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Exogenous nitric oxide (as sodium nitroprusside) ameliorates arsenic-induced oxidative stress in watercress (*Nasturtium officinale* R. Br.) plants

Shahram Namdjoyan [a,*], Hossein Kermanian [b]

[a] Department of Biology, Shahre Qods Branch, Islamic Azad University, Shahre Qods, Tehran, Iran
[b] Department of Pulp and Paper, Energy and New Technology Faculty, Zirab Rachis, Shahid Beheshti University, Iran

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ABSTRACT

This research investigated the effects of exogenous sodium nitroprusside (SNP) supplementation as a nitric oxide (NO) on alleviating arsenic-induced oxidative damage in watercress (*Nasturtium officinale* R. Br.) plants. In arsenic-treated plants, dry weight of roots and shoots and chlorophyll content of leaves markedly decreased, while application of 100 μM SNP, alleviated the inhibitory effect of arsenic on plant growth and chlorophyll content in arsenic-treated plants. Treatment with 100 μM SNP considerably reduced root-to-shoot translocation of arsenic and increased slightly the protein and proline content in watercress tissues. Level of oxidative markers (lipoxygenase activity and malondialdehyde (MDA) and H_2O_2 content) sharply increased in arsenic-treated plants. Upon application of SNP this trend was alleviated; however, the activities of enzymatic antioxidants increased when plants were subjected to arsenic stress in combination with SNP. Results obtained in the present study suggest that exogenous NO could alleviate negative effects of arsenic on watercress plants probably through its ability to the stimulation of reactive oxygen species (ROS)-scavenging enzymes activity and/or direct scavenging of superoxide onion.

1. Introduction

Aresenic (As) is a member of the nitrogen family with both metallic and nonmetallic properties (Azizur Rahman and Hasegava, 2011). It is a naturally occurring toxic element in the environment. Although the occurrence of As in the environment is mainly from minerals and geogenic sources, human activities such as mining, burning of fossil fuels, use of As containing chemicals in agriculture also cause As distribution in the environment (Sun et al., 2009; Liu et al., 2010). As-contaminated soils and waters are of great concern worldwide due to arsenic's toxicity as a carcinogen (Tripathi et al., 2007). Arsenite (As III) and arsenate (As V) are the major inorganic forms of As toxicity and these anions are readily taken up by the plants (Tripathi et al., 2007; Ismail, 2012). Phytoextraction has the potential to clean up As-contaminated sites (Wei et al., 2010). In plants, As toxicity include inhibition of the sulfhydryl groups found in some enzymes and tissue proteins (Ozturk et al., 2010), disruption of plant water status, inhibition of nutrient uptake (such as Fe, Mn, Cu and P) and negative effect on chlorophyll metabolism. At cellular level, As stimulates the production of reactive oxygen species (ROS) such as $O_2^{\bullet-}$ and H_2O_2 and induces oxidative stress as evidenced by enhanced lipid peroxidation (Ozturk et al., 2010; Ismail, 2012). Moreover, lipid peroxidation can be initiated enzymatically by lipoxyganase (LOX) and is believed to be an important factor of growth inhibition in plants exposed to stress conditions (Kazemi et al., 2010). The production of ROS must be carefully regulated to avoid oxidative damage. plant cells are normally protected against this oxidative damage by a broad spectrum of radical scavenger systems, including antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants such as glutathione and ascorbic acid (Namdjoyan et al., 2011). Nitric oxide (NO) is a diffusible gaseous free radical (Laspina et al., 2005). This bioactive molecule involved in signaling process within plants (Wendehenne et al., 2001) and plays a central role in a variety of physiological processes such as growth (Arasimowicz and Floryszak-Wieczorek, 2007), seed germination (Ismail, 2012), iron availability (Zhang et al., 2012) and adaptive responses to environmental stresses (Kazemi et al., 2010). Also, application of the NO donor sodium nitroprusside (SNP) shown to be involved in mediation of environmental stresses such as salinity (Lopez-Carrion et al., 2008), and heavy metals (Ismail, 2012). Nitric oxide is itself a reactive nitrogen species and its effects on different types of cells have proved to be either cytoprotective or cytotoxic, depending on its concentration and on the position of action (Lamattina et al., 2003; Kazemi et al.,

* Corresponding author. Tel.: +98 213 336 05 14; fax: +98 213 336 05 14.
E-mail addresses: shahram_biotech@yahoo.com (S. Namdjoyan), H_Kermanian@sbu.ac.ir (H. Kermanian).

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2010). Despite the toxicity associated with arsenic, some plants are known to be able to survive high concentrations of As in the substrate (Ma et al., 2001). Watercress (*Nasturtium officinale* R. Br.), an edible and medicinal aquatic plant, has recently been discovered to hyperaccumulate As (Ozturk et al., 2010).

The objectives of this study were to (1) examine the effect of exogenous NO on plant growth and As uptake by watercress plant and (2) investigate the effect of exogenous NO on alleviation of the As-induced toxicity and determine the effect of exogenous NO on modulating the antioxidant enzymes in the roots and shoots of watercress plants.

2. Materials and methods

2.1. Plant growth and treatment

Watercress (*N. officinale* R. Br.) seeds were obtained from a local market. The seeds were surface-sterilized with 2% (v/v) sodium hypochlorite for 5 min and then washed thoroughly several times with sterile distilled water, and then the seeds were germinated on a wet filter paper at 25 °C for 7 days. Initially, seedlings of uniform size were transferred to plastic pots (volume 500 ml) filled with perlite (3 plants per pots) and watered with half-strength Arnon and Hoagland nutrient solution (Arnon and Hogland, 1940) for 7 days. The seedlings were then watered with full-strength Hoagland solution. Sodium nitroprusside (SNP) was used as NO donor and sodium arsenate ($Na_2HA_5O_4{\cdot}7H_2O$) as a source of As. Twenty-one-day old plants were transferred to fresh medium supplemented with one of the following treatment solutions: (a) distilled water alone (control); (b) 100 μM SNP; (c) 25 μM As; (d) 25 μM As + 100 μM SNP; (e) 50 μM As; (f) 50 μM As + 100 μM SNP; (g) 75 μM As; (h) 75 μM As + 100 μM SNP; (i) 100 μM As; (j) 100 μM As + 100 μM SNP. Hoagland solution was adjusted to pH 6.5 and applied twice a week. Plants were grown in controlled-environment chamber at 16/8 light/dark photoperiod and photon flux density 200 μmol m^{-2} s^{-1}, Day/night temperature of 26/22 °C and 65 ± 5% relative humidity. After 7 days of growth with the above conditions, the plants were harvested and the roots and shoots were separated and washed with deionized distilled water. For the estimation of plant dry mater and As concentration, the plants were dried at 80 °C for 48 h. For the enzyme activity determination, fresh plant material was frozen in liquid nitrogen and stored at −70 °C until use.

2.2. Arsenic determination

Roots and shoots were separated, weighed and placed in a drying cabinet at 80 °C until a constant weight was reached. Plant material then ground into powder. Dry powders of root and shoot tissue (0.5 g) were digested with 10 ml of pure HNO_3 on a hot block digester (Environmental Express, Mt. Pleasant, S.C.) using USEPA Method 3050A. Total As concentrations in digested solution were determined by atomic absorption spectrophotometry (SpectrAA-200, Varian, Australia) (Srivastava et al., 2005). The detection limit of the instrument for As in solution was 0.02 mg L^{-1}. Standard reference materials from the National Institute of Science and Technology (US NIST, Gaithersburg, MD) were used to assess method accuracy and precision (within 100 ± 20%).

2.3. Chlorophyll contents determination

Fresh leaves (0.2 g) were homogenized with 80% acetone, filtered and made up to final volume of 20 ml. Total chlorophyll contents were calculated from the absorbance of extract at 645 and 663 nm using the formula of Lichtenthaler (1987).

2.4. Analysis of lipid peroxidation

Lipid peroxidation in roots and leaves was determined by estimation of the MDA concentration following the method of Heath and Packer (1968) with slight modification. Fresh samples (0.5 g) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 × *g* for 5 min. To every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added. The mixture was heated at 95 °C for 30 min and then cooled quickly on the ice bath. The resulting mixture was centrifuged at 10,000 × *g* for 15 min, and the absorbance of the supernatant was taken at 532 and 600 nm. The nonspecific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol g^{-1} FW (fresh weight).

2.5. Hydrogen peroxide (H_2O_2) determination

For determination H_2O_2 concentration, root and shoot tissues (0.2 g) were extracted with 3 ml of 0.1% (w/v) TCA in an ice bath and centrifuged at 12,000 × *g* for 15 min (Velikova et al., 2000). An aliquot (0.5 ml) of supernatant was added to 0.5 ml of phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance of the mixture was read at 390 nm. H_2O_2 content was determined using the extinction coefficient 0.28 μM^{-1} cm^{-1} and the amount expressed as μmol g^{-1} FW.

2.6. Total protein and proline determination

The protein content was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard protein.

Proline content was determined according the method of Bates et al. (1973). Plant samples (0.2 g) were homogenized with 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 × *g* for 10 min, 2 ml of supernatant were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin for 1 h at 100 °C. The developed colour was extracted in 4 ml toluene and measured colourimetrically at 520 nm against toluene. A standard curve with L-proline was used for the final calculations. Content of proline was expressed as μmol g^{-1} FW.

2.7. Enzyme activity assays

Root and shoot fresh samples from different treatments (500 mg) were homogenized with a mortar and pestle in 5 ml of ice-cold potassium phosphate buffer (50 mM, pH 7.5) containing 1 mM ethylenediamine tetra acetic acid (EDTA), 1% polyvinylpyrrolidone (pvp), with the addition of 5 mM ascorbate in the case of APX assay. The protein contents in the supernatant were determined according to the method of Bradford (1976), with BSA as a standard. The supernatant was used for the enzyme activity measurement.

SOD (EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridivich (1971). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the nitroblu tetrazolium (NBT) reduction rate at 560 nm.

CAT (EC 1.11.1.6) activity was assayed in the reaction mixture containing 25 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 10 mM H_2O_2, and the enzyme. A decrease in the absorbance of H_2O_2 within 1 min at 240 nm (E = 39.4 mM^{-1} cm^{-1}) was recorded (Aebi, 1984).

APX (EC 1.11.1.11) activity was measured according to the method of Nakano and Asada (1987) by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mM^{-1} cm^{-1}). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0)