Environmental and Experimental Botany 69 (2010) 167–174

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Arsenic accumulation and biological responses of watercress (*Nasturtium officinale* R. Br.) exposed to arsenite

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ARTICLE INFO

Article history:
Received 14 October 2009
Received in revised form 23 February 2010
Accepted 12 March 2010

Keywords:
Nasturtium officinale
Arsenite
Accumulation
Biological response
DNA damage

ABSTRACT

The objective of the present study was to investigate biological responses of watercress (*Nasturtium officinale* R. Br.) under arsenic stress. Watercress samples were exposed to 1, 3, 5, 10, and 50 μM of arsenite (As(III)) for 7 days. Arsenic accumulation in the leaves of watercress was investigated, and its influence on the rates of lipid peroxidation, ion leakage, photosynthetic pigmentation, proline content, enzymatic antioxidant performance, and DNA damage was examined. Watercress was capable of accumulating large amounts of arsenic in the leaves. The highest accumulation of As (1012 μg g^{-1} dw) was found in the leaves of the plants exposed to 50 μM of As(III). Plant growth was stimulated at 1 μM As(III) application, while higher concentrations unfavorably affected plant growth. It was observed that exposure to As(III) significantly increased the ion leakage and lipid peroxidation compared to the control. An increase in protein and proline content was observed, followed by a gradual decline at higher concentrations. Stress conditions caused up-regulation of the antioxidant enzyme activity in dose dependent manner. The results indicated that the changes that occur in the random amplified polymorphic DNA (RAPD) profiles after an As(III) treatment, include the presence of certain modifications in the band intensity and the gain or loss of bands. The results of the present study confirmed that *N. officinale* is capable of overcoming the occurrence of As(III)-induced stress, especially, due to conditions of moderate exposure.

1. Introduction

Inorganic arsenic compounds have been employed in industrial and agricultural activities and also in the control of aquatic weed. The phytotoxicity of arsenic depends on its oxidation state. Arsenite (As(III)) and arsenate (As(V)) are inorganic, phytoavailable forms of arsenic and are highly toxic to plants (Mkandawire et al., 2004). These inorganic forms are interconvertible, depending on the redox condition of the aquatic ecosystem.

Arsenic exists predominantly as As(V) in natural waters. However, Zhao et al. (2009) have stated that under aerobic conditions, As(III) may also exist in the rhizosphere due to activity of microbes and root exudates. Consequently, plants growing in aerobic environment may encounter a mixed pool of As(III) and As(V) (Wang et al., 2010). Zhao et al. (2009) determined that arsenic exists in the plant predominantly as As(III), even though plants had been exposed to As(V). Therefore, it can be concluded that, following uptake, As(V) is reduced efficiently to As(III) in plant cells. As(V) and phosphate are chemically similar, allowing arsenate to act as a phosphate analogue, thereby permitting transport into the cell (Meharg and Macnair, 1990). In contrast, As(III) is transported in its natural form, $As(OH)_3$. However, Wang et al. (2010) determined that *Pteris vittata* was more efficient in taking up As(III) than As(V) in the presence of phosphate.

As(III) is considered as phytoavailable and the most phytotoxic arsenic species (Mkandawire et al., 2004). As(III) is powerful inhibitors of the sulfhydryl groups found in some enzymes and tissue proteins. They attack plant cell membranes, causing an inhibition of cellular function and death (Sizova et al., 2002). Relative growth rate (RGR) is an important parameter in evaluating the physiological effects of toxic chemicals on plants (Cedergreen, 2008). Stress condition may enhance protective processes such as accumulation of compatible solutes and increase in the activities of detoxifying enzymes. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Ohkawa et al., 1979). Mishra and Dubey (2006) determined that when rice seedlings are subjected to arsenic stress, the normal rate of activity of their proteolytic enzymes and the level of proteins are change.

Another noteworthy reaction induced in the plants that are exposed to arsenic stress is the accumulation of certain specific metabolites, such as, free proline. Proline accumulates violently in several plants under stress, procuring the plants defense against damage by ROS. Proline plays important roles in osmoregulation,

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doi:10.1016/j.envexpbot.2010.03.006

protection of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity (Choudhary et al., 2007).

Some authors previously reported that at increasing rates of As concentrations, certain plants produce reactive oxygen species (ROS) which damage cell membranes, DNA, protein, lipid and chloroplast pigments (Cao et al., 2004; Patra et al., 2004). To minimize the harmful effects of ROS, plants have evolved an effective scavenging system composed of antioxidant molecules and antioxidant enzymes (Meharg, 1994). The major enzymatic antioxidant pathways of plants include superoxide dismutase (SOD), found in almost all cellular compartments. SOD is a metalloenzyme that catalyzes dismutation of superoxide anion into oxygen and hydrogen peroxide. Such enzymes provide a defense system for the survival of aerobic organisms (Beyer et al., 1991).

Several studies have used the comet, micronucleus or chromosome aberration assays to measure genotoxic effects of toxic chemicals on plants. Recently, the development of molecular marker technology has provided new tools for detection of genetic alteration in response to toxic chemicals tolerance by looking directly at the level of DNA sequence and structure. Over the last years the random amplified polymorphic DNA (RAPD) analysis has been used also for determination of genome rearragements caused by genotoxic factors including toxic chemicals (Atienzar et al., 1999). RAPD assay is a PCR-based technique that amplifies random DNA fragments of genomic DNA with single short primers of arbitrary nucleotide sequence under low annealing conditions (Williams et al., 1990). The assay was successfully applied to detect genomic DNA alterations induced by several DNA damaging agents, such as Cd, Pb and UV radiation in plants (Liu et al., 2009; Cenkci et al., 2010; Atienzar et al., 2000). It is suggested that alterations to RAPD profiles due to genotoxic exposure can be regarded as changes in genomic template stability (GTS, a qualitative measure of genotoxic effect) (Atienzar et al., 1999).

It is known that As accumulation potential of aquatic macrophytes is very high (Alvarado et al., 2008). As(V) uptake and toxicity have been well documented in plants (Smith et al., 2008; Mrak et al., 2008), whereas As(III) uptake and toxicity have been less studied (Mkandawire et al., 2004). Watercress, *Nasturtium officinale* R. Br., is an edible aquatic plant. As a medicinal plant, watercress has been traditionally considered a diuretic, purgative and tonic, and consumed as a salad green. The properties of *N. officinale* that entail metal accumulation were extensively studied in the past. However, even then the available knowledge regarding the As accumulation characteristics of watercress exposed to As(III) and the resultant effect of accumulation is not sufficient.

First, the present study focused on the accumulation properties of As. Second, this study aimed to demonstrate the biological responses of watercress against the As, based on the varied concentrations of As(III) application. The results of the investigations may help to explain the effects of arsenite on aquatic plants, and the consequent biologic responses of these plants. Furthermore, the findings may be useful when this plant is used as a phytoremediator in polluted water.

2. Materials and methods

2.1. Sample collection and cultivation

N. officinale seedlings were collected in April of 2008 from the Karasu Stream in Kayseri, Turkey. Prior to the experiment, containers were disinfected by immersion in 1% (v/v) NaClO for 3–5 min. Containers were then rinsed three times with distilled water (Hou et al., 2007). Collected samples were washed in tap water and acclimatized for 3 days in a climate chamber with a water temperature of 15 °C, a relative humidity of 70% and light/dark photoperiod of 16 h light/8 h dark. Containers were mildly aerated.

2.2. Experimental design

The experiments conducted in the present study were set-up in triplicate, wherein each replicate constituted approximately 4 g of the evaluated plants. The As(III) solutions utilized in the present study were prepared from $NaAsO_2$. Each plant sample of watercress were exposed to four varied test concentrations (1, 3, 5, 10, and 50 μM) of As(III) maintained in 10% Hoagland's solution in separate 400 mL conical flasks (Srivastava et al., 2006). The plants that were not exposed to As(III) served as the control groups of this experiment. The flasks that comprise the plant and As concentrates were placed in a climate chamber under the aforementioned conditions for 7 days. Flasks were not aerated during experiment. The change that occurred in the volume of the solution within the flasks due to evapotranspiration was compensated for by the addition of double distilled water. At the end of the exposure experiment, the resultant plant samples were collected and sieved with a plastic griddle. Each plant was rinsed with deionized water, drained, and then blotted on paper towels for 2 min.

2.3. Quantification of arsenic and determination of RGRs

The relative growth rate (RGR) of *N. officinale* was calculated in each treatment using the equation:

$$\mathrm{RGR}\,(\%/\mathrm{day}) = \frac{[\ln(W_2) - \ln(W_1)]}{t} \times 100\% \quad (1)$$

W_1 and W_2 are the initial and final fresh weights (g), respectively, and t is the length of the experimental period.

Leaves of plants sample was dried at 70 °C. Each sample was then digested with 10 mL of pure HNO_3 using a CEM Mars 5 (CEM Corporation Mathews, NC, USA) microwave digestion system. The digestion conditions were as follows: the maximum power was 1200 W, the power was at 100%, the ramp was set for 20 min, the pressure was 180 psi, the temperature was 210 °C and the hold time was 10 min. After digestion, the solution was evaporated to near dryness in a beaker. The volume of each sample was adjusted to 10 mL using 0.1 M HNO_3. The total concentration of arsenic was determined using an inductively coupled plasma mass spectrometer (Agilent, 7500a). The stability of the device was evaluated every ten samples by examining an internal standard. Reagent blanks were also prepared to detect any potential contamination during the digestion and analytical procedure. Peach leaves (NIST, SRM-1547) were used as the reference material for all of the performed analytical procedures. The samples were analysed in triplicate. All chemicals used in this study were analytical reagent grade (Merck, Darmstadt, Germany).

2.4. Determination of ion leakage and lipid peroxidation

The ion leakage induced by the As(III) was estimated by measuring the electrical conductivity (EC) (Devi and Prasad, 1998). Leaves of arsenic-exposed plants were washed with double-deionized water. Then, 500 mg of plant material was transferred to 100 mL of deionized water for 24 h to facilitate maximum ion leakage. The EC of the water was then recorded with WTW model conductivity meter. For determination of lipid peroxidation, leaf material (500 mg) was homogenized with 3 mL of 0.5% TBA in 20% TCA (w/v). The homogenate was incubated at 95 °C for 30 min, and ice was used to stop the reaction. The samples were centrifuged at 10,000 × g for 10 min, and the absorbance of the resulting supernatant was recorded at 532 and 600 nm. The amount of malondialdehyde (MDA) (extinction coefficient of 155 $mM^{-1}\,cm^{-1}$) was calculated by subtracting the non-specific absorbance at 600 nm from the absorbance at 532 nm (Heath and Packer, 1968).