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Changes in pigments and photosynthetic parameters of cowpea under two inorganic arsenicals

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Abstract: *A net house experiment was conducted to investigate the relative effects of two inorganic forms of arsenic namely, arsenate (As V) and arsenite (As III), on some physiological parameters in leaves of cowpea (Vigna unguiculata L. Walp) cv. BCKV-1. Cowpea plants were grown in pot culture and were treated with different doses of As (V) and As (III) as 0 (control) 10, 20, 30 and 40 mg L^{-1}. The application of arsenic in either form showed an adverse effect on pigment and protein contents, net photosynthesis, transpiration rate, stomatal conductance especially at higher doses of 30 and 40 mg L^{1}. A higher reduction in photosynthetic pigments, protein, transpiration rate and stomatal conductance were recorded in As (III) treatments than As (V), while net photosynthetic rate and intrinsic water use efficiency (IWUE) were most severely affected in As (V).*

Key words**:** *Arsenate, arsenite, cowpea, photosynthesis, pigments*

I. Introduction

Arsenic, a widespread metalloid, may play an essential role in animal nutrition [1] possibly through methionine metabolism, but is generally considered as a highly toxic and non-bioorganic element to plants and animals and occurs naturally in the environment through geological activities as well as many anthropological activities like use of various arsenical pesticides, wood preservatives, industrial waste and growth promoter for plants and animals [2]. Arsenic toxicity in humans has recently received increasing attention due to large scale contamination in regions such as Bengal delta basin bound by rivers Bhagirathi and Padma and northwest China [3]. However, main focus of attention, until recently, was devoted almost exclusively on arsenic contaminated drinking water. However, it is now well known that soil arsenic level is building up gradually due to continuous and massive lifting of contaminated ground water for crop-irrigation purposes. These arsenic enriched soils are now being considered as major sources of contamination in the food chain and water supplies and this is of great environmental concern because arsenic is known to be a potential carcinogen and mutagen [4]. Soil arsenic levels are very much related with local well water arsenic concentration which suggests that source of soil contamination in the irrigation water [5]. Thus use of arsenic contaminated groundwater through irrigation creates hazards to both in soil environment and in crop quality. The presence of excessive heavy metals and/ or metalloids in the soil can pose dual problem: contamination in the food-chain through the intake of contaminated harvested crops and yields are reduced due to adverse effect on plant growth [6,7]. Twenty percent loss of crop (cereal) production due to high concentration (20ppm) arsenic in plant body has been reported [8].

In terrestrial plants, both organic and inorganic arsenic species have been found [9,10], with the inorganic species, arsenate [As (V)] and arsenite [As (III)] being the most dominant. Plants take up arsenic mainly as arsenate via the phosphate uptake system [11,12], whereas arsenite (As^{III}) is actively taken up via a glycerol-transporting channel which transport water and neutral solutes in bacteria, fungi, plants and animals called as aquaporins, in the roots [13,14]. Arsenic is analogous to phosphorus; both have similar electron configurations and chemical properties and compete for the same uptake carriers in the root plasmalemma [15]. Once inside the cytoplasm, arsenate and arsenite react in different ways. For instance, arsenate competes with phosphate and replaces phosphate in ATP to form unstable ADP-As and leads to disruption of energy flows in cells [16] whereas arsenite causes inactivity of enzymes particularly having sulphydryl (-SH) group. Though arsenic is not a redox metal yet there is significant evidence that exposure of plants to inorganic arsenic results in the generation of reactive oxygen species (ROS), which are connected with the valence changes that the element readily undergoes from arsenate to arsenite in plants [15]. The ROS, such as superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet -}$) and hydrogen peroxide (H_2O_2), are strong oxidizing agents that cause oxidative damage to biomolecules such as lipids and proteins and eventually cause cell death [17,18]. Exposure to arsenic As causes damage to cellular membranes and therefore leakage of electrolyte [19]. Arsenic inhibits the growth together with fresh and dry biomass production [20] and causes physiological disorders [21], as well as

reduction in photosynthetic rate [22] and crop productivity [23]. Arsenic causes damage to the chloroplast membrane of the leaves, the most essential organ for the manufacture of photo-assimilate as well most sensitive part of plant to stress, and disorganized the membrane structure. The damages of chloroplasts structure during the treatments with high arsenic level imply functional changes of the integrated photosynthetic processes.

However, the comparative physiological responses of plants to inorganic arsenicals (arsenite and arsenate) are very scanty. The objective of this study was to investigate the effect of arsenate and arsenite on photosynthesis and its apparatus in leaves of cowpea. Cowpea is an important and popular crop grown all over the world. In India it is being cultivated since the Vedic period. The entire plant is of economic importance, the pods harvested are rich in protein, while the foliage is used as green manure and fodder for animals.

II. Materials And Methods

The experiment was carried out with cowpea (*Vigna unguiculata* (L). Walp) cv. BCKV-1 grown in a net house. The net house was used only to protect the experiment from some unwanted disturbances. Therefore, the conditions inside the net house did not differ from those outside. Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for three minutes followed by thorough washing in glass-distilled water, and then soaked in arsenate and arsenite solutions of following concentrations as 0 (glass distilled water as control), 10, 20, 30, 40 mg L^{-1} for four hours. Sodium arsenate (Na_2HAsO_4, $7H_2O$) and arsenic trioxide (As_2O_3) were used as sources of arsenate (As V) and arsenite (As III), respectively. Seeds were then placed in earthen pot packed with 5 kg of alluvial soil and compost (3:1 ratio). Then 250 ml solution of above concentrations was given in each pot kept in net house. Untreated pots were maintained as control (added with 250ml glass distilled water) to compares the results. The plants were irrigated with tap water as and when needed. Plants were analyzed for all parameters after 35 days of sowing.

Biochemical analysis: Chlorophylls and carotenoids contents in the leaves were extracted by 80% (v/v) acetone following percolation method of Hiscox and Israelstam [24] and determined spectrophotometrically at 646 nm, 663 nm (chlorophylls) and 470 nm (carotenoids), after extraction had been completed as indicated by discoloration of leaf samples. The amount of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids were calculated according to the Lichtenthaler and Wellburn [25] formulae.

Protein content in the extract was determined according to Lowry *et al*. [26]. Protein was precipitated with 20% (w/v) chilled TCA and the mixture was allowed to stand for atleast one hour at 4^0C in a refrigerator. The contents were centrifuged and the supernatant was discarded. The residue was washed with glass distilled water to remove TCA. The water washed residue was treated with 80% (v/v) acetone to remove pigments. The clean residue was dissolved in 0.1N NaOH at 80^0C in a water bath for 10 min. After centrifugation a suitable aliquot was drawn and reacted with 0.5 ml Folin Ciocalteu Reagent (FCR). The optical density of the mixture was measured at 640 nm. The readings were referred to standard curve prepared from crystalline Bovin serum albumin and the concentration of protein was expressed as mg/g fresh weight of tissue.

Photosynthetic parameters: The net photosynthesis rate (Pn), transpiration rate (E) and stomatal conductance (C) of the youngest, fully developed intact leaves were measured with a CI-340 portable photosynthesis system (CID Inc., USA). The measurements were made at ambient CO_2 concentrations between 09:00 and 11:00 h on a clear sky day. Intrinsic water use efficiency (IWUE) was computed by using the data available (Pn/C). Leaf gas exchange was measured on a mature, but not senescent leaf in the middle of plant with a maximum PAR of 1080 µmol $m^{-2}s^{-1}$ in the leaf cuvette of the analyzer.

Statistical analysis: The analyses were done in three replications for each treatment. The results shown in tables 1-2 are mean values (± SD) of 27 plants. Experimental data were analyzed statistically by using the windows-based SPSS 12.0 package at 95% significance level.

III. Results And Discussion

The results from table 1 show that chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids contents in the leaves decreased gradually with increase in both arsenate and arsenite stresses. Chlorophyll to carotenoids ratio (chl/car) was found to be higher in all the arsenate as well as arsenite treatments, compared to control. The reductions in photosynthetic pigments were higher in As (III) treatments as compared to As (V). The chlorophyll to carotenoids ratio (chl/car) was also higher in arsenite treated plants than that in the arsenate treated plants.

The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthesis rate. The decrease in photosynthetic pigments under both arsenate and arsenite treatments may be due to their toxic effect and it is the sign of absence of adaptive adjustments of pigment synthesis to high arsenic levels. Stancheva *et al.* [27] reported that the photosynthetic pigments are some of the receptor points of the toxic arsenic effect. A reduction of the pigment content in the case of increasing levels of heavy metals and metalloids was also established by Rahman *et al*. [28]. The lower amounts of photosynthetic pigments in the arsenite treatments might be due to more toxicity of this form than that of the arsenate [29].

There is significant evidence that exposure to inorganic arsenic species results in the generation of reactive oxygen species. This probably occurs through the conversion of arsenate to arsenite, a process which readily occurs in plants. So the lower ratio (chl/car) in arsenate than arsenite treated plants may be due to the fact that there is an oxidative stress, which is a marker of the tissue ageing, as a result of the stress factors of the environment [30]. The higher ratio of (chl/car) in arsenic treated plants than in control might be due to lower degradation of chlorophyll which could be attributed to induced anti-oxidative activities under arsenic stress.

The total soluble protein content was found to decrease with the increase in both arsenate and arsenite levels in the growing media (Table 1). It was comparatively lower in arsenite than the corresponding arsenate treated plants. The reduced amount of soluble protein content in the leaves of arsenic treated plants was most probably a result of the reduced biosynthesis or accelerated catabolic processes, as well and these results are in confirmation with the earlier report on arsenic toxicity as observed in spinach [31]. The soluble protein content in plant cells is an important indicator of their physiological state. The protein degradation to amino acids is in fact an adaptation of the cells to the carbohydrate deficiency under heavy metal stress [32].

Table 2 shows that leaf photosynthetic activity was markedly reduced than in control in both arsenate and arsenite treated plants. However, the reduction in photosynthetic activity was more severe in arsenate than it was with arsenite. The data (Table 2) also revealed that both transpiration ratio and stomatal conductance were reduced under arsenic treatments. Intrinsic water use efficiency (IWUE), measured in terms of net photosynthesis to stomatal conductance ratio (Pn/c), and was also decreased with the increasing arsenic stresses. The (Pn/C) ratio recorded a lower value under arsenic treatments than in control in most of the cases. IWUE was measured in the sense that it had been recognized as a measure of carbon gain per unit of water loss and is inversely proportionate to the ratio of intercellular and environmental CO_2 concentrations [33]. It is evident that a higher IWUE and a higher photosynthetic rate can improve yield under stress [33,34].

The reduction in transpiration intensity and stomatal conductance was more in arsenite than arsenate treatments, whereas IWUE was more severely affected under arsenate treatments. The reduced photosynthetic rate can be due to many factors. The photosynthetic reactions are closely related with stomatal behaviour (through diminishing or cessation of CO_2 uptake) and others to thylakoid (through photosynthetic electron transport, ATP synthesis). The insufficient water supply to tissues may induce photo-inhibition, but in some cases plants prevent this by decreasing the rate of electron transport, as a result of both photosystem I (PSI) and photosystem II (PSII) activity [35].

The decreased photosynthesis rate under stress conditions could be due to both stomatal and mesophyll limitations. The mesophyll factors could be of different nature, such as disturbances in the pigment apparatus, light and biochemical reactions from the Calvin Cycle. The process of photosynthesis is found to be affected by arsenic stress [20, 36] and other stresses like drought [37]. The greater reduction in photosynthesis rate under As (V) treatment could be attributed to higher disturbances in the pigment apparatus by a higher level of reactive oxygen species (ROS) generated under systems predominated with arsenate compared to arsenite. The negative effect of arsenic on the transpiration rate, stomatal conductance as well as IWUE could probably be the result of the disturbed uptake caused by malfunctioning in stomatal behaviour.

IV. Conclusion

The study illustrates the effect of two inorganic forms and concentrations of arsenic on some plant physiological parameters. Arsenic (As) generates a considerable stress in cowpea plants, regardless of the forms of it, and as a result, the photosynthetic pigments, protein, net photosynthesis, transpiration intensity, stomatal conductance and IWUE were suppressed. Application of As (III) recorded the lower values of photosynthetic pigments, protein, transpiration rate and stomatal conductance compared to As (V). But net photosynthetic rate and IWUE were more severely affected by As (V).

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TABLE 1: Effect of two inorganic forms of arsenic on photosynthetic pigments and protein contents in cowpea plants grown under sand culture (Data expressed as mg g^{-1} fresh wt.)

Treatment	Dose (mg L^{-1})	Chl-a	Chl-b	Total chl	Carotenoids	Chl/Car	Protein
Control	0	1.204 ± 0.020	0.327 ± 0.014	1.531 ± 0.011	0.357 ± 0.028	4.289 ± 0.318	53.7 ± 1.54
As (III)	10	0.819 ± 0.023	0.260 ± 0.012	1.080 ± 0.013	0.224 ± 0.024	4.813 ± 0.520	45.4 ± 1.33
	20	0.775 ± 0.013	0.252 ± 0.010	1.027 ± 0.022	0.220 ± 0.033	4.676 ± 0.720	45.6 ± 1.28
	30	0.778 ± 0.005	0.239 ± 0.012	1.017 ± 0.012	0.163 ± 0.039	6.226 ± 0.302	44.2 ± 1.00
	40	0.767 ± 0.005	0.207 ± 0.008	0.974 ± 0.004	0.137 ± 0.033	7.113 ± 0.405	39.7 ± 1.40
As (V)	10	1.171 ± 0.068	0.317 ± 0.018	1.488 ± 0.086	0.343 ± 0.039	4.343 ± 0.283	52.7 ± 0.96
	20	0.991 ± 0.003	0.246 ± 0.024	1.237 ± 0.023	0.285 ± 0.022	4.346 ± 0.355	52.5 ± 1.09
	30	0.847 ± 0.011	0.245 ± 0.004	1.092 ± 0.010	0.214 ± 0.028	5.101 ± 0.076	46.8 ± 1.84
	40	0.807 ± 0.015	0.240 ± 0.005	1.047 ± 0.020	0.222 ± 0.037	4.708 ± 0. 441	44.4 ± 0.76

Values in the table indicate mean of three replications (±standard deviation)

TABLE 2: Effect of two inorganic forms of arsenic on photosynthetic parameters in cowpea plants grown under sand culture

Treatment	Dose (mg L^{-1})	Net photosynthesis (Pn) (µmolCO_2 m^{-2} S^{-1})	Transpiration (E) (mmol H_2O m^{-2} S^{-1})	Stomatal conductance (C) (mmol H_2O m^{-2} S^{-1})	Intrinsic water use efficiency (Pn/C)
Control	0	12.45 ± 0.88	5.25 ± 0.118	102.93 ± 1.98	0.121 ± 0.0015
As (III)	10	9.77± 0.48	2.54 ± 0.077	78.20 ± 3.24	0.124 ± 0.0060
	20	2.87 ± 0.27	1.63 ± 0.040	71.95 ± 1.45	0.039± 0.0060
	30	1.80 ± 0.18	1.48 ± 0.021	54.55 ± 1.24	0.033 ± 0.0027
	40	2.39 ± 0.26	1.44 ± 0.012	46.70 ± 0.99	0.051 ± 0.0106
As (V)	10	7.83 ± 0.24	3.50 ± 0.027	93.62 ± 0.84	0.084 ± 0.0045
	20	1.59 ± 0.06	2.59 ± 0.031	89.67 ± 1.18	0.018 ± 0.0009
	30	0.84 ± 0.05	2.36 ± 0.027	65.95 ± 1.35	0.013 ± 0.0017
	40	0.54 ± 0.03	1.29 ± 0.024	49.13 ± 1.02	0.011 ± 0.0021

Values in the table indicate mean of three replications (± standard deviation)