

Effect of Sodium Arsenite and Arsenate on Growth and Pigment Content of Mungbean Seedlings

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Abstract Effect of sodium arsenite (NaAsO_2) and sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) on the growth and pigment content of mungbean (*Vigna radiata* (L.) Wilczek cv B-105) seedlings was investigated. With the increase in concentration of sodium arsenate and sodium arsenite (0.5 μM , 1 μM and 2 μM) significant decrease in seedling length i. e., shoot length and root length and primary leaf area was observed. Photosynthetic metabolism was markedly affected. Main pigments like chlorophyll a, b, total chlorophyll content, and carotenoid content of leaves reduced appreciably in the sodium arsenate and arsenite treated seedlings. Non cyclic electron transport or Hill activity of chloroplasts of leaves also reduced appreciably in the arsenical treated seedlings. Although sub lethal, 2 μM concentration proved to be most sensitive concentration for arsenic applica-

tion by giving least values for seedling length and pigment content. Sodium arsenite was found to be much more toxic than sodium arsenate in all respects.

Keywords *Vigna radiata*, Sodium arsenite, Sodium arsenate, Pigments, Hill activity.

Introduction

Arsenic pollution has gained at present an alarming global importance. Groundwater contamination by As has been reported from many countries. Arsenic (As) a ubiquitous, carcinogenic trace metalloid [1] found in virtually all environmental media [2]. Arsenic exists in the environment in various organic and inorganic and inorganic forms (species) [3]. The most important inorganic species are arsenate-As, (V) and arsenite -As (III) . Its toxicity to plants depends on its valence state. Due to greater cellular uptake, As III is reported to be much more soluble, mobile and toxic than As (V) [4].

Arsenic arrests growth in higher plants by destroying its photosynthetic machinery. It was found that dimethyl arsenic acid (DMAA) reduced net photosynthesis and photosynthetic capacity, leaf area and dry matter [5]. Reduction in net photosynthesis in

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rice plants caused by As toxicity has also been reported [6]. There are a number of studies investigating the effect of As on different plant species including rice [7, 8] but little work has been done on mungbean crops. leguminous plants are found to be highly sensitive to arsenic [9]. Pulse crop mungbean, is also an important crop and principal source of protein in Indian diet. The mature seeds of mungbean contain as high as 24% protein. Yield of mungbean is bound to suffer if as toxicity becomes prevalent in the productive cropland where it is grown.

There is insufficient information on the effect of arsenic pollution on photosynthetic machinery of young mungbean plants so, the objective of the present investigation was to study some physiological and biochemical parameters of the seedlings under As induced stress and to find out how sodium arsenate and arsenite disrupt photosynthetic machinery in mungbean seedlings.

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Materials and Methods

Plant material and arsenic treatments

Fresh, viable and uniform mungbean (*Vigna radiata* (L.) Wilczek cv B-105) seeds, collected from Bidhan Chandra Krshi Viswavidyalaya, Nadia, West Bengal, were treated with 0.1% w/v HgCl_2 solution for 2 min for surface sterilization and washed repeatedly with distilled water.

Two salts of arsenic viz. sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and sodium arsenite (NaAsO_2) (Merck) were used for arsenic treatment. Mungbean seeds were treated with arsenic salts at concentrations—0.5 μM , 1 μM and 2 μM for 5 days. Twenty seeds were placed in each petriplate of 9 cm diameter lined with double layered filter paper (Whatman No. 2). Freshly prepared 10 ml of treatment solu-

tion was applied to respective petriplate after washing the previous solution daily. The petriplates were kept in climatic room under controlled conditions of temperature ($25^\circ\text{C} \pm 2^\circ\text{C}$) and relative humidity (65%). Number of germinated seeds was counted daily and data were recorded after every 12 h for 5 days regularly. The set which received only distilled water for 5 days served as control. The above mentioned concentrations of As salts are comparable to soil conditions and are environmentally relevant.

Morphological studies

After 5 days, arsenate induced damaging effects were observed and root length and shoot length of growing mungbean seedlings were measured in all the sets. Seedling length of 20 seedlings were determined after excising the cotyledons and averaged. Estimation of leaf area (primary leaves) was carried out using graph paper. Data were collected from 20 plants at a time that were selected randomly from the same set and then averaged. Treatment with distilled water only for 5 days served as control. All the experiments were repeated 5 times and analyzed statistically.

Estimation of chlorophyll content

In order to understand the pigment status of the seedlings which is of utmost importance for photosynthetic process primary leaves of the seedlings of all the sets were collected after 5 days of growth and quantitative estimations of chlorophyll content (Chl *a* chl *b* and total chl) was done. Estimation of chlorophyll content was carried out by extracting the fresh green leaves twice with 80% alkaline acetone (containing 0.1M sodium carbonate) and centrifuged at $6000 \times g$ for 20 mins. After centrifugation, the combined supernatants were collected and a definite volume was made up with alkaline acetone for all the sets.

Chlorophyll was estimated spectrophotometrically according to the method of Arnon [10]. The optical density values of the clear supernatant containing leaf extracts in 80% alkaline acetone were

Table 1. Effect of various concentrations of sodium arsenate, As(V) and sodium arsenite, As (III) on shoot and root length and primary leaf area of 5 day old mungbean seedlings. Figures in parantheses are % decrease (–) over control. CD – Critical Difference.

Treatments	Shoot length (cm)		Root length (cm)		Primary leaf area (sq cm)	
	As (V)	As (III)	As (V)	As (III)	As (V)	As (III)
Control	12.5	12.5	4.5	4.5	1.20	1.20
0.5µM	7.0 (-44)	5.8 (-54)	1.5 (-67)	0.8 (-82)	0.82 (-25)	0.72 (-35)
1µM	4.7 (-62)	3.6 (-71)	0.8 (-82)	0.5 (-89)	0.64 (-42)	0.52 (-53)
2µM	2.4 (-81)	1.5 (-92)	0.4 (-91)	0.3 (-93)	0.40 (-64)	0.36 (-67)
SE (mean)	0.3	0.31	0.1	0.1	0.25	0.22
CD ($p=0.05$)	0.44	0.46	0.21	0.2	0.82	0.76

recorded in the Hitachi U-2000 spectrophotometer at 645 nm and 663 nm.

Estimation of carotenoid content and Hill activity

Estimation of carotenoid content (carotene and xanthophyll pigments) were carried out by extracting the fresh green leaves twice with 80% alkaline acetone (containing 0.1M sodium carbonate) and centrifuged at $6000 \times g$ for 20 mins. Carotenoid was estimated spectrophotometrically according to the method of Davies [11]. The optical density values for carotene and xanthophyll content were recorded in the Hitachi U-2000 spectrophotometer at 425 nm and 450 nm respectively and the data were expressed in terms of optical density (OD) per g fresh weight. Photosynthetic non-cyclic electron transport of isolated chloroplasts also known as Hill activity was measured spectrophotometrically according to Vishniac [12], using the artificial electron acceptor

dye 2,6-dichlorophenolindolphenol (DCPIP) under the light intensity of 7500 lux.

Statistical analysis

The experiments were carried out in a completely randomized design (CRD) with 5 replicates ; each replica comprising a single petridish containing an average of 20 seeds. All the experiments were analyzed statistically using ANOVA Table (and expressed in Critical difference at 5%)

Results and Discussion

Effect of arsenicals on the growth parameter of seedlings

From the present work, it is well established that both the arsenicals are very toxic for the growth of mungbean seedlings. These salts inhibited growth at very small concentration such as 0.5 µM. Roots were

Table 2. Effect of sodium arsenate, As (V) and sodium arsenite, As (III) on chlorophyll a chlorophyll b and total chlorophyll content (mg/g fresh weight) of leaves of 5 day old mungbean seedlings. Figures in parantheses are % decrease (–) over control. CD – Critical Difference.

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophyll	
	As (V)	As (III)	As(V)	As (III)	As (V)	As (III)
Control	0.66	0.66	0.36	0.36	1.02	1.02
0.5µM	0.43 (-35)	0.32 (-51)	0.22 (-39)	0.18 (-50)	0.65 (-36)	0.50 (-51)
1µM	0.33 (-50)	0.23 (-65)	0.14 (-61)	0.12 (-67)	0.47 (-54)	0.35 (-66)
2µM	0.14 (-79)	0.10 (-85)	0.08 (-78)	0.06 (-83)	0.22 (-78)	0.16 (-84)
SE (mean)	0.02	0.02	0.02	0.02	0.03	0.03
CD ($p=0.05$)	0.15	0.13	0.061	0.052	0.12	0.11

characteristically stubby and brittle and root tips gradually turned brown. In the present work, length of mungbean seedlings demonstrated an inverse relationship to the applied molar concentrations of sodium arsenate and arsenite. Inhibition of elongation of mungbean seedlings started at a concentration of 0.5 μ M and it was remarkably pronounced at 2 μ M (Table 1). The inhibition increased at higher doses, the effect being more pronounced on root than shoot. Present investigation reveals that Arsenic is more toxic for root growth than for shoot growth. Similar reports regarding roots were also noted in alfalfa, lettuce and wheat, rice and other plants due to As toxicity [6,13]. The applied concentrations of sodium arsenate were found to have significant effect on the area of pair of primary leaves. Maximum reduction in leaf area occurred due to the highest concentration of sodium arsenate and sodium arsenite (2 μ M) and there was a stepwise reduction of leaf area with increasing concentrations of sodium arsenate and sodium arsenite respectively. Simultaneous reduction of leaf area of the treated seedlings is naturally a consequence of inhibition of cell division and cell enlargement [14].

Effect of arsenicals on total chlorophyll content

Sodium arsenate and arsenite have a drastic effect on seedling growth and photosynthetic machinery of germinating mungbean seedlings. Different concentrations of sodium arsenite and arsenate were found to have significant effect on chlorophyll *a* and chlorophyll *b* content. Total chlorophyll content of mungbean seedlings declined with increasing con-

centrations of sodium arsenate and sodium arsenite in comparison to the control (Table 2). Decrease in chlorophyll content may be related to reduced photosynthesis in As toxicated mungbean seedlings. Reduction in net photosynthesis in rice plants caused by As toxicity has been reported earlier [5, 6]. Arsenic may be functional in blocking either the synthesis or the activity of enzyme proteins responsible for chlorophyll biogenesis. Maximum decrease in total chlorophyll content was observed under the influence of maximum concentration -2 μ M of sodium arsenate (78%) and arsenite (84%) in comparison to control. Due to reduced photosynthesis, decline in reducing sugar content was observed with higher concentrations of As (V) in mungbean seedlings [15].

Effect of arsenicals on carotenoid content and Hill activity

The carotenoid pigment content in the leaves of treated seedling decreased with increasing concentrations of sodium arsenate and arsenite as shown in Table 3. An appreciable reduction of carotenoid content of the mungbean seedlings was found due to sodium arsenite in comparison to sodium arsenate. It is pertinent to suggest that decrease in the amount of the carotenoids in the present work, accelerates the decaying of the chlorophylls. Again, reduction in chlorophyll content may be related to reduced photosynthesis in As toxicated mungbean seedlings. Carotenoid contents were also reduced in bean plants (*Phaseolus vulgaris* L.) due to arsenic toxicity [16].

A stepwise reduction of Hill activity was evi-

Table 3. Effect of sodium arsenate, As (V) and sodium arsenite, As (III) on carotene content (OD/g fresh weight) xanthophyll content (OD/g fresh weight) and Hill activity (μ mole DCPIP reduced/g Chl/h) of leaves of 5 day old mungbean seedlings. Figures in parantheses are % decrease (-) over control. CD – Critical Difference.

Treatments	Carotene content		Xanthophyll content		Hill activity	
	As (V)	As (III)	As (V)	As (III)	As (V)	As (III)
Control	0.98	0.98	0.48	0.48	1.62	1.62
0.5 μ M	0.76 (-22)	0.70 (-29)	0.36 (-25)	0.31 (-35)	0.84 (-48)	0.74 (-54)
1 μ M	0.64 (-35)	0.61 (-38)	0.24 (-50)	0.20 (-58)	0.68 (-58)	0.62 (-62)
2 μ M	0.44 (-55)	0.40 (-59)	0.18 (-62)	0.16 (-67)	0.44 (-73)	0.40 (-75)
SE (mean)	0.02	0.02	0.01	0.01	0.02	0.02
CD ($p=0.05$)	0.062	0.064	0.038	0.035	0.071	0.071

dent due to increasing concentrations of sodium arsenate and arsenite in comparison to the control (Table 3). In this context, the metalloid, As may be assumed to have caused impaired development and general depression of photosystem II reactions and O₂ evolving centers. Results obtained from arsenic treated sets signify the possible damage to non-cyclic electron transport. Arsenicals probably inhibit the photolysis of water with simultaneous decrease in the rate of electron flow from excited chlorophyll molecules to the artificial electron acceptor dye DCPIP thus resulting in the decline of the Hill activity was also noticed. With higher concentration of As (V) also, reduction in Hill activity was also noticed which led to poor sucrose metabolism due to damaged photosynthetic apparatus of the mungbean seedlings [17].

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