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Effect of sodium arsenite, As (III) toxicity on photosynthetic metabolism and carbohydrate content of mungbean seedlings

Arpita Swarnakar
Department of Botany, Bangabasi College, University of Calcutta, Kolkata, West Bengal, India

Abstract
Sodium arsenite ($NaAsO_2$) is a potent inhibitor of germination and growth of mungbean (*Vigna radiata* (L.) Wilczek cv. B-105) seedlings. With the increase in concentration of sodium arsenate (0.5µM, 1µM and 2µM) significant decrease in seedling length and primary leaf area was observed. Photosynthetic metabolism was markedly affected. Photosynthetic pigments like Chlorophyll a, b, total chl. content, carotenoid content and non cyclic electron transport or Hill activity of chloroplasts reduced appreciably in the sodium arsenite treated seedlings. Perturbations in carbohydrate synthesis were evidenced by the reduction in reducing sugar and non-reducing sugar content of the seedlings whereas the starch content was found to be elevated. Thus, Arsenite induced stress leads to lesser growth and development of the important pulse crop, Mungbean.

Keywords: sodium arsenite, germination, pigment, sugar, *Vigna radiata*

1. Introduction

Arsenic (As) pollution has gained at present an alarming global importance. Groundwater contamination by As has been reported from many countries. Among many countries in different parts of the world affected by groundwater arsenic contamination, the largest population at risk is in Bangladesh followed by our state, West Bengal in India. Arsenic is known to have many toxic effects in humans and is ranked first, in the priority list of hazardous substances compiled by the US Environmental Protection Agency (USEPA) [1]. Study shows that, besides groundwater, food is also an important pathway of arsenic in to human system [2]. Arsenic enters in food chain directly by drinking contaminated water or by crops growing on arsenic contaminated soil.

Arsenic arrests growth in higher plants by destroying its photosynthetic machinery. Plants grown in presence of arsenite show reduced seed germination and growth [3]. It was found that dimethyl arsenic acid (DMAA) reduced net photosynthesis and photosynthetic capacity, leaf area and dry matter [4]. Reduction in net photosynthesis in rice plants caused by As toxicity has also been reported [5]. In rice seedlings arsenic toxicity also caused perturbations in carbohydrate metabolism leading to accumulation of soluble sugars by altering enzyme activity [6]. Due to greater cellular uptake, As III is much more soluble, mobile and toxic than As (V) [7]. Leguminous plants are found to be highly sensitive to arsenic [8]. Pulse crop Mungbean, is also an important crop and principal source of protein in Indian diet. Yield of Mungbean is bound to suffer if As toxicity becomes prevalent in the productive crop land where it is grown. There is insufficient information on the effect of arsenite on photosynthetic machinery of young mungbean plants. So, the objective of the investigation was to study the physiological and biochemical parameters of the mungbean seedlings regarding photosynthetic and carbohydrate metabolism due to stress induced by As (III) toxicity.

2. Material and Methods

2.1 Plant material and Arsenite treatments

Fresh, viable and uniform mungbean (*Vigna radiata* (L.) Wilczek cv. B -105) seeds, collected from Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, were treated with 0.1% w/v $HgCl_2$ solution (Merck, India) for 2 min for surface sterilization and washed repeatedly with distilled water. In order to study the effect of Sodium arsenite on the germination of Mungbean, the seeds were placed in petriplates of 9 cm diameter lined with double layered filter paper (Whattman No. 2). Sodium arsenite (Merck, India), $NaAsO_2$, was used as test solution at concentrations – 0.5µM, 1µM and 2µM. Germination parameters were studied each day with fresh test solutions to calculate germination percentage. Germination percentage (GP) is an estimate of the viability of a population of seeds. A seed was considered as germinated when 2 mm of radicle had emerged out of seed coat. Germination percentage was calculated according to the given formula [9].

$$\text{Germination percentage} = (\text{Germinated seeds} \div \text{Total seeds}) \times 100$$

For studying the effect of As (III) on morphological parameters, about twenty seeds were placed in petriplates and Sodium arsenite, $NaAsO_2$, was used as test solution at concentrations – 0.5µM, 1µM and 2µM. The petriplates were kept in climatic room under controlled conditions of temperature (25 ºC ± 2 ºC) and relative humidity (65%). The seeds soaked in distilled water served as control. The pH of the solution was 6.5 throughout. The above mentioned arsenite concentrations are comparable to soil conditions and are environmentally relevant. All the experiments were repeated 5 times and analyzed statistically.

2.2 Morphological studies

After 5 days, seedling length was observed and root length and shoot length of growing mungbean seedlings were measured in all the sets. Seedling length of 10 seedlings were determined after excising the cotyledons and averaged. Estimation of leaf area of a seedling was carried out by removing the first pair of leaves (primary leaves) followed by sketching the boundary of each leaf on a graph paper. By counting the number of squares with in the boundary, the leaf area was expressed in terms of sq. mm. Data were collected from 10 plants at a time which were selected randomly from the same set and then averaged.

2.3 Quantitative Estimation of Photosynthetic Pigments

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 total chlorophyll content and carotenoid content was done. Estimation of chlorophyll, 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×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 spectrophotometric ally according to the method of Arnon [10]. The optical density values of the clear supernatant containing leaf extracts in 80% alkaline acetone were recorded in the Hitachi U-2000 spectrophotometer at 645 nm and 663 nm. The chlorophyll contents were expressed in terms of mg chlorophyll present per gram fresh tissue. 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×g for 20 mins. Carotenoid was estimated spectrophotometric ally [11]. The optical density values of were recorded in the Hitachi U-2000 spectrophotometer at 425 nm and 450 nm and the data were expressed in terms of optical density per g fresh leaf. Photosynthetic non-cyclic electron transport of isolated chloroplasts also known as Hill activity was measured spectrophotometric ally [12], using the artificial electron acceptor dye 2, 6-dichlorophenolindolphenol under the light intensity of 7500 lux.

2.4 Quantitative estimation of Carbohydrate Content

In order to understand the carbohydrate metabolism of the seedlings which is required for normal growth and development, seedlings of all the sets were collected after 5 days of growth and quantitative estimations of reducing sugar content, non-reducing sugar content and starch content were done. The amount of total soluble sugar was estimated by phenol sulphuric acid reagent method [13]. The estimation of reducing sugar was made by copper reduction method [14]. This value includes hexoses and non-fermentable reducing substances. The amount of non- reducing sugar was measured by subtracting the value of reducing sugar from the value of total soluble sugar. Estimation of starch was done by the method of McCready *et al.* [15].

2.5 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).

3. Result and Discussion

3.1 Effect of Sodium Arsenite on the Growth of Seedlings

Sodium arsenite has a drastic effect on seedling germination and growth of mungbean seedlings. The applied concentrations of sodium arsenite were found to have significant effect on the percentage of germination of Mungbean seedlings. Percentage of seed germination was found to decrease significantly with increasing concentrations of arsenite. Minimum seedling germination (22%) was recorded with 2µM concentration of As (III) (Table 1). Sodium arsenite posed more stress on germination percentage, as inorganic As (III) is more toxic than As (V) [16]. Considerable decrease in germination percentage was recorded with increasing level of arsenic in sunflower [17], where higher concentrations of heavy metals suppressed the seed germination parameters.

Seedling length including both epicotyl and shoot and hypocotyl and root demonstrated an inverse relationship to the applied molar concentrations of Sodium arsenite. Inhibition of elongation of mungbean seedlings started at 0.5µM (lowest dose of as used here), became much more pronounced at1µM (intermediate dose) and was drastic at 2µM (Table 1). Present investigation shows that Arsenic is more toxic for root growth than for shoot growth (Table 1). Similar reports regarding roots were also noted in alfalfa, lettuce and wheat, rice and other plants due to as toxicity [5, 18]. Reduction of leaf area of primary leaves was also noted due to the highest concentration of sodium arsenite, 2µM and there was a stepwise reduction of leaf area with increasing concentrations of sodium arsenite. Simultaneous reduction of leaf area of the treated seedlings is naturally a consequence of inhibition of cell division and cell enlargement [19]. With the increase in concentration of Sodium arsenate also, primary leaf area of mungbean was reduced considerably along with water content of the seedlings [20]. Along with reduction in primary leaf area simultaneous decrease in total number of stomata or Stomatal Index was also noticed due to As (III) toxicity.

Table 1: Effect of various concentrations of Sodium arsenite, As (III) on shoot and root length and primary leaf area of 5 day old mungbean seedlings. Figures in paranthesis are % decrease (-) over control. C.D. - Critical Difference.

Treatment	Germination percentage (GP)	Shoot length (cm)	Root length (cm)	Seedling length	Primary leaf area (sq.cm)
Control	100	12.5	4.5	17	1.20
0.5µM,As(III)	48	5.8(-54)	0.8 (-82)	6.69(-61)	0.72 (-35)

1μM,As(III)	30	3.6(-71)	0.5 (-89)	4.1(-76)	0.52 (-53)
2μM,As(III)	22	1.5(-92)	0.3 (-93)	1.8(-89)	0.36 (-67)
S.E.(mean)	3	0.31	0.1	0.4	0.22
C.D. (P=0.05)	0.44	0.46	0.2	0.51	0.76

3.2 Effect of Sodium Arsenite on Pigment Content

Sodium arsenate was found to have significant effect on pigment status and thereby photosynthetic efficiency of mungbean seedlings. There was a linear decrease in the levels of total chlorophyll, carotenoids (carotene and xanthophylls) under increasing concentrations of sodium arsenite (Table 2). 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 treated mungbean seedlings. Reduction in net photosynthesis in rice plants caused by Arsenic toxicity has been reported earlier [5]. Arsenic is assumed to 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 of sodium arsenite used, 2μM which accounted for 84% reduction over control.

A stepwise reduction of Hill activity and possible damage to non-cyclic electron transport was evident due to increasing concentrations of sodium arsenite in comparison to the control (Table 2). In this context, the metalloid, as may be assumed to have caused impaired development and general depression of photosystem II reactions and O_2 evolving centres. From the present work, it is apparent that As (III) used here reduced the photolysis of water with simultaneous decrease in the rate of electron flow from excited chlorophyll molecules to the artificial electron acceptor dye DCPIP as evidenced by the rate of enhancement of DCPIP. With higher concentration of arsenate also, reduction in Hill activity was noticed which led to poor sucrose metabolism due to damaged photosynthetic apparatus of the mungbean seedlings [20].

Table 2: Effect of Sodium arsenite, as (III) on photosynthetic pigments- chlorophyll a, Chl. b, Hill activity and Carotenoids content of 5 day old mungbean seedlings. Figures in paranthesis are % decrease (-) over control. C.D. - Critical Difference

Treatment	Chl a (mg /gfwt.)	Chl b (mg /gfwt.)	Total Chl (mg / gfwt.)	Carotene content ($A_{425}g^{-1}$f wt)	Xanthophyll content ($A_{450}g^{-1}$f wt)	Hill activity
A)Control	0.66	0.36	1.02	0.98	0.48	1.62
0.5μM, As(III)	0.32(-51)	0.18(-50)	0.50(-51)	0.70(-29)	0.31(-35)	0.74(-54)
1.0μM, As(III)	0.23(-65)	0.12(-67)	0.35(-66)	0.61(-38)	0.20(-58)	0.62(-62)
2.0μM, As(III)	0.10(-85)	0.06(-83)	0.16(-84)	0.40(-59)	0.16(-67)	0.40(-75)
S.E.(mean)	0.02	0.02	0.03	0.02	0.01	0.02
C.D. (P=0.05)	0.13	0.052	0.11	0.064	0.035	0.071

3.3 Effect of Sodium arsenite on carbohydrate metabolism

The main photosynthate of plants-carbohydrate metabolism of germinating mungbean seeds was immensely affected by Arsenite. Reducing sugar content of mungbean seedlings showed a declining trend with increasing concentrations of sodium arsenite (Table 3). Reduction in reducing sugar content can be easily correlated with growth inhibition. This could be an effect of reduced leaf area, photosynthetic pigment content and ultimately lesser photosynthetic rate due to as toxicity. It is also to be noted that lower level of reducing sugar in the treated plants finds correlation with lower metabolic status in these plants. Decline in reducing sugar content might also be due to reduced α-amylase activity, which resulted in reduced hydrolysis of reserve polysaccharides [21]. However, concomitant with the increase in concentration of arsenate, starch content of the seedlings were found to be increasing. It may be assumed that mungbean seedlings under arsenate toxicity accumulated more of starch as reserved polysaccharide and for osmotic adjustment. Elevated levels of starch content have also been reported in arsenate treated rice seedlings [22]. The perturbation of carbohydrate level under arsenate stress condition might have impaired the growth and metabolism of mungbean seedlings which is also relevant from the morphological data.

Table 3: Effect of Sodium arsenite, As (III) on Reducing sugar content, non-reducing sugar content and Starch content of 5 day old mungbean seedlings. C.D-Critical Difference.

Treatment	Reducing sugar content (mg/g f wt)	Reduction (%)	Non-Reducing sugar content (mg/g f wt)	Reduction (%)	Starch content (mg/g f wt)	Promotion (%)
A)Control	3.78	-	5.14	-	78	-
0.5μM As(III)	2.14	43	3.04	41	81	4
1μM As(III)	1.10	71	2.25	56	84	8
2μM As(III)	0.86	77	1.56	70	89	14
S.E.(mean)	0.04	-	0.04	-	3.5	-
C.D (P=0.05)	0.41	-	0.45	-	7.86	-

4. Conclusion

Arsenic has an inhibitory, toxic effect on growth, metabolism and thereby, productivity of plants. Sodium arsenite affects the seed germination and early growth parameters of mungbean seedlings, particularly the root growth. Arsenite alters the morphological and physiological characters of the seedling which ultimately leads to stunted growth and death. Arsenite also damaged the photosynthetic machinery by causing reduced chlorophyll and carotenoid contents and also led to perturbations in carbohydrate metabolism. Arsenite is much more toxic than arsenate. The concentration of Arsenic contaminated water in polluted sites often exceeds the concentration used in the present research work. This has practical importance and is of much concern in agricultural systems, as it hampers plants early development and leads to lesser productivity of crops.

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6. References

1. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Arsenic. Georgia, 2011, 500
2. Norra S, Berner ZA, Agarwala P, Wagner F, Chandrasekharam D, Stüben D. Impact of irrigation with As rich groundwater on soil and crops: a geochemical case study in West Bengal Delta Plain, India. App. Geochem. 2005; 20(10):1890-1906.
3. Rahman MM, Rahman MA, Maki T, Hasegawa H. Phytotoxicity of arsenate and salinity on early seedling growth of rice (*Oryza sativa* L.): A threat to sustainable rice cultivation in South and S E Asia. Bull. Environ. Contam. Toxicol. 2012; 88:695-702.
4. Marin AR, Pezeshki SR, Masschelen PH, Choi HS. Effect of dimethyl arsenic acid (DMAA) on growth, tissue arsenic and photo synthesis of rice plants. J Plant Nutr. 1993; 16(5):865-880.
5. Azizur RM, Hasegawa H, Rahman MM, Islam MN, Miah MAM, Tasmen A. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere. 2007; 67:1072-1079.
6. Jha AB, Dubey RS. Carbohydrate metabolism in growing rice seedlings under arsenic toxicity, J Plant Physiol. 2004; 161:867-872.
7. Giri AK, Patel RK. Phytoaccumulation potential and toxicity of arsenic ions by *Eichhornia crassipes* in hydroponic system. J Bioremed. Biodegrad. 2012; 3:137.
8. Adriano DC. Arsenic. Trace Elements in the Terrestrial Environment. Springer-Verlag, New York, 1986, 46-72.
9. Tanveer A, Rehman A, Javaid MM, Abbas RN, Sibtain M, Ahmad A, *et al*. Allelopathic potential of *Euphorbia helioscopia* L, against wheat (*Triticum aestivum* L.) chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medic.). Turk. J Agric. For. 2010; 34:75-81.
10. Arnon DI. Copper enzyme in isolated chloroplasts. Polyphenol Oxidase in *Beta vulgaris.* Plant Physiol. 1949; 24:1-15.
11. Davies BH. Analysis of Carotene pigments. Chemistry and Biochemistry of Plant Pigments. Academic Press. New York.1965; 489-531.
12. Vishniac W. Methods for the study of Hill reaction. Methods of Enzymology. Academic press. New York. 1957; 4:342-355.
13. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956; 28:350-356.
14. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing Sugars. Anal. Chem. 1972; 31:426-428.
15. McCready RM, Guggolz J, Silviera V, Owens HS. Determination of starch and amylase in vegetables. Anal. Chem. 1950; 31:426-428.
16. Winkel L, Berg M, Amini M, Hug SJ, Johnson CA. Predicting ground water arsenic contamination, in S E Asia from surface parameters. Nature Geosci. 2008; 1:536-542.
17. Ahmad MSA, Hussain M, Ashraf M, Ahmad R, Ashraf MY. Effect of nickel on seed germ inability of some elite sunflower (*Helianthus annuus* L.) cultivars. Pak. J Bot. 2009; 41(4):1871-1882.
18. Rofkar RJ, Dwyer DF. Effects of light regime, temperature and plant age on uptake of arsenic by *Spartina pectinata* and *Carex stricta*. International J of Phyto. 2011; 13:528-537.
19. Zidan I, Azaizen II, Neuman PM. Does salinity reduce growth in maize roots epidermal cells by inhibiting their capacity for cell wall acidification? Plant Physiol. 1990; 93:7-11.
20. Swarnakar A. Enhancement Effect of Gibberellic Acid and Kinetin on Sucrose metabolism in mungbean seedlings under Arsenate toxicity. American J of Biosc. Bioengg. 2017; 5(1): 50-55.
21. Swarnakar A. Relative toxicity of Sodium Arsenite on seed germination and development of Mungbean seedlings. Indian Biologist. 2017; 49(1):9-13.
22. Zaidi PH, Singh BB. Effect of growth regulators on IAA-oxidase and Peroxidase activity in soybean under salinity. Indian J Plant Physiol. 1995; 38(4):337-339.
23. Choudhury B, Chowdhury S, Biswas AK. Regulation of growth and metabolism in rice (*Oryza sativa* L.) by arsenic and its possible reversal by phosphate. J Plant Interact. 2011; 6:15-24.