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Effect of arsenate and arsenite on some physiological parameters of rice (*Oryza sativa* L.)

Minsura Begum* and S Mondal

Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India

****Corresponding author e-mail: smondalbckv@rediffmail.com***

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ABSTRACT

The present investigation was undertaken to study the effect of different concentration of arsenic on some physiological parameters of rice in the early growth phases. Arsenic was applied as $Na_2HAs_3O_4$ *and* As_2O_3 *at concentrations of 5, 10, 15 ands 20 mg* l^{-1}*. The results revealed that under the influence of arsenic, there occurs stress in the plants, which results in the significant decrease in amylase activity, promptness index, germination stress indices, growth efficiency, tolerance index, plant height stress index. Relative water content in the root and shoot also changed in the negative aspect, whereas relative membrane lipid peroxidation changed to positive aspect. The inhibitory effect of arsenite was more pronounced in reducing physiological parameters as compared to arsenate stress.*

Key words: *Arsenic, amylase, germination indices, relative water content, relative membrane lipid peroxidation*

Arsenic is a naturally occurring toxic metalloid and widely distributed in the soil, water, air and all living matters. The main sources of arsenic contamination are the non-ferric metallurgy plants and the application of arsenic containing herbicides. Arsenic is of great environmental concern due to its extensive contamination in groundwater and carcinogenic toxicity. Long-term use of arsenic contaminated groundwater for irrigation has resulted in elevated soil arsenic in agricultural lands (Ullah 1998). The solubility, mobility, bioavailability and toxicity of arsenic in soil-crop system also depend on its chemical form, primarily the oxidation state (Woolson 1977). Arsenic in groundwater is generally present as dissolved, deprotonated/protonated oxyanions, namely arsenites or arsenate, or both, besides the organic forms. From the physiological point at the cellular level, arsenic toxicity results in excessive ROS production, which can cause oxidative damage to biomolecules such as lipid, protein and nucleic acids, and disrupt cellular metabolism (Cakmak and Horst 1991). Arsenic has been shown to disturb several physiological and biochemical processes which could hamper normal growth and development of plants (Tang and Miller 1991). We used both of these two inorganic species in our study to see whether there is any differential toxicity effect shown by the arsenic species on rice cultivars. In this present study, we evaluated the effect of arsenate and arsenite 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 distilled water and soaked separately in different arsenic solution. 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, on which the soaked seeds were spread for germination. Control set was prepared similarly using distilled water. All petridishes were kept in the incubator maintained at 28 ± 10C temperature in triplicate. Germination counts were obtained at every 24 hours interval for eight days. Seeds with radical emergence equal to or greater than 2 mm were considered as successful germination. Amylase activity was determined at three days after treatment. On the eighth day, seedlings were removed from petridish and length of shoot and root were measured separately and mean values were determined. On the basis of dry mass of seedlings, plants tolerance indices were calculated by adopting the formula given by Maiti *et al.* (1994). Promptness index (PI), germination stress index (GSI), growth efficiency (GE) and plant height index (PHSI) was calculated as described by Bouslama and Schapaugh (1984).Promptness index (PI) = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.50) + nd_8 (0.25)

Where, nd_2, nd_4, nd_6 and nd_8 = percentage of seeds observed to germinate on the 2nd, 4th, 6th and 8th day of observation, respectively.

$$\text{Germination stress index} = \frac{\text{PI of stressed seed (PSI)}}{\text{PI of controle seed (PIC)}} \times 100$$

Plant height stress index(PHSI)=

$$\frac{\text{Pl. ht. of stressed seedlings (PHS)}}{\text{Pl. ht. of controle seedling (PHS)}} \times 100$$

$$\text{Tolerance index (TI)} = \frac{\text{Dry wt. of seedlings under stress (g)}}{\text{Dry wt. of seedlings under controle (g)}}$$

$$\text{Growth Efficiency(GE)} = \frac{\text{Dry weight of seedlings (g)}}{\text{Substrate consumed (g)}}$$

Total amylase activity was measured as per modified method of Filner and Varner (1967). In a chilled glass mortar and pestle, 200 mg germinated rice seeds were homogenized with 3 ml of 20% aqueous glycerol at cold condition (0^0C). Homogenates centrifuged at 5000 rpm for 10 minutes at 0^0C. The residue was re-extracted with 1 ml of 20% (v/v) aqueous glycerol. The supernatants were combined and volume was made to 5 ml by adding aqueous glycerol 20% (v/v) and used as the source of enzyme. Reaction mixture containing 1 ml of enzyme, 2 ml of 0.1(M) phosphate buffer (pH-7.0), 1 ml of 0.1% (w/v) starch solution was taken and incubated at 37^0C for 15 minutes and the reaction was stopped by adding 1.0 ml HCl containing I-KI reagent. Optical density was recorded at 620 nm in Spectronic 20 Genesys spectrophotometer using a reagent blank. Amount of starch degraded was calculated with the help of a standard curve prepared with different concentrations of soluble starch in the similar way. Total amylase activity was expressed as µg starch degraded g^{-1} fresh weight of seeds min^{-1}.

Determination of relative membrane lipid peroxidation and relative water content: Surface sterilized rice seeds were placed in pot for germination, seedlings were grown for 14 days and were transplanted to modified Hoagland's solution. Seedlings were transplanted to plastic buckets containing 250 ml of solution pH being maintained to 5.8- 6.0. The solutions were renewed after 7 days. On 28th day, such plants were transferred to Hoagland's solution contaminated with arsenate or arsenite. After 3 days of treatment, seedlings were removed and relative water content and relative membrane peroxidation were determined.

Relative water content (RWC) was determined by employing the formula given by Barrs and Weatherley (1950).

$$RWC = \frac{FW-DW}{TW-DW} \times 100$$

Where,

RWC = Relative water content

FW = Fresh weight of sample

DW = Dry weight of sample

TW = Turgid weight of sample

Lipid peroxidation in the leaves and roots were measured to assess the membrane damage. The level of lipid peroxidation was measured in terms of thiobarbituric acid reactive substance (TBARS) content, a product of lipid peroxidation following the method of Heath and Packer (1968).

Oxidative stress index was evaluated in terms of relative membrane lipid peroxidation as

Membrane lipid peroxidation(%)=

$$\frac{\text{Accumulation of TBARS under treatment relative}}{\text{Accumulation of TBARS under untreated controle}} \times 100$$

RESULTS AND DISCUSSION

Amylase activity in the germinating rice seeds decreased significantly with increase in the concentration of arsenate and arsenite. The values of amylase activity for arsenite was lesser than that for the corresponding concentration of arsenate indicating again more poisonous effect of arsenite than arsenate in the processes of mobilization of stored food of seeds for seedling growth(Table 1). In arsenate treated seeds at 20 mg l^{-1} amylase activity decreased to 73.33%, whereas in arsenite treated seeds 90.50% decrease in amylase activity over control (355.80%) was observed. Decrease in amylase activity due to arsenic suggests delay in the degradation of starch results significant impact on alteration in starch content or on its mobilization and hamper germination of seeds. Similar to our observations a marked inhibition in the activities of amylase was observed by Jha and Dubey (2004, 2005).

Promptness index (PI), germination stress index (GSI), tolerance index (TI), growth efficiency (GE) and plant height stress index (PHSI) of the seeds treated with different species and concentration of arsenical toxicant declined significantly with increase of their concentration in comparison to untreated seeds. Decline in growth indices was more striking in arsenite treated seeds than the corresponding concentration of arsenate. Seedlings of the seeds treated with arsenite concentration $\geq$ 15 mgl^{-1}could not survive. PI is indicator of speed of germination of the seeds. Decrease in PI by arsenate was 3.25, 20.72, 31.51, 44.52% at 5, 10, 15 and 20 mgl^{-1} respectively; however arsenite caused 52.74 and 84.76% at 5 and 10 mg l^{-1}. GSI is the reflection of germination impairment of the seeds. These results clearly showed that germinational processes of rice seeds in the presence of arsenic in the nutrient solutions at the concentrations of 5 mg l^{-1} and above were either fully or partly inhibited at their operational levels. Arsenic toxicants also had detrimental effect on post germinational growth which was sufficiently indicated in the values of tolerance index (TI). All the values of TI except that at 5 mg l^{-1} were $\leq$ 1 indicating lower dry matter mobilizations in the seedlings of the arsenic treated seeds. In arsenate treated seeds maximum and minimum percent of GE recorded in 5 mg l^{-1} (47.43) and 20 mg l^{-1} (16.99) respectively, whereas in arsenite at 5 mg l^{-1} (18.84) was recorded. PHSI was found to be the highest in non stressed seeds (36.490), whereas in 10 mg l^{-1} of arsenate and arsenite stressed seeds PHSI value was found to be 30.330 and 19.813, respectively. Lower germination of rice seed at higher concentration of arsenite than arsenate could be an important consideration for wetland rice culture because of presence of arsenite (Onken and Hossner 1995, 1996). Significant reduction in rice shoot and root length with increasing arsenic concentration suggests that rice shoot and root length can also be used as a good indicator for arsenic tolerance and toxicity. Reduced shoot length due to application of arsenic in this study also corroborates with the result of Marin *et al.* (1992), who found significant reduction of rice shoot length when arsenite or mmAA was applied at a relatively lower dose of 0.8 mg As l^{-1}. So, it was established that arsenite causes more negative effect on growth parameters of rice than that of arsenate treatment. A decline in germination index under arsenic stress remained in agreement with the earlier observations by Abedin and Meharg (2002), Li *et al.* (2006) and Liu *et al.* (2006).

Table 1. Effect of arsenic on amylase activity (µg of starch degraded /g fresh wt /min), indices of growth and germination of rice

Treatment (mg l^{-1})		Amylase activity	Promptness index (PI)	Germination stress index (GSI)	Growth efficiency (GE)%	Tolerance index (TI)	Plant height stress Index (PHSI)
Control	0	355.80	243.33	1.00	55.15	1.00	1.00
AS-V	5	312.10	235.42	96.75	47.43	0.880	36.490
	10	228.45	192.92	79.30	33.43	0.742	30.330
	15	215.91	166.67	68.55	24.02	0.579	23.710
	20	94.91	135.00	55.53	16.99	0.295	19.820
AS-III	5	298.14	115.00	47.31	18.84	0.588	32.670
	10	289.18	37.08	15.27	3.16	0.279	19.813
	15	276.84	0.00	0.00	0.00	0.00	0.00
	20	59.29	0.00	0.00	0.00	0.00	0.00
	C.D (P= 0.05)	4.035	12.006	5.023	2.069	0.043	4.165

Oxidative stress index (measured in terms of relative membrane lipid peroxidation) in arsenate and arsenite treated roots and shoots increases significantly with increase in the toxicity level. During arsenate stress maximum percent of RMLP observed at 20 mg l^{-1} (543.48 and 571.15 in root and leaf respectively), minimum observed at 5 mg l^{-1} (264.94 and 184.44) respectively, whereas in aresnite stress, maximum RMLP found in 20 mg l^{-1} (897.06 and1189.10) and minimum found in 5 mg l^{-1} (229.06and 277.10%). So, stresses during early germination may result in the induction of oxidative stress in germinating tissues which increases the vulnerability of newly assembled membrane systems to oxidative damage. Similar results of increasing relative membrane lipid peroxidation with increasing stress also reported by Bhattacharjee and Mukherjee (2006) (Table 2).

Arsenic stress significantly lowered the RWC in root and shoot as compared to control condition. During arsenate stress the decrease in RWC in root was 1.86, 30.97, 33.65 and 50.35% over control at 5, 10, 15 and 20 mg l^{-1} respectively, whereas in arsenite 38.75, 69.33, 76.89 and 88.95% decrease over control. In leaf the decrease in RWC by arsenate were 3.00, 4.66, 7.26 and 8.55%, arsenite reduced by 43.80, 51.77, 79.20 and 85.94% over control. Similar, results also reported by Stoeva *et al.* (2005).

In conclusion, arsenic inhibited the activities of α -amylase that might delay mobilization of endospermic reserves leading to impairment in germination of seeds and growth indices. Within the plants arsenic caused oxidative stress, results in increasing in relative membrane lipid peroxidation and decreasing relative water content in rice plants. Arsenite caused more negative effects in plant physiological and biochemical responses as compared to arsenate stress.

Table 2. Effect of arsenic on relative water content (RWC), relative membrane lipid peroxidation (RMLP) in root and leaf of rice seedling

Treatment(mg l^{-1})		RMLP (%) root	RMLP (%) leaf	RWC (%) root	RWC (%)leaf
Control	0	1.00	1.00	76.56	94.00
AS(V)	5	264.94	184.44	75.14	91.18
	10	277.72	223.98	52.85	89.62
	15	367.78	270.41	50.80	87.18
	20	543.48	571.15	38.01	85.96
AS(III)	5	229.06	277.10	46.89	52.83
	10	522.83	613.80	23.48	45.34
	15	714.83	889.64	17.69	19.55
	20	897.06	1189.10	8.46	13.22
	C.D(0.05)	60.39	40.63	1.041	3.052

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