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Response of Rice (*Oryza sativa* L.) to Arsenate and Arsenite Stress

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ABSTRACT

Arsenic is a potential contaminant of groundwater as well as soil in many parts of the world. Elevated soil arsenic levels resulting from long-term use of arsenic contaminated ground water for irrigation may inhibit seed germination and seedling establishment of rice, the country's main food crop. An experiment was conducted to determine response of rice (*Oryza sativa* L) to arsenate and arsenite stress. For this purpose seeds were treated with 0, 5, 10, 15 and 20 ppm of arsenate and arsenite solution individually. Germination percent, root, shoot and seedling length, dry weight and indices of growth showed significant ($P<0.05$) decrease with increase in concentration of arsenate and arsenite as compared to control. Arsenite was found to be more toxic than arsenate on germination and early growth of rice seedling.

Introduction

Arsenic (As) is a toxic heavy metal dispersed in the environment through a variety of anthropogenic activities like industrial, mining and agricultural etc. In the natural environment, it pollutes the soil and contaminates water, thus posing a serious threat to biota, including plants, animals and humans (Mahimairaja *et al.,* 2005). Groundwater contamination with arsenic is reported from many regions of the world, the most severe problems occur in Bangladesh, West Bengal, China and Taiwan (WHO, 2001). Contaminated groundwater is not only the main source of drinking water but is also extensively used for irrigating crops. 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 (Alam and Sattar, 2000). In terrestrial plants, both organic and inorganic As species have been found, with the inorganic species (Arsenate [As (V)] and arsenite [As (III)]) being the most dominant. Arsenate is the predominant As species in aerobic soils, whereas arsenite dominates under anaerobic conditions. Arsenic availability to plants is greatly influenced by its forms in soil. Agricultural application of arsenicals has introduced many different kinds of arsenic compounds to the soil environment. Presence of arsenic in irrigation water or in soil at an elevated level could hamper normal growth and development of plants. Plants can develop toxicity symptoms while they are exposed to excess arsenic either in soil or in solution culture such as: Inhibition of seed germination (Liebig, 1966); decrease in plant height (Carbonell-Barrachina *et al.,* 1995); reduction in root growth (Tang and Miller, 1991), wilting and necrosis of leaf blades (Frans *et al.,* 1988), reduction in leaf area and photosynthesis decrease in shoot growth (Carbonell-Barrachina *et al.,* 1998). Arsenite and arsenate are inter convertible depending on the redox condition of the soil (Masscheleyn *et al.,* 1991) with arsenite dominating in flooded paddy soils.

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 response of rice to arsenate and arsenite stress.

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 petridish for germination. Control set was prepared similarly using glass distilled water. All petridishes were kept in the incubator maintained at 28 ± 1^0C 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. On the eighth day, seedlings were removed from petridishes and length of shoot and root were measured separately and mean values were determined. Seedling vigor was calculated by multiplying the germination % with total length (cm) of the seedling (Woodstock 1969, Abdul-Baki and Anderson 1973)

Results and Discussion

The germination response of the rice genotype, Satabdi, to the treatments with different concentrations and forms of arsenic at each of 2^{nd}, 4^{th}, 6^{th} and 8^{th} day differed significantly. Germination of rice seeds were seriously affected by the presence of arsenic in the nutrient solutions at the concentrations of 5 ppm and above in arsenite treated seeds and above 5 ppm in arsenate treatment. Arsenite contaminated seeds showed no germination at and beyond 15 ppm. The percent germination over control decreased significantly (p=0.05) with increasing concentration of arsenite and arsenate and germination was delayed by 1-2 days with arsenic stress (Table 1). There is 1-2 fold decrease in germination percent by arsenite stress over arsenate. Complete inhibition in germination observed above 10 ppm in arsenite treated seeds. 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 (Masscheleyn *et al.,* 1991 and Marin *et al.,* 1993). The results of decrease in germination under arsenic stress are in agreement with the earlier observations by Abedin and Meharg (2002).

Significant decrease in root length, shoot length, ratio of root and shoot length, seedling length, dry weight and seedling vigor was observed due to increase in arsenic concentrations and arsenite was found to be more toxic than arsenate in reducing seedling growth. Shoot length and root length of the seedlings were very seriously affected by the presence of arsenic toxicants in the nutrient solution. Even in the presence of 5 ppm arsenate or arsenite in the nutrient solution the total length of root and shoot was less than 40% of that of seedlings of untreated seeds. The inhibitory effect was more pronounced on root length than on shoot length. Reduced shoot height 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 height when arsenite or mmAA was applied at a relatively lower dose of 0.8mg As/l. The reduction of shoot height due to arsenic exposure can be an important consideration for rice cultivation as reduced shoot height will decrease rice leaf area, net photosynthesis (Marin *et al.,* 1993), and ultimately rice yield. Reduction of both root and shoot length is a typical response to toxic metals.

Reduced root length and shoot length growth in response to arsenic exposure has been reported by a number of investigators (Abedin and Meharg, 2002 and Singh *et al.,* 2007). It is evident from table 2 that arsenic both as arsenate and arsenite strongly inhibited dry weight of the plant.

Table.1 Effect of arsenate and arsenite on germination percentage (%) of rice seeds at 2^{nd}, 4^{th}, 6^{th} and 8^{th} days after treatment

Concentrations (ppm)		**Germination %**			
		2^{nd}	**4^{th}**	**6^{th}**	**8^{th}**
Control	0	91.67	100.00	100.00	100.00
AS(V)	5	86.67 -(5.46)	98.33 -(1.67)	100.00 (0.00)	100.00 (0.00)
	10	71.67 -(21.82)	78.33 -(21.67)	81.67 -(18.33)	81.67 -(18.33)
	15	61.67 -(32.73)	68.33 -(31.67)	71.67 -(28.33)	71.67 -(28.33)
	20	51.67 -(43.64)	55.00 -(45.00)	55.00 -(45.00)	60.00 -(40.00)
AS(III)	5	28.33 -(69.09)	51.67 -(48.33)	61.67 -(38.33)	68.33 -(31.67)
	10	8.33 -(90.91)	18.33 -(81.67)	20.00 -(80.00)	20.00 -(80.00)
	15	0.00 -(100.00)	0.000 -(100.00)	0.00 -(100.00)	0.00 -(100.00)
	20	0.00 -(100.00)	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 (±)	1.44	3.73	2.39	2.28
	C.D (0.05)	4.33	11.18	7.18	6.84

Table.2 Effect of arsenate and arsenite on seedling length, root and shoot length ratio (cm), seedling vigor and dry wt (mg/seedling) of rice seedling at 8 days after treatment

Concentrations ($mg\ l^{-1}$)		Seedling length (cm)	Root length/ shoot length	Seedling vigor	Dry wt. (mg/seedling)
Control	0	20.37	1.82	2037	7.00
AS(V)	5	7.43 -(63.53)	0.36 -(80.22)	743 -(63.53)	6.16
	10	5.87 -(71.18)	0.25 -(86.27)	479.40 -(76.46)	5.19
	15	4.83 -(76.29)	0.18 -(90.11)	346.17 -(83.00)	4.05
	20	3.80 -(81.35)	0.11 -(93.96)	228.00 -(88.80)	2.07
AS(III)	5	5.67 -(72.17)	0.32 -(82.42)	387.43 -(80.98)	4.12
	10	4.04 -(80.17)	0.19 -(89.56)	80.80 -(96.03)	1.96
	15	0.00	0.00	0.00 -(100.00)	0.00
	20	0.00	0.00	0.00 -(100.00)	0.00
For comparison mean of		5.89	0.38	468.18	3.51
	S.Em(±)	21.21	1.16	21.21	20.15
	C.D(0.05)	63.59	3.10	63.59	60.14

The reduction in dry weight of plant materials may be due to the poor functioning of physiological processes related to growth in presence of arsenic both as arsenate and arsenite. These decrease in dry weight with increase in arsenic concentration also reported by Rahman *et al.,* (2008).

So, from this investigation it can be concluded that both arsenate and ardenite reduces growth of rice seedling and ardenite was found to be more toxic than arsenate in decreasing germination and seedling growth.

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