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Accumulation, speciation, and coordination of arsenic in an inbred line and a wild type cultivar of the desert plant species *Chilopsis linearis* (Desert willow)

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ABSTRACT

This study investigated the absorption of arsenic (As), sulfur (S), and phosphorus (P) in the desert plant *Chilopsis linearis* (Desert willow). A comparison between an inbred line (red flowered) and wild type (white flowered) plants was performed to look for differential responses to As treatment. One month old seedlings were treated for 7 days with arsenate (As_2O_5, As^V) at 0, 20, and 40 mg As^V L^{-1}. Results from the ICP-OES analysis showed that at 20 mg As^V L^{-1}, red flowered plants had 280 ± 11 and 98 ± 7 mg As kg^{-1} dry wt in roots and stems, respectively, while white flowered plants had 196 ± 30 and 103 ± 13 mg As kg^{-1} dry wt for roots and stems. At this treatment level, the concentration of As in leaves was below detection limits for both plants. In red flowered plants treated with 40 mg As^V L^{-1}, As was at 290 ± 77 and 151 ± 60 mg As kg^{-1} in roots and stems, respectively, and not detected in leaves, whereas white flowered plants had 406 ± 36, 213 ± 12, and 177 ± 40 mg As kg^{-1} in roots, stems, and leaves. The concentration of S increased in all As treated plants, while the concentration of P decreased in roots and stems of both types of plants and in leaves of red flowered plants. X-ray absorption spectroscopy analyses demonstrated partial reduction of arsenate to arsenite in the form of As-$(SX)_3$ species in both types of plants.

1. Introduction

Arsenic (As) is a metalloid element found in the environment as organic and inorganic complexes (Smedley and Kinniburgh, 2002). Inorganic As sources are geological and anthropogenic (Welch et al., 2000; Doušová et al., 2006). The two most common forms of inorganic As are arsenite (As_2O_3) and arsenate (As_2O_5) (Oremland and Stolz, 2003). Arsenic occurs naturally in minerals as arsenopyrite (FeAsS) (Ganne et al., 2006) and in sediments from volcanic activity (Welch et al., 2000). This metalloid was commonly found in older pesticides, herbicides, and insecticides utilized in agriculture. Arsenic is also introduced into the environment through industrial activities, as a wood preservation constituent, a byproduct of mining and smelting processes, coal combustion, and hide tanning waste, among others (Doušová et al., 2006). These anthropogenic sources of As increase the inorganic forms present in the environment (Wang and Mulligan, 2005). According to Dudka and Miller (1999), As concentrations in soil up to 40 mg kg^{-1} are not considered a health threat. In the US, the limit for As in residential soil established by the EPA is 20 mg kg^{-1} (EPA, 2006). However, there are sites with higher As concentrations which raises public concern due to environmental and health risks (Welch et al., 2000; Polizzotto et al., 2006); As is known to be a human carcinogen (Abernathy et al., 1999; ATSDR, 2005). Some sites in El Paso, TX have reported concentrations of As in soil in a range between 24 and 186 mg kg^{-1} (EPA, 2003).

One way to reduce the risk of As contamination is by removing it from soil. Phytoremediation is an *in situ* remediation method to remove pollutants from the environment by the use of plants and is relatively cheaper than other remediation techniques (Barcelo and Poschenrieder, 2003; Gardea-Torresdey, 2003). In order to make phytoremediation a more suitable technology, researchers have investigated the As absorption, metabolism, and toxicity in many plant species (Meharg and Hartley-Whitaker, 2002). However, the Chinese brake fern *Pteris vittata* L., and some relative species are the only identified As hyperaccumulators (>1000 mg As kg^{-1} in the above ground biomass (Ma et al., 2001; Srivastava et al., 2006)). Interestingly, not all species within the *Pteris* genus are able to hyperaccumulate As (Caille et al., 2005). Moreover,

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the As uptake capacity and tolerance may also vary among members of the same species, as reported in two cultivars of winter wheat *Triticum aestivum* L. (Geng et al., 2006).

Desert willow (*Chilopsis linearis* (Cav.) sweet) is a deciduous desert tree that grows in alkaline soils with a pH range of 6–9 and tolerates full sun exposure and drought conditions. This species is native to southwestern US and northern Mexico. This plant was previously shown to be suitable for gold phytomining (Gardea-Torresdey et al., 2005a). Additionally, *C. linearis* plants have been observed to readily grow in copper mine tailings at Globe, Arizona, indicating some adaptive capabilities to stressful environments (arsenic contamination) (Haque et al., 2008). However, As accumulation has not been studied in *C. linearis*.

The objectives of this study were to determine the As accumulation and its effect on the absorption of some essential nutrients by plants of the inbred line "Louis Hamilton" (red flowered plant) and wild type plants of *C. linearis* (white flowered). Previous studies showed a differential response of the inbred line when exposed to heavy metals (data not shown). Thus, it would be possible that similar results could be observed under arsenic exposure. The null hypothesis was that no differences would be found between the two types of plants. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to determine the As and nutrient accumulation, and X-ray absorption spectroscopy (XAS) to study the speciation and local coordination environment of As inside the plant. XAS techniques requires minimal sample preparation which ensures little to no change of the structural environment of elements within the tissues (Gardea-Torresdey et al., 2005b).

2. Results

2.1. Arsenic accumulation

All plants survived exposure to As^V for 7 days and did not present signs of either chlorosis or necrosis in leaves. However, some of the plants treated with 40 mg As^V L^{-1} showed signs of necrosis in roots. The accumulation of As demonstrated a similar trend for both types of plants, with the highest concentration in roots followed by stems and leaves. At 20 mg As^V L^{-1}, roots of the red flowered plants concentrated more As (280.3 ± 11.5 mg As kg^{-1} dry wt) than the roots of the white flowered plants (196 ± 30 mg As kg^{-1} dry wt) (Fig. 1(I)). Likewise, stems of the red flowered and white flowered plants concentrated 98.3 ± 7.0 and 103.2 ± 13.7 mg As kg^{-1} dry wt, respectively. However, none of the plants showed As in leaves (As detection limit 0.014 mg L^{-1}) (Fig. 1(I)).

Arsenic accumulation increased significantly only in the white flowered plants when the concentration of As in media increased. In contrast with the 20 mg As^V L^{-1} treatment, at 40 mg As^V L^{-1} white flowered plants accumulated more As in stems and roots than the red flowered plants. Additionally, the translocation of As to the leaves was observed only in the white flowered plants (177 ± 40.6 mg As kg^{-1} dry wt) (Fig. 1, II).

2.2. Effect of As on sulfur and phosphorus absorption

The concentration of S showed a consistent increasing trend in roots, stems, and leaves in both types of plants treated with As compared to control plants (Table 1). However, the increase was not significant between plants treated with 20 mg As^V L^{-1} and the 40 mg As^V L^{-1}. Conversely, the exposure to As significantly reduced the concentration of P in roots and stems of the two phenotypes of *C. linearis*. The decrease was not significant between As treatments. However, none of the treatments reduced the concentration of P in leaves of the white flowered plants.

2.3. Effect of As on the production of low molecular weight thiols (LMWT)

The concentration of LMWT was determined to obtain an insight about their participation in the detoxification of As in *C. linearis*. Results from the red flowered phenotype showed concentrations of LMWT in roots, stems, and leaves below detection limits (0.107 units of absorption or 0.457 mmol kg^{-1} dry tissue). From the XAS (Table 2) and ICP-OES (Fig. 1, II) results, the amount of As bound to sulfur in roots of red flowered plants at the 40 mg As^V L^{-1} treatment was 1.60 mmol kg^{-1} dry tissue. This amount of As was coordinated in the form of an As-$(SX)_3$ type compound (Table 2). This indicates that an As–SH ratio of 1–3 (4.8 mmol SH kg^{-1}) is required to fulfill the molar ratio. However, the concentration of LMWT in this treatment was below the detection limit (0.457 mmol kg^{-1}; <10%). In roots of the white flowered plants, a concentration of 1.83 ± 0.27 mmol SH kg^{-1} dry tissue was found in the 40 mg As^V L^{-1} treatment. The amount of As present as As-(SX_3) complex in this treatment was 3.22 mmol As kg^{-1}dry tissue. This is only 19% of the total thiol pool required to fulfill the As–SH ratio.

2.4. X-ray absorption spectroscopy analysis

Fig. 2a shows the X-ray absorption near the edge structure (XANES) spectra of As in the white flowered plant roots, stems, and leaves. As can be observed in this figure, there are two species of As in the samples. One of the species has a whiteline feature at ~11.87 keV, which is indicative of an As-$(SX)_3$ complex, comparable to the As_2S_3 model compound as shown in Fig. 2c. The second feature in the XANES spectra has a whiteline at ~11.88 keV which is comparable to the Na_2HAsO_4 model compound (or a hydrate arsenate molecule). The As^{III} and As^V model compound contributions are shown in Fig. 2c to better indicate the position of the edge features. The XANES results indicate that there was only a partial reduction of As^V (the treatment) in the white flowered plants. These results were confirmed by the LC-XANES (linear combinations-XANES) fittings shown in Table 2. Fig. 2c shows an example of the LC-XANES fitting results obtained in this study indicating the features in the spectra from both species and how well the fittings match to the experimental data. The relative ratios of the As-$(SX)_3$ and the AsO_4^{3-} complexes found within the different portions of the plants are shown in Table 2. In roots of white flowered plants there was 59.5% of As-$(SX)_3$ and 40.5% of AsO_4^{3-}. However, in stems of these plants the result was 42.5% of As-$(SX)_3$ and 57.5% of AsO_4^{3-}. While in the leaves there was 66.0% of As-$(SX)_3$ and 34.0% of AsO_4^{3-}.

Fig. 2b shows the XANES spectra of As in the red flowered plant roots. Similar to the results observed for the white plants, in the red flowered plants As exists as As-$(SX)_3$ and AsO_4^{3-} type species. This is indicated by the whiteline features in the XANES at 11.87 keV (As-$(SX)_3$) and 11.88 keV (AsO_4^{3-}). The results of the LC-XANES fittings are shown in Table 2. In roots of red flowered plants, 41.8% of the As was as As-$(SX)_3$ and 58.2% as AsO_4^{3-}. However, in the stems of these plants, only 18.6% of the As was as As-$(SX)_3$ but 81.4% as AsO_4^{3-}.

3. Discussion

In this investigation the absorption of As and some nutrients in plants of an inbred line and a wild type of the desert species *C. linearis* was examined. Plants exposed to the 20 mg As^V L^{-1} treatment accumulated more As in roots with non-detectable levels in leaves (Fig. 1, I). Arsenic accumulation significantly increased only in the white flowered plants when the concentration of As in the media increased to 40 mg As^V L^{-1} (Fig. 1, II). Red flowered plants, though

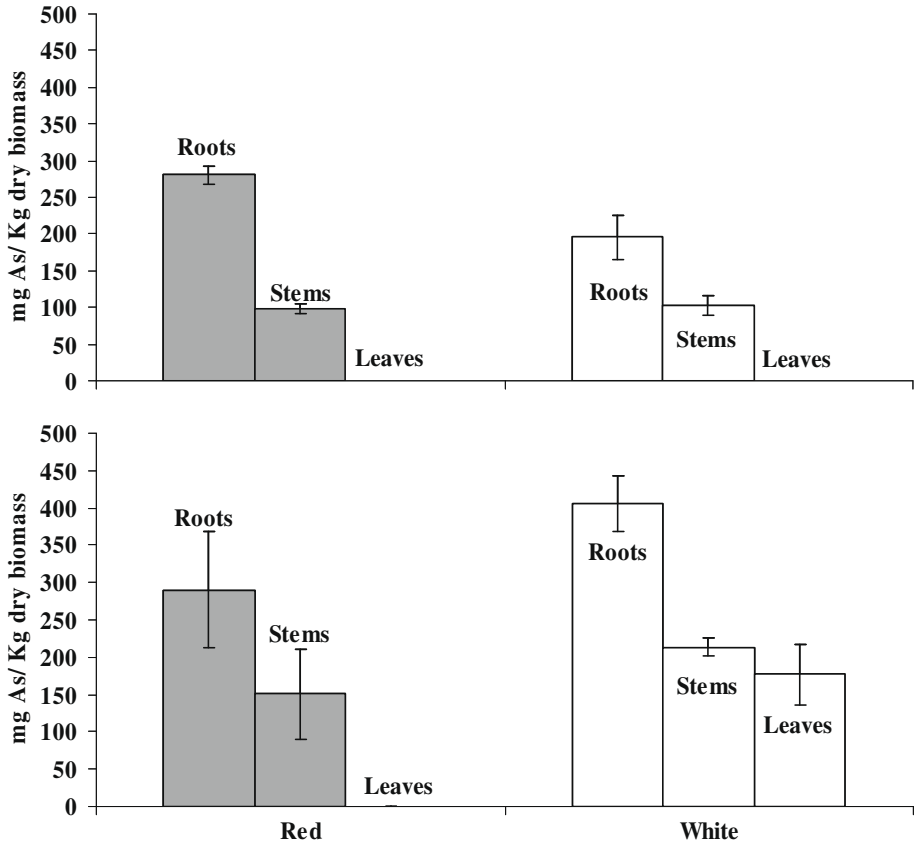

Fig. 1. Arsenic accumulation in *C. linearis* plants after 7 days of exposure in hydroponics to (I) 20 mg L^{-1} and (II) 40 mg L^{-1} of arsenic supplied as arsenate. Bars represent ± standard error ($n = 3$). Values with the same letter in the same tissue are not significantly different (Tukey $\alpha = 0.05$).

Table 1
Concentrations of sulfur and phosphorus in roots, stems and leaves of red and white flowered *C. linearis* plants after 7 days of exposure to arsenate in hydroponics. Values are means of three replicates ± Std. Error (mg kg^{-1} dry wt). Values with the same lower case letter in the same tissue are not significantly different (Tukey $\alpha = 0.05$). Upper case letters stand for significant differences between phenotypes within tissues (Tukey $\alpha = 0.05$).

As treatment (mg L^{-1})	Sulfur			Phosphorus		
	Roots	Stems	Leaves	Roots	Stems	Leaves
White						
Control	1160 ± 32 a,A	851 ± 26 a,AB	903 ± 45 a,A	16289 ± 1136 a,C	10360 ± 286 a,B	5652 ± 284 a,A
20	1472 ± 44 b,CD	955 ± 27 a,b,B	1187 ± 12 a,b,C	12537 ± 63 b,A	8179 ± 355 b,A	3665 ± 1526 a,A
40	1404 ± 43 b,BC	969 ± 4 b,B	1294 ± 4 b,C	13420 ± 1163 b,AB	7706 ± 37 b,A	5826 ± 229 a,A
Red						
Control	1261 ± 25 a,AB	781 ± 16 a,A	846 ± 24 a,A	14767 ± 521 a,AB	10745 ± 249 a,B	6556 ± 43 a,A
20	1554 ± 37 b,CD	860 ± 25 b,AB	984 ± 14 b,AB	12181 ± 493 b,B	8787 ± 298 b,A	669 ± 114 b,A
40	1603 ± 20 b,D	940 ± 41 b,B	1129 ± 76 b,BC	12002 ± 97 b,B	8668 ± 486 b,A	5774 ± 281 b,A

Table 2
LC-XANES fitting results of *C. linearis* white and red flowered plants treated with 40 mg L^{-1} of arsenic supplied as arsenate.

Sample	$\%As_2S_3$	$\%Na_2HAsO_4$
C. linearis white roots As^V	59.5	40.5
C. linearis white stems As^V	42.5	57.5
C. linearis white leaves As^V	66.0	34.0
C. linearis red roots As^V	41.8	58.2
C. linearis red stems As^V	18.6	81.4

As accumulation was higher in the 20 mg kg^{-1} treatment compared to the white flowered, did not significantly increased arsenic accumulation as the concentration in media increased. Similar results have been reported in two winter wheat cultivars where As was accumulated mainly in root tissues of both cultivars, but the Lovrin 10 cultivar accumulated more arsenic than the Jing 411 cultivar (Geng et al., 2006). In addition to this, only the white flowered plants translocated detectable concentrations of As to leaves. Differences in the As accumulation and tolerance between members of the same species have been observed also in the *Pteris* and *Triticum* genus (Caille et al., 2005; Geng et al., 2006).

Sulfur is a very important nutrient involved in As detoxification and tolerance. Reduced S in the form of thiol groups (–SH) is present in cysteine residues of the important peptide glutathione (GSH) and the metal-induced binding peptides phytochelatins (PCS) and metallothioneins. All of these are used by plants to bio-inactivate heavy metals and As (Cai et al., 2004; Zhang et al., 2004; de la Rosa et al. 2005). The reduction of arsenate to arsenite and further coordination to S containing groups, specially LMWT (PCS and GSH), has been reported as a successful strategy for tolerance and hyperaccumulation of As in *P. vittata* (Pickering et al., 2006). The reduction of As^V also can account for the toxicity of As inside plant tissue, since

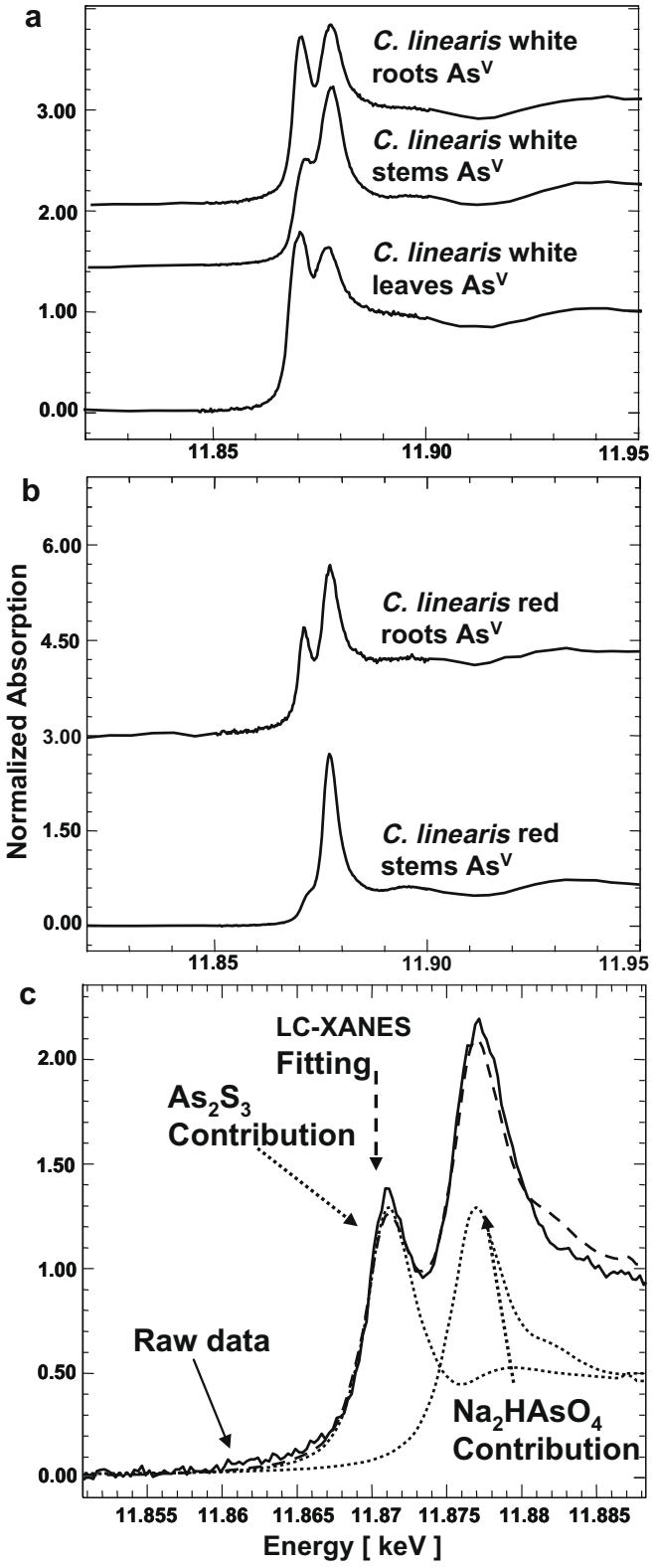

Fig. 2. XANES spectra from 11.8 to 11.95 keV of *C. linearis* plants treated with 40 mg L^{-1} arsenate (a) white flowered roots, stems, and leaves, (b) red flowered roots, and stems, and (c) LC-XANES fitting of As_2S_3 and $NaHAsO_4$ from 11.85 to 11.90 keV in the white flowered stems.

As in the 3^+ oxidation state may have inhibitory effects on the activity of cysteine containing enzymes and severely impair normal cellular function (Hughes, 2002). Results from the two *C. linearis* phenotypes exposed to arsenate showed increased accumulation of S compared to the control plants (Table 1). The analysis of LMWT content showed a concentration below 0.457 mmol kg^{-1} dry tissue in roots, stems, and leaves of the red flowered plants. Concentrations of 0.041 ± 0.007 mmol kg^{-1} have been reported in the hyperaccumulator *P. vitatta* control plant leaflets (Cai et al., 2004). As can be observed, in *P. vittata* the concentration of LMWT in control plants is also below the detection limit. In this study, a detectable concentration of LMWT was only found in roots of the white flowered plants at the 40 mg As^V L^{-1} treatment. The percentage of SH found as LMWT was only 19% of the total thiol pool required to fulfill the 1–3 As–S molar ratio found from the XAS data analysis. A similar result was found by Cai et al. (2004) when studying the role of LMWT in the detoxification of As in *P. vittata*. Exposure to As induced production of LMWT in *P. vittata* but the concentrations found were not sufficient to complex all of the As present in the tissues. These results prove that not all the As is binding to LMWT and that there is another source of SH groups in *C. linearis* red flowered plants that is complexing the As in a similar fashion as LMWT. The fraction of As complexed by LMWT could be only the amount of As that is stored in the vacuoles where this complexes are more stable due to acidic pH (Meharg and Hartley-Whitaker, 2002).

The nutritional importance of P in plants relies in the essential role that phosphate functional groups play in highly relevant cellular processes such as signal transduction, energy supply, and synthesis of DNA and RNA (Mengel and Kirkby, 2001). It has been reported that arsenate is incorporated inside the cellular cytoplasm through phosphate transport channels. However, the affinity of the arsenate/phosphate plasma membrane carriers is much higher for phosphate than arsenate; therefore, phosphate is reported as a very efficient competitive inhibitor of arsenate accumulation (Meharg and McNair, 1992). In this study, the results for P accumulation showed a general decreasing trend (Table 1), suggesting that arsenate is competing with phosphate for the carriers in the plasma membrane thus decreasing P accumulation. A similar result was observed in roots of the marsh grass *Spartina alterniflora* Loisel exposed to arsenate under hydroponic conditions (Carbonell et al., 1998). In this study, leaves of the white flowered plants exposed to As were able to maintain normal levels of P compared to control leaves. Accumulation of As was found also only in leaves of the white flowered plants. This behavior was discussed previously by Wang et al. (2002) in *P. vittata* treated with different concentrations of As and P. These researchers investigated the relationship between phosphate and arsenate. They found that the effect of arsenate on P concentration in fronds of this plant was relatively negligible because arsenite is the primary oxidized form of arsenate, which is loaded and transported through the xylem. At this point, phosphate does not compete with As in the long-distance transport from roots to the shoots. This was observed in Table 2 which shows no differences between the two of *C. linearis* phenotypes for the P concentrations in leaves.

Results from the XAS analysis indicate that the *C. linearis* white flowered plants move As throughout the plant as indicated by the presence of the AsO_4^{3-} species in roots, stems, and leaves. These results also suggest that the As-$(SX)_3$ is the storage molecule for As within the *C. linearis* white flowered plants. Similar results have been observed for peas, tumbleweed, and Indian mustard (Pickering et al., 2000; de la Rosa et al., 2006; Castillo-Michel et al., 2007). In Mesquite, another desert plant species, the speciation of As has shown a total reduction or arsenate to As^{III}–S (Aldrich et al., 2007). However, in *C. linearis*, only a partial reduction and chelation to sulfur was observed.

The XANES results from the red flowered plants differ from those of the white flowered plants. Although, the general trend of S speciation (As–S) in both the red and white flowered plants is high in roots, followed by stems, the red flowered plants did not present detectable amounts of As in leaves. The amounts of

the As-$(SX)_3$ complex were lower and the amounts of the AsO_4^{3-} complex were higher in the red flowered plants. This indicates a difference in the effectiveness of the two plants of the same species to manage and detoxify arsenate contamination. Alternatively, this may be indicating some difference in the effect of As on various biological mechanisms in these two *C. linearis* phenotypes, for example the synthesis of LMWT. Another well known example of this is the substitution of AsO_4^{3-} into the PO_4^{3-} mechanisms within plants (Zaray et al., 2004).

4. Concluding remarks

In summary, the results of this investigation demonstrated increased accumulation of As with increasing concentration of arsenate in media for the white flowered plants only. Nonetheless, *C. linearis* plants were tolerant to relatively high concentrations of As when supplied as arsenate (40 mg As^V L^{-1}). Absorption of S increased while the absorption of P decreased upon exposure to As^V. The XAS analysis demonstrated a partial reduction of arsenate to arsenite in the tissues of both *C. linearis* phenotypes. However, the relative ratios of the As-$(SX)_3$ and the AsO_4^{3-} complexes in the white and red flowered plants were different, as well as the production of LMWT. The results suggest that the wild type species (white flowered) is a better accumulator of As. These results suggest that the null hypothesis must be rejected. This wild type plant should be further studied for As phytoremediation purposes in the southwestern area of the US.

5. Experimental

5.1. Plant materials and arsenic treatments

C. linearis seeds were collected from the desert garden at The University of Texas at El Paso. This site has no previous report on As contamination. Approximately 10–12 seeds were placed at the edge of previously autoclaved paper towels (30 min at 120 °C and 1.0 kg cm^{-2} pressure) under a laminar flow hood. The towels were bent and rolled to cover the seeds and transferred to 250 mL mason jars containing sterilized deionized water for two weeks. Next, H_2O was replaced by half strength modified Hoagland's nutrient solution (pH 5.8) for another two weeks. Germination and growth of the seedlings took place at room temperature (22–26 °C) using a 16-h photoperiod (four 34 W Phillips lamps).

After one month of growth, the seedlings were exposed to As at 0, 20 and 40 mg L^{-1} of arsenate (As_2O_5) in hydroponic solution containing full strength modified Hoagland's nutrient solution (0.35 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.1 mM $CaCl_2 \cdot 2H_2O$, 0.91 mM $Mg(NO_3)_2 \cdot 6H_2O$, 0.97 mM KH_2PO_4, 0.255 mM KNO_3, 23.13 μM H_3BO_3, 3.9 μM $MnCl_2 \cdot 4H_2O$, 0.07 μM MoO_3, 0.44 μM $CuSO_4 \cdot 5H_2O$, 10 μM $Fe(NO_3)_3 \cdot 9H_2O$, 0.37 μM $Zn(NO_3)_2 \cdot 6H_2O$).

5.2. Elemental analysis

After 7 days of exposure to treatments, plants were rinsed with 0.1 M HNO_3 and deionized H_2O, separated into roots, stems and leaves, and lyophilized. Prior to lyophilization, the samples were first immersed in liq. N_2 for 45 min until completely frozen. Samples were then placed into a LabConco Freeze Zone 4.5 System (LabConco Corporation, Kansas City, MO) and lyophilized at −45 °C and 0.069 mbar pressure. For determination of As, S, and P, freeze-dried tissue (100–800 mg) were acid digested in a microwave oven (CEM MarsX, CEM Corporation, Mathews, NC) using 6 mL of trace pure 65–70% HNO_3 following the USEPA 3051 method (Kingston and Jassie, 1988). The volume was adjusted to 10 mL after digestion. Samples were examined with and ICP-OES Optima 4300 DV with an AS-90 plus autosampler rack; Perkin–Elmer, Shelton, CT). The following ICP-OES parameters were used: nebulizer flow, 0.80 L min^{-1}; power, 1450 W; peristaltic pump rate, 1.5 mL min^{-1}; flush time, 15 s; delay time, 20 s; read time, 10 s; wash time, 60 s. Every sample was read three times and three replicates for each sample were analyzed.

5.3. Low molecular weight thiols

Acid-soluble thiols were determined as described by de la Rosa et al. (2005). Briefly, extraction was carried out by grinding (using mortar and pestle) 25–35 mg of freeze-dried tissue in 2 mL ice-cold solution containing 5% (w/v) sulfosalicylic acid and 6.3 mM DTPA. Subsequently, samples were immediately frozen using liq. N_2. Next day, samples were thawed to room temperature, vortex, and centrifuged for 10 min at 11,000 rpm in a refrigerated centrifuge (Eppendorf centrifuge 5417R, Westbury, NY). Supernatants were assayed immediately after centrifugation. Concentrations of LMWT were determined by using reduced glutathione (GSH) dissolved in the extraction solution (Standards from 0–500 μM GSH). Supernatants (200 μL) or standard solutions (100 μL) were mixed with 0.525 M K_2HPO_4 previously added to the microplate wells. Later, 50 μL of DTNB solution (1.75 mM 5,52-dithiobis(2-nitrobenzoic acid), 0.143 M K_2HPO_4, 6.3 mm DTPA, pH 7.5) were added and absorbance at 412 nm (at room temperature) was measured in a microplate reader ~20 min after DTNB. Background-sample blanks were prepared as described using 50 μL of 0.143 M K_2HPO_4 instead of the DTNB solution. The absorbance corresponding to LMWT in solution was found by subtracting the absorbance of the background-sample blank from the absorbance of DTNB. Values were calculated using the molecular extinction coefficient calculated from the calibration curve. The lower detection limit obtained from 0 μM GSH absorbance media was found to be 0.107 units of absorbance.

5.4. XAS sample preparation

Lyophilized root, stem, and leaf samples were ground using mortar and pestle to obtain a homogenous mixture and packed into 1.0 mm thick aluminum sample holders with Kapton Tape® windows. Salts of As_2S_3 and Na_2HAsO_4 (Sigma–Aldrich, St Louis, MO) were used as model compounds.

5.5. XAS data collection

The samples were analyzed on beamline 2–3 at Stanford Synchrotron Radiation Laboratory (SSRL; Palo Alto, CA) at room temperature using a 13-element germanium fluorescence detector. The beamline setup was as follows: a Si (220) φ0 double crystal monochromator, a current ranging from 80–100 mA, beam energy of 2.0 GeV, and a 1.0 mm slit. In addition, the samples were analyzed using an internal As(0) foil (E_0 11.868 keV) for calibration purposes. A minimum of three sweeps per sample were averaged and used for data analysis.

5.6. XAS Data analysis

Arsenic samples were analyzed using the standard data reduction process and the WinXAS© software (Ressler, 1998). The data were first calibrated based on the first inflection point of the As(0) foil E_0 11.868 keV using a second degree derivative of the absorption edge. After energy calibration, the sample spectra were then background corrected using a one-degree polynomial fitting to the pre-edge region and normalized to one absorption unit. After normalization, the XANES spectra of individual samples and model compounds were then extracted by sectioning the XAS spectra

from 11.80 to 11.95 keV. After that, the XANES spectra were fitted using LC-XANES fitting procedures as previously reported (Ressler et al., 2000).

5.7. Statistical analysis

A one way ANOVA and a post hoc Tukey test with a 0.05 significance level was used for the statistical analysis using the SPSS 15.0 software package for windows.

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