (actin 2)	NM_112764.3	F-CTTGCACCAAGCAGCATGAA R-CCGATCCAGACACTGTACTTCCTT
AOX1A (alternative oxidase 1A)	NM_113135.3	F-CTGGACCACGTTTGTTC R-ACACCCCAATAGCTCG
AOX1D (alternative oxidase 1D)	NM_102968.2	F-TACCGCACTCTTCGAC R-GGCTGGTTATTCCCACT
APX1 (L-ascorbate peroxidase 1)	NM_001123772.1	F-TGCCACAAGGATAGGTCTGG R-CCTTCCTTCTCTCCGCTCAA
APX2 (L-ascorbate peroxidase 2)	NM_111798.3	F-TTGCTGTTGAGATCACTGGAGGA R-TGAGGCAGACGACCTTCAGG
CAD1 (glutathione γ-glutamylcysteinyltransferase 1)	NM_123774.3	F-TGGTGTTGAATGCTCTTTCTATCG R-GGTTCGCAGCAATCCAACAT
CAT1 (catalase 1)	NM_101914.3	F-AAGTGCTTCATCGGGAAGGA R-CTTCAACAAAACGCTTCACGA
CAT2 (catalase 2)	NM_001036714.5	F-AACTCCTCCATGACCGTTGGA R-TCCGTTCCCTGTCGAAATTG
CSD2 (copper/zinc superoxide dismutase 2)	NM_128379.3	F-GAGCCTTTGTGGTTCACGAG R-CACACCACATGCCAATCTCC
EF1A (elongation factor 1-alpha 4)	NM_001125992.1	F-TGAGCACGCTCTTCTTGCTTTCA R-GTGGTGGCATCCATCTTGTTACA
GR (glutathione reductase, chloroplastidic)	NM_115323.3	F-GCCCAGATGGATGGAACAGAT R-TAGGGTTGGAGAATGTTGGCG
GR1 (glutathione-disulfide reductase, cytosolic)	NM_001125216.1	F-CTCAAGTGTGGAGCAACCAAAG R-ATGCGTCTGGTCACACTGC
GSH1 (glutamate-cysteine ligase)	NM_001203879.1	F-CCCTGGTGAACTGCCTTCA R-CATCAGCACCTCTCATCTCCA
GSH2 (glutathione synthetase 2)	NM_122620.3	F-GGACTCGTCGTTGGTGACAA R-TCTGGGAATGCAGTTGGTAGC
GSTF8 (glutathione S-transferase phi 8)	NM_180148.4	F-GCGAGAGTCAAAGAGCACCT R-TCTTTAAAGGCATGTTCGGC

Table 2 Suppl. Young and fully expanded leaf anatomy of *Arabidopsis thaliana* plants cultivated in nutrient solution containing 0 and 108 µM arsenic for five days. Means ± SE, $n = 5$,. * and ** indicate significant differences at $P < 0.05$ and 0.01, respectively.

Leaf stage	Arsenic [µM]	Thickness [µm]					Number of palisade parenchyma cells
		epidermis		parenchyma		total	
		adaxial	abaxial	palisade	spongy		
Young	0	35.14	27.30	190.02	203.06	455.52	42
	108	34.38	27.61	194.95	161.15**	418.10**	44
Fully expanded	0	38.18	29.02	218.40	217.45	503.05	41
	108	33.41**	22.73*	194.90**	174.14**	425.18**	38

Table 3 Suppl. Effects of arsenic on the metabolic profile of roots, fully expanded leaves, and young leaves of *Arabidopsis thaliana* plants cultivated in nutrient solution containing 0, 27, 108, and 216 µM arsenic for five days. Different letters indicate significant differences ($n = 4$ or 5; $P < 0.05$, Tukey's test). Values are given relative to those in the control (0 µM arsenic) for each organ, except for fructose, glucose, sucrose and starch which are given in [mmol kg^{-1}(d.m.)].

As	Roots				Fully expanded leaves				Young leaves			
[µM]	0	27	108	216	0	27	108	216	0	27	108	216
Saccharides												
Fructose	15.1 ab	20.2 a	11.3 b	3.4 ab	16.7	12.0	18.9	13.8	13.6	20.6	13.8	14.1
Glucose	63.5 ab	75.0 a	61.1 ab	18.7 b	56.7 a	48.2 a	195.4 b	126.2 ab	47.8 a	171.2 b	203.0 b	220.6 b
Glucose-6-P	1.0 ab	2.6 a	2.6 ab	0.7 b	1.0	0.4	0.6	0.8				
Maltose	1.0 a	3.1 ab	12.8 b	39.1 c	1.0 a	0.3 b	0.6 ab	0.3 ab	1.0	0.8	0.5	0.6
Myo-inositol	1.0	1.5	2.0	2.0	1.0 a	0.7 b	0.6 b	0.8 ab	1.0	1.2	0.8	1.0
Ononitol/pinitol	1.0 a	1.6 a	5.7 b	6.2 b	1.0	0.7	0.5	0.5	1.0 a	0.8 a	0.4 b	0.4 b
Raffinose	1.0	1.4	38.5	22.9	1.0	1.5	2.3	0.6	1.0 a	4.6 ab	6.2 b	3.9 ab
Starch	38.8	43.9	42.6	56.5	116.0 a	92.5 a	106.3 a	54.3 b	115.2 a	111.9 a	102.0 a	74.1 b
Sucrose	67.3	64.0	118.5	84.0	52.8	48.7	68.2	70.2	49.6 ab	54.0 a	56.0 a	24.5 b
Organic acids												
2-Oxoglutarate					1.0 a	4.1 ab	11.5 b	8.9 ab				
Citrate	1.0 a	1.1 a	3.3 b	1.2 a	1.0	0.9	0.8	0.4	1.0 a	0.9 ab	0.6 ab	0.4 b
Erythreonic acid	1.0 a	6.4 bc	8.3 c	2.9 ab	1.0	1.3	2.6	2.1	1.0 a	1.5 a	2.6 b	2.2 ab
Fumarate	1.0	1.8	1.7	4.9	1.0 a	1.0 a	0.4 b	0.4 b	1.0 a	1.3 b	0.9 a	0.5 c
Glycerate	1.0 a	73.7 b	25.2 ab	5.0 ab	1.0	0.8	1.2	0.8	1.0	0.7	0.9	0.9
Glycolate	1.0 a	1.3 a	2.1 ab	4.0 b	1.0 a	4.1 ab	7.9 b	1.9 ab	1.0	0.6	1.3	1.5
Malate	1.0	1.6	2.1	1.1	1.0 a	2.4 a	5.9 b	5.1 b	1.0 a	1.4 a	2.4 b	2.7 b
Pyruvate	1.0 a	3.3 ab	3.9 b	2.7 ab	1.0	1.0	0.9	0.8				
Succinate	1.0 a	1.4 ab	2.6 b	1.2 ab	1.0 ab	1.5 a	0.9 ab	0.3 b	1.0 ab	1.3 a	0.7 ab	0.5 b
Threonic acid	1.0 a	4.9 ab	8.7 b	6.6 b	1.0	0.7	1.1	0.7	1.0	0.9	1.0	0.8
N compounds												
Alanine	1.0	1.3	2.1	0.8	1.0 a	0.7 a	14.3 a	130.5 b	1.0	0.0	4.8	4.7
Allantoin	1.0 a	1.0 a	0.9 ab	0.3 b								
β-alanine	1.0 a	2.5 ab	4.3 b	2.9 ab	1.0 a	1.7 ab	2.0 ab	5.0 b	1.0 a	0.9 a	1.2 a	2.7 b
Ethanolamine					1.0 a	0.5 a	5.2 b	4.5 b	1.0	0.3	1.6	2.5
GABA	1.0	1.2	2.1	1.0	1.0	0.7	0.9	0.9	1.0 a	1.2 ab	1.5 bc	1.7 c
Glycine	1.0	1.2	1.3	0.8	1.0 a	3.3 a	8.3 a	21.0 b	1.0 a	0.4 a	4.4 a	10.2 b
Leucine	1.0 a	2.5 ab	4.5 b	2.8 ab								
Lysine	1.0 a	1.8 ab	2.8 b	2.3 ab								
Phenylalanine	1.0 a	3.5 b	2.0 ab	2.4 ab	1.0	1.0	1.2	3.1	1.0 a	0.5 ab	0.3 b	0.8 ab
Proline	1.0	0.7	28.3	113.8	1.0 a	18.8 a	574.8 a	2303.8 b	1.0 a	0.8 a	1300.6 ab	2611.9 b
Pyroglutamate	1.0	1.1	1.1	0.6	1.0	0.7	1.0	0.8	1.0 a	0.6 ab	0.6 b	0.5 b
Valine	1.0 a	3.0 b	3.2 b	3.7 b	1.0 a	41.7 ab	81.8 ab	179.7 b	1.0 a	1.0 a	33.4 a	98.2 b

Table 4 Suppl. Macro and micronutrient content in shoot and root tissues of *Arabidopsis thaliana* plants cultivated in nutrient solution containing 0, 27, 108, and 216 μM arsenic for five days. Different letters indicate significant differences ($n = 5$; $P < 0.05$, Tukey's test).

	As	Macronutrients [(g kg^{-1} (d.m.)]					Micronutrients[(mg kg^{-1} (d.m.)]				
	[μM]	Ca	K	Mg	P	S	Cu	Fe	Mn	Mo	Zn
Shoots	0	29.4 a	16.4 a	7.3 a	3.37 a	6.8 a	2.35 c	122.3 b	91.1 b	3.6 a	19.1 a
	27	30.4 a	14.8 a	7.5 a	2.84 ab	6.8 a	2.16 c	122.0 b	96.4 ab	3.2 ab	12.8 b
	108	31.0 a	15.5 a	7.6 a	2.26 b	6.8 a	2.47 ab	139.5 ab	104.1 a	3.2 bc	11.3 b
	216	30.6 a	16.6 a	7.7 a	2.17 b	7.3 a	2.58 a	160.9 a	98.6 ab	2.7 c	14.1 b
Roots	0	9.7 c	25.4 a	5.7 a	4.32 a	8.1 ab	22.57 a	2283 bc	97.1 b	15.7 a	60.5 a
	27	11.3 bc	16.2 b	5.5 a	4.08 a	9.4 a	16.62 b	1859 c	113.7 b	5.0 b	21.1 b
	108	13.4 b	10.5 bc	4.3 b	3.32 b	6.1 ab	19.01 ab	2498 b	324.2 a	6.1 b	16.5 b
	216	14.5 a	5.0 c	4.0 b	3.20 b	5.7 b	23.27 a	3062 a	87.6 b	5.4 b	25.0 b