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Research Article

EFFECT OF DIFFERENT ARSENIC CONCENTRATIONS ON SOME PHYSIOLOGICAL PARAMETERS OF RICE (*Oryza sativa* L.)

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Abstract: The objective of the study was to investigate the effect of different arsenic concentrations on some physiological parameters of rice (*Oryza sativa* L.). Arsenic was applied as arsenate and arsenite at concentrations of 5, 10, 15 and 20 mg/L. Results revealed that germination percentage, net photosynthesis, chlorophyll content and catalase activity decreased significantly with increase in the concentrations of arsenic. The inhibitory effect of arsenite was more pronounced in reducing pigment content, germination percentage net photosynthesis and catalase activity than arsenate contaminated plants. The increase in lipid peroxidation, peroxidase activity with decline in catalase activity in rice is a typical reaction of plants to oxidative stress.

Keywords: Arsenic, Catalase, Chlorophyll pigment, Germination, Lipid peroxidation, Net photosynthesis, Relative water content

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Introduction

Arsenic is a naturally occurring toxic metalloid and widely distributed in the soil, water, air and all living matters. Anthropogenic activities, including metal smelting, coal combustion, dyes, and hide tanning wastes, chemical weapons and arsenic pesticides have contributed to elevated arsenic in the environment. In terrestrial plants, both organic and inorganic arsenic species have been found, with the inorganic species, arsenate (As V) and arsenite (As III) being the most dominant. Arsenate is the predominant arsenic species in aerobic soils, whereas arsenite dominates under anaerobic conditions. Arsenic avaibility to plants is greatly influenced by its forms in soil. Rice is the most important crop of India and second most important crop of the world. Long term use of arsenic contaminated groundwater for irrigation has resulted in elevated soil arsenic levels in agricultural lands [1]. Arsenic induced oxidative stress leads to the production of reactive oxygen species(ROS).The activities of antioxidative enzymes are inducible by oxidative stress due to exposure to various types of stresses, and represent a general plant response to adverse conditions [2].These enzymes helps in scavenging ROS and thus prevent cellular damage. Arsenic caused a reduction of the photosynthesis rate [3]. This study focuses on the effect different arsenic concentrations on some physiological parameters of rice (*Oryza sativa* L.).

Materials and Methods

Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for two minutes, washed repeatedly with glass distilled water and divided into several batches of 20 seeds. Seeds were then soaked separately in different arsenic solution (5, 10, 15 and 20 ppm). Sodium arsenate ($Na_2HAs_3O_4$, $7H_20$) and arsenic oxide (As_2O_3) were used for preparation of arsenate (ASV) and arsenite (ASIII) solution. Ten (10) ml of each solution was used to soak Whatman No.1 filter paper in each of the sterilized petridishes of 9 cm diameter, on which the soaked seeds were spread at the rate of 20 numbers per petridishes for germination. Control set was prepared similarly using glass distilled water. All petridishes were kept in the incubator maintained at 28°C temperature. The experiment was conducted in triplicate. Germination counts were obtained at every twenty four hours interval for eight days. Seeds with radical emergence equal to or greater than two (2) mm were considered as successful germination. Germination percent of seeds were calculated for each treatment at eighth day of incubation. The experiments were carried out under laboratory conditions in hydroponic system with balance nutrient solution during the year 2012.Surface sterilized rice seeds were placed were placed in pot containing 5 kg sand for germination. Seedlings were grown for 14 days and were transplanted to modified Hoagland's solution Ph being 5.6-5.8. The solutions were renewed after 7 days. On 28th days, such plants were transferred to Hoagland's solutions contaminated with arsenate and arsenite. After 3 days of treatment seedlings were removed. Pigments from the leaves of such rice plants were extracted by 80% acetone (v/v) following percolation method of Hiscox and Israelstam(1978) [4]. After extraction was completed as indicated by discolouration of leaf samples, chlorophyll was determined spectrophotometrically by taking absorbance at 646,663 and 470 nm. The amount of total chlorophyll was calculated according to Lichtenthaler and Wellburn (1983) formulae [5].

Estimation of relative water content (RWC)

Relative water content (RWC), defined as water content of tissue as a percentage of that of the fully turgid tissue of 28th day old seedlings by employing the formula given by Barrs and Weatherley (1950) [6].

Determination of lipid peroxidation

The level of lipid peroxidation in the roots was measured in terms of thiobarbituric acid reactive substances (TBARS) content, a product of lipid peroxidation following the method of Heath and Packer (1968) [7]. Freshly harvested seedling sample (0.5 g) were extracted in 4.0 ml of 0.5% thiobarbituric acid(TBA) made in 20% TCA. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 rpm for 10 min, the absorbency of the supernatant was recorded at 532 nm using a UV-VIS spectrophotometer. The value for non-specific absorption at 600 nm was subtracted from the value recorded at 532 nm. The TBARS content was calculated using its extinction coefficient of 155 mM-1 cm-1 and expressed as nmol (TBARS) g-1 fresh weight.

Table-1 Germination percentage (%) of rice at different concentration of As-V and As- III at 2nd, 4th, 6th and 8th days after treatment

Treatment	Dose	Germination %			
		2nd	4th	6th	8th
Control	0 mg/L	91.67	100.00	100.00	100.00
AS(V)	5 mg/L	86.67 -(5.46)	98.33 -(1.67)	100.00(0)	100.00(0)
	10 mg/L	71.67 -(21.82)	78.33 -(21.67)	81.67 -(18.33)	81.67 -(18.33)
	15 mg/L	61.67 -(32.73)	68.33 -(31.67)	71.67 -(28.33)	71.67 -(28.33)
	20 mg/L	51.67 -(43.64)	55.00 -(45)	55.00 -(45)	60.00 -(40)
AS(III)	5 mg/L	28.33 -(69.09)	51.67 -(48.33)	61.67 -(38.33)	68.33 -(31.67)
	10 mg/L	8.33 -(90.91)	18.33 -(81.67)	20.00 -(80)	20.00 -(80)
	15 mg/L	0.00 -(100)	0.000 -(100)	0.00 -(100)	0.00 -(100)
	20 mg/L	0.00 -(100)	0.00 -(100)	0.00 -(100)	0.00 -(100)
Mean	44.45	52.22	54.45	55.74	44.45
For comparison mean of	S.Em (±)	1.443	3.727	2.394	2.282
	C.D (0.05)	4.326	11.174	7.177	6.842

*Values in parenthesis indicate the percent reduction over control

Table-2 Relative water content (RWC) in the roots and leaves of rice seedling under different arsenic treatment

Treatment	Dose	Relative root water content (%)	Relative leaves water content (%)
Control	0 ppm	76.56	94.00
AS(V)	5 ppm	75.14 -(1.86)	91.18 -(3.00)
	10 ppm	52.85 -(30.97)	89.62 -(4.66)
	15 ppm	50.80 -(33.65)	87.18 -(7.26)
	20 ppm	38.01 -(50.35)	85.96 -(8.55)
AS(III)	5 ppm	46.89 -(38.75)	52.83 -(43.80)
	10 ppm	23.48 -(69.33)	45.34 -(51.77)
	15 ppm	17.69 -(76.89)	19.55 -(79.20)
	20 ppm	8.46 -(88.95)	13.22 -(85.94)
For comparison mean of	S.Em(±)	0.347	1.018
	C.D(0.05)	1.041	3.052

*Values in parenthesis indicate the percent reduction over control.

Table-3 Rate of photosynthesis (Pn) (µmol CO_2 m^{-2} s^{-1}), total chlorophyll (mg/g fr. wt), Lipid peroxidation (nmol of TBARS content /g fresh weight), Catalse activity (U/ min/ g fr.wt), Peroxidase activity (U/ min/ g fr.wt) under different arsenic treatments.

Treatment	Dose	Net photosynthesis (Pn)	Total Chl	Lipid peroxidation	Catalse activity	Peroxidase activity
CONTROL	0 ppm	8.40	1.96	54.00	0.690	5.473
AS-V	5 ppm	7.65 -(8.89)	1.95 -(0.82)	143.07	0.657 -(4.78)	6.267 (14.50)
	10 ppm	6.90 -(17.86)	1.76 -(10.39)	149.97	0.540 -(21.74)	10.730 (96.04)
	15 ppm	4.89 -(41.80)	1.49 -(24.09)	198.60	0.450 -(34.78)	15.510 (108.23)
	20 ppm	3.08 -(63.32)	1.03 -(47.58)	293.48	0.310 -(55.07)	19.300 (191.11)
AS-III	5 ppm	7.02 -(16.36)	1.52 -(22.67)	123.69	0.620 -(10.15)	6.180 (12.91)
	10 ppm	6.07 -(27.75)	1.17 -(40.50)	282.33	0.370 -(46.38)	10.080 (84.18)
	15 ppm	0.00(leaves dry)	0.901 -(54.10)	386.01	0.150 -(78.26)	11.397 (183.38)
	20 ppm	0.00 (leaves dry)	0.539 -(72.54)	484.41	0.053 -(92.32)	15.933 (252.62)
For comparison mean of	S.Em (±)	0.323	0.056	4.59	0.011	0.558
	C.D (P=0.05)	0.969	0.148	13.94	0.033	1.563

*Values in parenthesis indicate the percent reduction over control.

Determination of catalase activity

Catalase activity was estimated following the method of Kar and Misra, (1976) [8]. One g of the sample was ground with 5 ml of 0.1 (M) phosphate buffer of pH 7.0 in a prechilled mortar and pestle. It was then centrifuged at 15,000 rpm for 30 minutes at 4°C and the supernatant was stored as enzyme source. Three ml of phosphate buffer of pH 7.0, 2 ml of 0.05 (M) H_2O_2 and 1 ml of the enzyme source were pipette out in a conical flask and incubated for 3 minutes. After 3 minutes, the reaction was stopped by addition of 10 ml of 0.7 (N) H_2SO_4. This reaction mixture was then titrated against 0.01 (N) $KMnO_4$ to find out the residual H_2O_2, the end point being the appearance of light violet colour which persisted for at least 15 seconds. The blank was prepared by adding 1 ml of the enzyme source to 3 ml phosphate buffer, 1 ml 0.05 (M) H_2O_2 and 10 ml of 0.7 (N) H_2SO_4 were added to it and immediately titrated against $KMnO_4$.

Determination of peroxidase activity

Peroxidase activity was estimated following the method of Shannon (1960). One g of Fresh sample was extracted with 10 ml Tris HCl buffer (pH 7.6) at 4°C in a pre cooled mortar pestle. The homogenate was centrifuged at 4°C at 10,000 rpm for 20 minutes. The supernatant so obtained was used as an enzyme source.0.1 ml of enzyme source was taken in a cuvette 2.8 ml of reaction mixture (which consists of 26 ml H_2O, 3 ml sodium acetate buffer and 1 ml of o-dianisidine) was added to it and thoroughly mixed. 0.1 ml of H_2O_2 was added and the change in absorbance per minute was monitored at 430 nm at intervals of 1 min upto 3 min by the method of Shannon (1960). Tris HCl buffer was used as a blank [9].

Photosynthetic parameters

Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for two minutes, washed repeatedly with glass distilled water and grown in field for 50 days. After 50 days plants were transferred in modified Hoagland solution contaminated with different concentrations of arsenate and arsenite for 3 days. After 3 days net photosynthesis rate (Pn), of the youngest, fully developed intact leaves were measured with a CI-340 portable photosynthesis system (CID Inc., USA). The measurements were obtained at ambient CO_2 concentrations between 10.0 and 12.0 h on a clear sky day after 8 days of sowing. Statistical Data analysis: The data of different parameters, were subjected to statistical analysis as per design(s) following the method described by Panse and Sukhatme (1989) to find out the significance between treatments used in the experiment [10].

Results and Discussion

The germination response under arsenate and arsenite treatments at 2nd, 4th, 6th and 8th days were observed to be significantly lower than the control. The percent germination over control decreased significantly (p=0.05) with increasing concentration of arsenite and arsenate and germination were delayed by 1-2 days with arsenic stress. This was more prominent with arsenite than arsenate (p=0.05). Germination decreased by 18.33, 28.33, 40% at 10, 15 and 20 mg L-1, respectively for arsenate. There is 1-2 fold decrease in germination percent by arsenite stress over arsenate. The decrease was 31.67, 80 % at 5 and 10 mg L-1 for arsenite, the decline in germination was cent percent 10 mg L-1 and above. Complete inhibition in germination observed above 10 mg L-1 in arsenite treated seeds. The results of decrease in germination under arsenic stress are in agreement with the earlier observations by Abedin and Meharg (2002), Li *et al*.,(2006) and Liu *et al*., (2006) [11-13]. Arsenite treated leaf and root showed significant decrease in relative water content as compared to arsenate stress. Plants exposed to arsenate, relative shoot water content were less affected over relative root water content at the same concentration, as the % decrease in relative root water content were 1.86, 30.97, 33.65, 50.35 at 5,10,15 and 20 mg L-1of arsenate respectively over the control; however, % decrease in relative shoot water content were3.00, 4.66, 7.26, 8.55% at the same concentration. Plants exposed to arsenite, relative water content in shoot were more affected except at 10 and 20 mg L-1than relative root water content. At 5 and 15 mg L-1of arsenite relative shoot water content were 43.80, 79.20% respectively, whereas relative root water content was 38.75, 76.89% at the same concentration. More negative affect of arsenite in reducing relative root and shoot water content than arsenate might be due to more toxicity of arsenite which causes water stress in plants. It is well known that photosynthesis is one of the most sensitive processes to the different types of stress. The photosynthetic rate (Pn) decreased significantly with the increase in arsenate and arsenite concentrations [Table-3].This was more evident at a concentration of 5, 10,15 and 20 mg/L decrease in net photosynthesis by arsenate was 8.89,17.86,41.80 and 63.32% respectively, below the control, arsenite decreased by 16.36 and 27.75% for 5 and 10 mg/L ,respectively. Such decline in rates of the physiological processes in plants exposed to arsenite was more pronounced and plants did not survive beyond five days and 15 mg/L concentration of the toxicant. Reduction in net photosynthesis in response to arsenic exposure also reported by Stovea *et al*., (2005). Arsenic affected the photosynthetic pigments, which in certain cases are able to limit the photosynthesis rate. Total chlorophyll content decreased by arsenate were0.82, 10.39,24.09, 47.58% below the control at 5, 10, 15 and 20 mg L-1; arsenite caused 22.67, 40.50,54.10, 72.54% below the control respectively at the same concentration. Reduction in the pigment content with increasing levels of heavy metals and metalloids was also established by Bogoeva (1998) [14]. The lower amounts of photosynthetic pigments in the arsenite treatments might be due to more toxicity of this form than that of the arsenate. Lipid peroxidation was measured in terms of TBARS, increased significantly with increasing concentration of arsenic and recorded in [Table-3]. The lipid peroxidation of root tissues of the rice seedlings under arsenite was higher than that under the corresponding concentration of arsenate stress. This increase in TBARS content may be due to the generation of free radicals that may distort the membrane architecture causing an oxidative damage as reported in other higher plants [15]. Catalase activity decreased significantly with increase in concentrations of arsenate and arsenite [Table-3]. Catalase activity reduced by 4.78, 21.74, 34.78 and 55.07% at 5, 10, 15 and 20 mg/L treatments, respectively, for arsenate. While it decreased by 10.15,46.38, 78.26 and 92.32% for arsenite. This decrease in catalase activity indicated the weakening of super oxide and H_2O_2 scavenging system due to arsenic stress and this enzyme was no way involved in providing protection against as toxicity. The gradual increase in lipid peroxidation with a simultaneous decrease in catalase activity suggested an imposition of oxidative stress. The observed decrease in catalase activities may be because of enzyme inhibition since heavy metal is known to be a potential enzyme inhibitor. Catalase activity affected by heavy metal was also reported by Choudhury and Panda, (2004) [16]. In roots the increase in activity were 14.50, 96.04, 108.23 and 191.11% respectively in 5, 10, 15 and 20 mg L-1arsenate treatment; in arsenite treated roots percent increase were 12.91, 84.18, 183.38, 252.62 respectively at the same treatment. At low level of toxicity (5 and 10 mg L-1) in arsenite treated plants peroxidase activity were lower as compared to arsenate treatment and at higher toxic level (10 and 15 mg L-1) reverse was found *i.e*., peroxidase activity were higher in arsenite treated roots [17].

Application of research: It is clearly indicated that both arsenate and arsenite were detrimental to germination, relative water content, net photosynthesis, total chlorophyll content, catalase activity and induces lipid peroxidation and peroxidase activity. Arsenite was found to be more toxic than arsenate in inducing oxidative stress in plants

Research Category: Arsenite concentrations

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