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Research article

Nitric oxide alleviates arsenic-induced toxic effects in ridged *Luffa* seedlings

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ABSTRACT

Hydroponic experiments were conducted to investigate whether exogenous addition of nitric oxide (NO) as sodium nitroprusside (SNP) alleviates arsenic (As) toxicity in *Luffa acutangula* (L.) Roxb. seedlings. Arsenic (5 and 50 μM) declined growth of *Luffa* seedlings which was accompanied by significant accumulation of As. SNP (100 μM) protected *Luffa* seedlings against As toxicity as it declined As accumulation significantly. The photosynthetic pigments and chlorophyll fluorescence parameters such as Fv/Fm, Fv/F0, Fm/F0 and qP were decreased while NPQ was raised by As. However, the toxic effects of As on photosynthesis were significantly ameliorated by SNP. The oxidative stress markers such as superoxide radical, hydrogen peroxide and malondialdehyde (lipid peroxidation) contents were enhanced by As, however, these oxidative indices were diminished significantly in the presence of SNP. As treatment stimulated the activities of SOD and CAT while the activities of APX and GST, and AsA content and AsA/DHA ratio were decreased. Upon SNP addition, along with further rise in SOD and CAT activity, APX and GST activity, and levels of AsA and AsA/DHA ratio were restored considerably. Overall results revealed that significant accumulation of As suppressed growth, photosynthesis, APX and GST activities and decreased AsA content, hence led to the oxidative stress. However, the addition of SNP protected seedlings against As stress by regulating As accumulation, oxidative stress and antioxidant defense system.

1. Introduction

Arsenic (As) is a highly toxic metalloid and its contamination in soil and water has become an important environmental concern [1]. The potential sources of As in soil and water include oxidation of pyrite and use of phosphate fertilizers, herbicides and insecticides etc. [1]. In soil, As is found predominantly in two inorganic forms i.e. arsenate (As^{V}) and arsenite (As^{III}) depending on soil conditions – whether soil is aerobic or anaerobic, respectively [2]. Arsenate is an analog of phosphate; therefore it is readily absorbed by plants through high affinity phosphate transporters [3]. Studies showed that availability and phytotoxicity of As to plants are substantially determined by factors such as As concentration in soil, pH, soil properties like redox potential and drainage, As species, soil phosphorus content and plant species [4,5]. There are increasing evidences that As severely affects various physiological and biochemical processes of plants leading to the reduced yield [4–7] As is also known to affect photosynthetic pigments, membrane system of chloroplasts, chlorophyll fluorescence and RUBISCO enzyme thereby reducing photosynthetic activity [8,9]. Further, As has also been shown to alter nutrient balance and their assimilation, oxidative phosphorylation and protein metabolism [10]. Thus, As causes adverse effects on plants from molecular to whole plant level.

Nitric oxide (NO) is a gaseous free radical, which acts as inter- as well as intracellular signaling molecule and affects many

Abbreviations: F0, minimal fluorescence; Fv (Fm–F0), variable fluorescence in dark adapted leaves; Fv/Fm, maximum photochemical efficiency of PS II; Fv/F0, the activity of PS II; Fm/F0, electron transport rate through PS II; qP, photochemical quenching; NPQ, non-photochemical quenching; APX, ascorbate peroxidase; CAT, catalase; GST, glutathione-*S*-transferase; SOD, superoxide dismutase; AsA + DHA, total ascorbate; AsA, reduced ascorbate; DHA, dehydroascorbate; ROS, reactive oxygen species; SOR, superoxide radical; H_2O_2, hydrogen peroxide; MDA, malondialdehyde.

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physiological and biochemical processes under normal as well as in stress condition [11,12]. The studies show that exogenous application of NO as sodium nitroprusside (SNP) alleviates toxicity of various abiotic stresses such as salt [13], UV-B radiation [14], heat [15], cadmium [16], light [17] etc. Despite the availability of ample literature, the understanding of relationship between metal stress and NO interaction; and physiological and biochemical mechanisms related with NO-mediated alleviation of metal stress are still not well known.

Luffa acutangula (L.) Roxb. (sponge gourd) is a widely grown vegetable in different parts of India. It is rich in mineral and vitamins and thus a popular vegetable. There exists a possibility that if *Luffa* was grown in As polluted soil then it could too have adverse effects as discussed above. Therefore, it becomes necessary to investigate the rate of As accumulation and its effects on physiological and biochemical processes in *Luffa*. To ascertain the above facts, the present study was undertaken to investigate (i) As accumulation and physiological and biochemical responses of *Luffa* under As stress, and (ii) the mechanisms related to NO-mediated alleviation of As stress.

2. Results

2.1. Growth and As accumulation

Growth measured in terms of fresh weight and length of root and shoot declined significantly ($P < 0.05$) following As treatments (Fig. 1). Treatment of *Luffa* seedlings with 5 and 50 μM As resulted in 15 and 26% decline in fresh weight, respectively. Under similar As treatments, the decrease in the length of root and shoot was 12 and 21% and 9 and 16%, respectively hence roots experienced greater As-sensitivity. On the addition of SNP, 5 and 50 μM As showed a decline of 7 and 14% in fresh weight, 6 and 12% in root length and 4 and 8% in shoot length, respectively (Fig. 1). *Luffa* seedlings grown under 5 and 50 μM As treatments accumulated 26.7 ± 0.59 and 218.6 ± 5.02 μg As g^{-1} dry weight in root and 10.8 ± 0.22 and 62.4 ± 1.43 in shoot, respectively. However, with SNP: 5 μM As + SNP and 50 μM As + SNP, the metal accumulation was decreased significantly ($P < 0.05$) as there was 14.9 ± 0.36 and 116.9 ± 2.68 μg As g^{-1} dry weight in root and 3.2 ± 0.08 and 7.2 ± 0.17 μg As g^{-1} dry weight in shoot, respectively (Table 1).

Besides NO, SNP may also produce other residual products such as sodium cyanide, ferrocyanide, ferricyanide, sodium nitrite and sodium nitrate. To determine the possible role of these products, 100 μM of each was tested against As toxicity. Results reveal that none of these residual products could alleviate As toxicity on growth significantly (Fig. 2).

2.2. Photosynthetic pigments

Data pertaining to the photosynthetic pigments i.e. chlorophyll *a*, chlorophyll *b* and carotenoids are presented in Table 2. Arsenic at both the concentrations significantly ($P < 0.05$) declined Chl *a* and Chl *b* contents. The decrease in Chl *b* was greater than Chl *a* hence, higher Chl *a*/Chl *b* ratio was observed. Arsenic also reduced ($P < 0.05$) carotenoids content, however, impact was less than that of chlorophylls (Table 2). Arsenic induced effects on photosynthetic pigments were alleviated significantly ($P < 0.05$) in presence of SNP in nutrient medium (Table 2).

2.3. Photosynthetic performance

The results pertaining to changes in chlorophyll fluorescence parameters: Fv/Fm, Fv/F0, Fm/F0, qP and NPQ in presence of As alone and together with SNP are shown in Fig. 3. Arsenic at 5 and

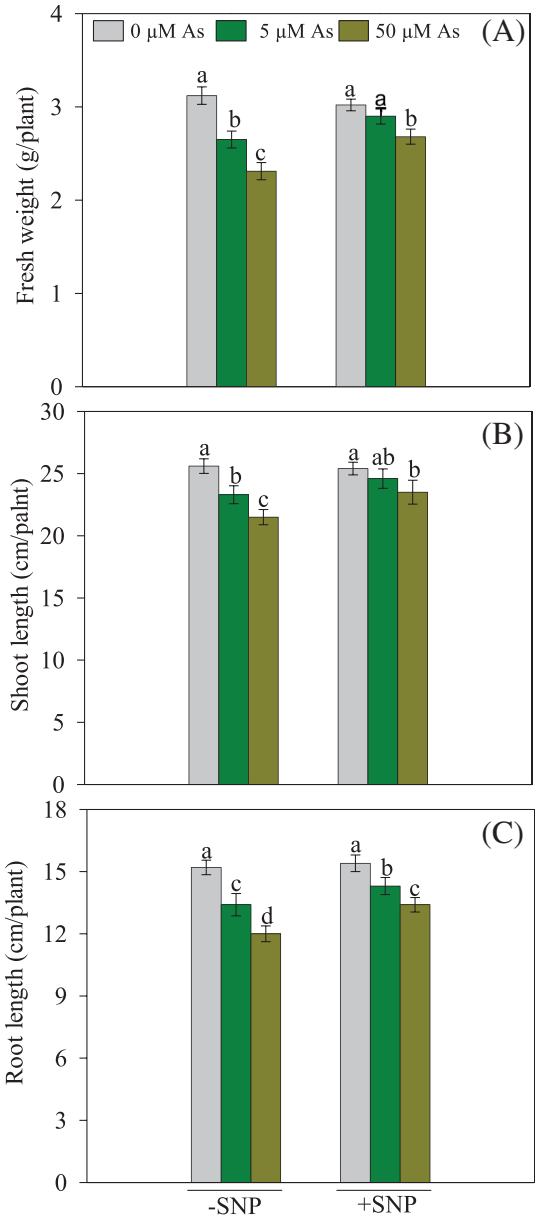

Fig. 1. Effect of NO on fresh weight (A), shoot length (B) and root length (C) of ridged *Luffa* seedlings under arsenic stress. Data are means ± standard error of six replicates. Bars followed by different letters show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test.

Table 1
Effect of NO on As accumulation in *Luffa* seedlings under As stress. Data are means ± standard error of six replicates. Values with different letters within same column show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test. nd = not detected.

Treatments	Arsenic content (μg As/g dry weight)	
	Root	Shoot
As (μM)		
0	nd	nd
5	26.7 ± 0.59b	10.8 ± 0.22c
50	218.6 ± 5.02d	62.4 ± 1.43d
As (μM) + SNP (100 μM)		
0 + SNP	nd	nd
5 + SNP	14.9 ± 0.36a	3.2 ± 0.08a
50 + SNP	116.9 ± 2.68c	7.2 ± 0.17b