Aquatic Toxicology 157 (2014) 70–80

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Arsenic accumulation and tolerance in rootless macrophyte *Najas indica* are mediated through antioxidants, amino acids and phytochelatins

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ARTICLE INFO

Article history:
Received 28 July 2014
Received in revised form
23 September 2014
Accepted 27 September 2014
Available online 7 October 2014

Keywords:
Amino acid
Antioxidants
Arsenate
Arsenite
Najas indica
Phytochelatin

ABSTRACT

Arsenic (As) accumulation and tolerance response of a submerged rootless macrophyte *Najas indica* were evaluated during arsenate (As^V; 10–250 μM) and arsenite (As^{III}; 1–50 μM) exposure. Higher As accumulation at As^{III} exposure and more tolerance upon As^V exposure resulted in more toxicity during As^{III} stress than As^V, which was evident through measurement of growth parameters and oxidative stress related parameters viz., lipid peroxidation (MDA content), electrical conductivity (EC) and hydrogen peroxide (H_2O_2) levels. Antioxidant enzymes and various amino acids were more prominent during moderate exposure of As^V, suggesting their possible role in As tolerance and detoxification. Various non-enzymatic antioxidant metabolites viz., ascorbic acid (ASC), glutathione (GSH), non-protein thiols (NPTs) and phytochelatins (PCs) biosynthesis involving phytochelatin synthase (PCS) activity increased more significantly during As^{III} stress. However, PCs content seems inadequate in response to As accumulation leading to lower PC-SH:As molar ratio and higher As phytotoxicity during As^{III} stress. *N. indica* may prove useful plant species for phytoremediation purpose in moderately As contaminated water bodies due to high As accumulation and tolerance potential.

1. Introduction

Arsenic (As) is a class one carcinogen present ubiquitously in the environment. Natural (geochemical sources) and anthropogenic activities such as mining, land irrigation, use of As based pesticides and waste disposal have locally led to severe As contamination (Zhao et al., 2010; Tripathi et al., 2012a,b). Arsenic is a non-essential element to biota, as no role of this element is known into metabolism, while toxicity of As to plants, humans and animals has been taken into account due to its recognition as food chain contaminant (Zhao et al., 2010; Finnegan and Chen, 2012). Remediation of As from aquatic environment has become a major issue (Tu and Ma, 2003; Zhao et al., 2010). Phytoremediation, a plant based technology for the removal of toxic contaminants from soil and water is an attractive approach (Mishra et al., 2008; Danh et al., 2014).

Inorganic arsenate (As^V) and arsenite (As^{III}) are the most predominant forms of As in oxic and anoxic soil, respectively (Tripathi et al., 2012a,b). Arsenate is a chemical analogue of phosphate and is taken up by plant roots through the high affinity phosphate uptake system, whereas As^{III} is taken up at considerable rates, mainly via aquaglyceroporins (Meharg and Hartley-Whitaker, 2002; Zhao et al., 2010). Once inside the plant, As^V can exert toxicity itself, through phosphate substitution in phosphorylation reactions, or after reduction to As^{III}, which is more cytotoxic than As^V due to its high affinity for protein —*SH* (Tripathi et al., 2012a,b).

Arsenic toxicity, range of accumulation and plant responses depends on the presence of particular As species. Arsenic interferes with various metabolic processes that cause physiological and morphological disorders leading to inhibited plant growth (Srivastava et al., 2007; Mishra et al., 2008; Tripathi et al., 2012a,b). Under arsenic stress, plants often produce reactive oxygen species (ROS), causing damage to DNA, proteins and lipids. To minimize the harmful effects of ROS, plants have evolved a range of detoxification strategies that comprises of phytochelatins (PCs) mediated

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http://dx.doi.org/10.1016/j.aquatox.2014.09.011
0166-445X/

detoxification (Mishra et al., 2008; Zhao et al., 2010; Tripathi et al., 2012a,b, 2013a) and antioxidants mediated ROS scavenging which is composed of antioxidant metabolites such as non protein thiols (NPTs), cysteine, glutathione (GSH), proline and ascorbic acid and also various antioxidant enzymes such as superoxide dismutase (SOD) and peroxidases (Srivastava et al., 2007; Rai et al., 2011).

Amino acids (AAs) may play an important role in plant stress resistance through osmotic adjustment and the accumulation of compatible osmolytes, detoxification of ROS and pH regulation. Studies demonstrated that As causes interruption in nutrient metabolism consequently leading to AAs loss, while some stress responsive AAs viz., proline, cysteine and glycine are known to be induced significantly during As exposure (Dwivedi et al., 2010; Tripathi et al., 2013a).

Considering this whole background of responses induced under As stress, the present study aimed to analyze the possible involvement of antioxidant system in combating As induced oxidative stress and role of PCs in its homeostasis and detoxification vis-á-vis As accumulation in an aquatic weed, *Najas indica*. *Najas* is a fully submerged, rootless floating plant with long narrow leaves of family Najadaceae. These features provide a large surface area for heavy metal adsorption and absorption potential (Singh et al., 2010) and may thus possibly be used as a remediator of As contaminated aquatic environments. It is hypothesized that this plant has adequate As detoxification mechanisms to sustain higher level of As accumulation. To better understand the mechanisms of As detoxification by *N. indica*, it is worthwhile to examine the As induced physiological and biochemical responses to combat As phytotoxicity.

2. Materials and methods

2.1. Plant material and treatment conditions

Plants of *N. indica* were obtained from Environmental Field Station, NBRI and were grown for six months in large hydroponic tubs (165 cm × 60 cm × 47 cm) filled with 1/4 soil. Before treatment, plants (two inches tip portion) were acclimatized for five days in laboratory conditions at 115 μmol m^{-2} s^{-1} light with a period of 16:8 (light:dark) h at temperatures of 25 °C day and 20 °C night in 10% Hoagland's solution (Hoagland and Arnon, 1950). Plant acclimatized in 10% Hoagland's solution were treated with different concentrations of As^V (0, 10, 50, 100 and 250 μM; prepared using Na_2HAsO_4) and As^{III} (0, 1, 5, 10 and 50 μM; prepared using $NaAsO_2$) maintained in 10% Hoagland's solution in 250 ml beakers under above mentioned laboratory conditions for a period of 1, 2, 4 and 7 days. Each beaker contained plants of equal size (approximately 4 g fw) were aerated for an hour every day. Plant grown without As for each set of experiment served as control. Three replicates were used for each concentration and duration. After harvesting, plants were washed with MilliQ water, blotted gently and used for the study of various physiological and biochemical parameters.

2.2. Arsenic quantification and quality control

For estimation of total As, dried plant material (100 mg) were powdered and digested in 2 ml HNO_3 at 120 °C for 6 h and then diluted with demineralized water (Dwivedi et al., 2010). The level of As was quantified by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500 cx). The standard reference material of As (Agilent, Part # 8500-6940) was used for the calibration and quality assurance for each analytical batch. Rice flour NIST 1568a was used as a reference material with known spiked samples and recovery of total As was 93.5% (±2.8; $n = 5$) and 89.5% (±3.1; $n = 5$) respectively. The detection limit of As was 1 μg l^{-1}.

2.3. Determination of As species

For As species, samples were powdered; 0.2 g was weighed in a centrifuge tube, and extracted with 1 ml of 1.52 mM NaH_2PO_4 buffer containing 0.198 mM Na_2EDTA, 3 mM $NaNO_3$, 10 mM CH_3COONa and 1% C_2H_5OH (pH 6.0) modified from Zheng et al. (2011). The extraction solutions were centrifuged and passed through a 0.45 μm nylon syringe filter. Samples were kept on ice and in the dark and analyzed within a few hours after extraction to minimize potential further transformation of As species. Arsenic species in the extracts were assayed by HPLC-ICP-MS (Agilent 7500cx ICP-MS, HPLC Agilent Technologies 1200 series). Chromatographic columns consisted of a Column 150 mm × 4.6 mm (anion exchange resin hydrophilic polyacrylete as basic resin, PEEK1, Agilent Technologies, Inc., Tokyo, Japan). The mobile phase consisting of 1.52 mM NaH_2PO_4 buffer containing 0.198 mM Na_2EDTA, 3 mM $NaNO_3$, 10 mM CH_3COONa and 1% C_2H_5OH (pH 6.0) was run isocratically at 1 ml min^{-1}. Standard compounds of As^V, As^{III}, dimethylarsinic acid (DMA^V) and monomethylarsonic acid (MMA^V) were used to obtain retention times. Matrix-matched DMA^V standards were used to calibrate the instrument. Arsenic species in samples were identified by comparisons with the retention times of the standard compounds and quantified using external calibration curves with peak areas. As such there are no reference materials with certified As concentrations for different species, but various studies have analyzed inorganic As in rice flour SRM 1568a. The mean recovery of inorganic As from the rice flour was 86.4% (±2.7; $n = 6$).

2.4. Estimation of LPO, EC, H_2O_2 and protein

The lipid peroxidation (LPO) was determined following the method of Heath and Packer (1968). The amount of malondialdehyde (MDA) was calculated by difference in absorbance at 532 and 600 nm using the ε of 155 mM^{-1} cm^{-1}. The ion leakage was measured in terms of electrical conductivity (EC) according to Devi and Prasad (1998). Metalloid exposed plants were washed with deionized water (Millipore, USA) and 500 mg of plant material was then transferred to 100 ml of deionised water for 24 h to facilitate maximum ion leakage and the EC of the water was recorded. For estimation of hydrogen peroxide (H_2O_2) levels, plants were homogenized in ice cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1% Triton X-100 and 1% PVP (w/v) and the level of H_2O_2 was measured according to Pick (1986). Protein content was estimated following the method of Lowry et al. (1951).

2.5. Estimation of thiols

NPTs content was measured following the method of Ellman (1959). For which, plant material (700 mg) was homogenized in 6.67% 50-sulfosalicylic acid. After centrifugation at 10,000 × *g* for 10 min at 4 °C, NPTs content was measured in the supernatant by reaction with Ellman reagent. The level of reduced glutathione (GSH) and oxidized glutathione (GSSG) was measured by following the protocol of Hissin and Hilf (1976). Plant material (500 mg) was frozen in liquid nitrogen homogenized in 0.1 M sodium phosphate buffer (pH 8.0) containing 25% meta-phosphoric acid. The homogenate as centrifuged at 20,000 × *g* for 20 min at 4 °C and total glutathione (GSSG and GSH) content was determined fluorometrically at 420 nm after excitation at 350 nm in the supernatant after 15 min incubation with o-phthaldialdehyde (OPT).

2.6. Estimation of antioxidant enzymes

Control and As exposed plants were homogenized in buffers specific for each enzyme under chilled conditions. Homogenate