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Screening of arsenic tolerance in rice at germination and early seedling stage as influenced by sodium arsenate

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Abstract

Arsenic contamination in rice would be a serious problem for human health. A screening program was conducted at germination and early seedling stage of rice cultivars which were grown under plate culture and hydroponic culture condition. Five different concentrations of arsenate viz.T_1 (1ppm), T_2 (3ppm), T_3 (6ppm), T_4(9ppm), T_5 (12ppm) and distilled water (control) were applied on seven cultivars of rice such as BR-26, BRRI-28, BRRI-29, BRRI-45, BRRI-50, BR-3 and BR-14.The germination percentage and tolerant percentage, root length and shoot length and biomass or seedling dry weight decreased significantly with the increase of arsenic levels.Among seven cultivars, BRRI-29 showed the maximum percentage of germination and tolerant percentage, BR-26 produced the highest root length and shoot length to highest arsenic concentration (12 ppm) whereas maximum biomass was obtained for BR-14.

Key words: Sodium arsenate, rice cultivars, germination, root length, biomass, seedling stage

INTRODUCTION

Arsenic contamination of groundwater is a severe problem in Bangladesh and this has affected at least 25 million people (Ravenscroft *et al.,* 2005). Next to drinking water, rice could be a potential source of arsenic exposure of the people living in the arsenic affected areas of Bangladesh (Hossain *et al.,* 2008, Panaullah *et al.,* 2009, William *et al*., 2009). Roberts *et al.*(2007) estimated that over 1000 tons of arsenic might be transferred to arable land each year from arsenic contaminated groundwater irrigation, creating a potential risk for future agricultural sustainability and food security of the country. Arsenic may enter into human body directly through drinking water and indirectly through foods, chiefly rice for Bangladeshi people. Rice covers about 75% of the total cropped areas in the country. Many areas have high groundwater and soil arsenic contents which are likely to be taken up by plants through roots and transported to the aerial portion. The concentration of arsenic in groundwater from the affected areas have very large range from <0.5 to >3200µg/L (Smedley & Killiburgh, 2002). In Bangladesh, large numbers of shallow tube wells and deep tubewells are being used to irrigate about 4.3

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million hac. which contribute significantly to the country's food grain production (Rashid *et al.,* 2004). As-contaminated groundwater is used for irrigation as well as for drinking. The World Health Organization provisional guideline value for drinking water is 0.01 mg/kg. There are concerns that arsenic may be absorbed by plants, particularly cereals, entering the grains and thus the food chain. Therefore arsenic poisoning of human beings and livestock occur frequently (Chun-xi *et al*., 2006). Arsenic (As) toxicity to rice can threaten food security in countries such as Bangladesh where rice is the major staple food and arsenic contamination of irrigation waters and soil is widespread (Panaullah *et al*., 2013).The concentration levels of different arsenic species in rice heavily depends on environmental conditions and to a lesser extend on genotype of the rice plants (EVISA, 2013).

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 (Abedin and Meharg, 2002); decrease in plant height (Marin *et al.,* 1992; Carbonel-Barrachina *et al.,* 1995; Abedin *et al.,* 2002b; Jahan *et al.,* 2003a)); reduction in root growth (Carbonel-Barrachina *et al.,* 1998; Abedin *et al.,*2002); decrease in shoot growth (Cox *et al.,* 1996; Carbonel-Barrachina *et al.,*1998); sometimes leading to death (Baker *et al.,* 1976; Marin *et al.,* 1992). In hyper accumulator species, arsenic increases antioxidant mechanisms, both enzymatic and non-enzymatic, leading to its detoxification and subsequent hyper accumulation in the tissue (Srivastava *et al.,* 2005; Singh *et al.,* 2006). In contrast, in non-hypera ccumulators, arsenic induces an oxidative stress resulting in cellular damage in terms of enhanced lipid peroxidation, H_2O_2 accumulation and up-regulation of several scavenging enzymes (Hartley-Whitaker *et al.,* 2001; Mascher *et al.,* 2002).Germination energy, germination percentage, germination index, vitality index, length and biomass of root and shoot all displayed decreasing trend with increasing concentrations of arsenic. Reduced root length growth in response to arsenic exposure has been reported by a number of investigators in other plants (Hartley-Whitaker *et al.,* 2001 and Carbonell-Barrachina *et al.,* 1998).

Germination and early seedling stage of rice is more sensitive than that of any other growth stages. Therefore an attempt has been taken to study the effect of arsenate on germination of seeds, and early growth of seedlings on seven rice cultivars.

MATERIALS AND METHODS

Rice Cultivars: Seven cultivars of rice (BR-26, BRRI-28, BRRI-29, BRRI-45, BRRI-50, BR-3 and BR-14) were collected from BRRI, Gazipur, Dhaka, Bangladesh.

Treatmeants: Following the previous research information (Carbonell-Barrachina and Burló-Carbonell, 1997) five concentrations of arsenic (As) (source $Na_2HAsO_4.7H_2O$) solution, T_1=1ppm, T_2=3ppm, T_3=6ppm, T_4=9ppm and T_5=12ppm As and T_0= distilled water were used as control treatment.

Both germination experiment and hydroponic culture were carried out in the Laboratory of Plant Physiology and Plant Biochemistry Lab. of Botany Department, Jahangirnagar University, during November, 2010 to April, 2011.

Germination Test: For germination experiment 20 seeds of each cultivar were placed separately on a blotter paper in a 9cm diameter Petri dishe covered with a lid. The Petri dishes were kept under laboratory condition where temperature fluctuated between 32±2ºC. Petri dishes and blotter papers were soaked with 20 ml arsenate solution of different concentrations (mentioned in the section treatments) and distilled water (as control) separately. The number of germinated seeds was recorded when both plumule and radical extended more than 2mm from the seed coat. Germinated seeds were counted on the 2nd day of germination and the results were expressed as percentage over control. Each treatment was replicated three times. The tolerance percentage was calculated after final germination with the following equation:

$$\text{Tolerance percentage} = \frac{\text{Germination \% in each treatment}}{\text{Germination \% in the control}} \times 100$$

Hydroponic culture for seedling growth: The seedling growth experiment was conducted by hydroponics culture under laboratory condition. Several plastic pots were taken and filled with 250 ml of nutrient solution containing 5mM $Ca(NO_3)_2$, 5mM KNO_3, 5mM K_2SO_4 and 5mM KH_2PO_4 in distilled water (Abbas *et al*., 2008). Five germinated seeds were placed in the hole of plastic nets attached with cork sheets and were then placed on the mouth of plastic pots. The outside of the plastic pots were painted with black colour. Deep coloured plastic beads were placed on the upper part of plastic net of each pot to prevent light from penetrating the solution.

Then the distilled water and different concentrations of arsenate (1, 3, 6, 9 and12 ppm As) were added to the nutrient solution. The pH was adjusted to 5. For each treatment three replications were used. The plastic pots with germinated rice seedlings were then placed at room temperature (32±2 ºC) for 10 days. Root length and shoot length of ten days old seedlings were measured and biomass (dry weight/ten seedlings) of whole seedlings was measured after drying for 72 hours at 70ºC.

Statistical Analysis: Completely randomized design (CRD) was used for the experiment with three replications having six treatments and six cultivars. Recorded data of different parameters were statistically analyzed with the help of Microsoft Excel 2003 and Duncan’s multiple range test (DMRT) was done by using SPSS Program 16. The mean treatment differences were evaluated by least significant difference test at 5% level of significance (Gomez & Gomez,1984).

RESULTS AND DISCUSSION

The effects of arsenate on germination percentage, tolerance percentage, root and shoot growth, and biomass of rice cultivars obtained from this experiment are described below under following heads:

Germination percentage and tolerance percentage: The mean values of germination percentages and tolerance percentage of seven varieties of rice are presented in Table1and 2. The results show a decreasing tendency of the germination percentage and tolerance percentage with the increase of concentration of arsenate (Table1 and 2). Among seven varieties of rice, BD-29 showed highest germination percentage for all treatments. At the highest arsenate level (T_5=12 ppm) germination Percentages of BRRI-29, BR-26, BR-3, BRRI-28, BRRI-45, BRRI-50 and BR-14 were 82.22, 68.89, 57.78, 71.11, 74.44, 76.67 and 76.67 respectively while their tolerance percentages were 87.06, 72.95, 63.42, 78.05, 85.89, 85.19 and 84.15 respectively. Therefore, BRRI-29 showdthe highest germination and tolerant percentage, while BR-14, BRRI-50, BRRI-45 and BRRI-28 showed moderate results and the lowest rate of germination and tolerant percentages were found in BR-3 and BR-26 respectively for the highest As treatment. From the CV % (Table1) it can be showed that the Germination Percentage of BR-29 is more homogeneous and germination percentage of BR-3 is more scattered. Arsenic is not essential for plants but higher concentrations of arsenic interfered with metabolic processes and inhibited seed germination, plant growth and development through arsenic induced phytotoxicity (Marin *et al.,* 1993a, Abedin & Meharg, 2002).They also reported that germination and early seedling growth of rice decreased significantly with increasing concentrations of arsenic. Seed germination and the early seedling growth are more sensitive to metal pollution because some of the defense mechanisms have not developed such as, starch, storage energy of germinating seeds does not degrade predominantly via the amylolytic pathway (Swain & Dekker, 1966a)

Root and Shoot growth: Root length was recorded 10 days after the germination of seeds. It was measured up to 12 ppm of arsenate treatment and found that the root length was decreased from 107.74 mm for the control to 70.46 mm for the 12 ppm arsenate treatment (Table 3). The reduction of root length had started from arsenate 1ppm treatment (Table 3). At the highest level of arsenate treatment (12ppm) the average values for root lengths found to be 60.7, 90.30, 61.60, 83.00, 61.80, 60.10 and 75.7mm for BRRI-29, BR-26, BR-3, BRRI-28, BRRI-45, BRRI-50 and BR-14 respectively. Thus BR-26 had relatively the longest root followed by BRRI-28, while BRRI-50 had the shortest root. From the CV (%) (Table 3) it can be seen that the root lengths of BR-3 are more scattered compare to other cultivars and that of BRRI-28 are more homogeneous.

Shoot length was recorded after 10 days of emergence of radicals. Almost all the varieties show healthy seedlings but with the increase in arsenate concentration the mean shoot length of all rice cultivars decreased significantly (Table 4). Seedling heights or shoot lengths were recorded upto arsenate 12ppm. For the control (0ppm) BRRI-28 produced the longest plants followed by BR-26. For the highest level of arsenate (12ppm) concentration the mean values for seedling heights were 53 mm for BRRI-29, 80.75 mm for BRRI-26, 51.90 mm for BR-3, 66.70 mm for BRRI-28, 59.75 mm for BRRI-45, 60.10

mm for BRRI-50 and 64.35 mm for BR-14. BR-26 produced the longest seedling while BR-3 produced the shortest for 12 ppm As. Arsenate decreased the shoot length from 98.64 mm (control) to 61.85 mm for 12 ppm arsenate treatment. It can be seen that among all cultivars for all treatments the mean (Cultivar mean) shoot length of BRRI-29 is the shortest (68.60 mm) and the longest is for BR-26 (94.90 mm). From the CV (%) (Table 4) it can be seen that the shoot lengths of BR-3 was more scattered than that of other cultivars and the shoot lengths of BRRI-28 and BRRI-45 were more homogeneous.

Table 1. Effect of different levels of arsenate on germination percentage of seven rice cultivars

Treatments	Cultivars							Treatment mean
	BRRI -29	BR-26	BR-3	BRRI -28	BRRI -45	BRRI -50	BR-14	
T0	94.44± 2.94c	94.44± 2.94c	91.11± 1.11b	91.11± 2.94c	86.67± 3.33b	90.00± 1.95b	91.11± 1.11d	91.27
T1	88.89± 2.94abc	78.89± 2.94bc	86.67± 3.33b	88.89± 78.89bc	78.89± 1.11ab	88.89± 1.11b	84.44± 4.48cd	85.08
T2	91.11± 2.22bc	73.33± 1.92ab	86.67± 3.45b	85.56± 1.11bc	82.22± 4.84ab	86.67± 1.95ab	81.1± 1.11bc	83.81
T3	84.44± 1.11ab	75.55± 1.11ab	82.22± 1.11b	78.89± 2.9ab	77.78± 2.94ab	83.33± 5.09ab	76.67± 1.92bc	79.84
T4	83.33± 1.93 a	68.89± 2.94 a	78.89± 1.11 b	78.89± 6.19ab	75.56± 4.01ab	80.00± 3.85ab	80.00± 3.58 ab	77.14
T5	82.22± 1.11a	68.89± 2.94 a	57.78± 13.92a	71.11± 2.94a	74.44± 2.22 a	76.67± 3.85a	76.67± 3.85a	70.48
Cultivar Mean	87.41	76.67	80.56	82.41	79.26	84.26	78.33	
LSD (p<0.05)	3.22	3.79	9.02	4.93	4.88	4.83	4.48	
CV%	6.35	12.65	17.74	10.36	8.10	8.12	13.08	

In a column followed by common small letters do not differ significantly at 5% level.

Carbonell-Barrachina *et al*. (1998) showed that the growth of root and shoot was inhibited by high concentrations (5-20mg/kg) arsenic treatment. The inhibition was stronger in the roots than in the shoots when treated with arsenic (Wang *et al*., 2002). The reasons are that the plant roots are the first point of contact for these toxic arsenic species in the nutrient media (Abedin & Meharg, 2002) and the uptake of nutrition is inhibited in roots, as a result the growth of the whole plant is constrained (Mitchell & Barr, 1995).

Biomass: At the low level of arsenate (T_1=1ppm), BR-26, BR-3, BRRI-45, BRRI-50 and BR-14 cultivars were started to decrease the biomass of seedlings. With the increase of arsenate levels the biomass decreased from 0.0793 gm/ten seedlings for the control and reduced to 0.0594 gm/ten seedlings with 12ppm (T_5) arsenate. The mean biomass at 12ppm (T_5) arsenate was 0.044 gm for BRRI-29, 0.0595 gm for BR-26, 0.0665 gm for BR-3, 0.0665 gm for BRRI-28, 0.063 gm for BRRI-45, 0.0445 gm for BRRI-50 and 0.0705 gm for BR-14 (Figure 1). Therefore BR-14 had highest biomass followed by BR-3, while BRRI-29 had the lowest biomass followed by BRRI-50. The result, however, that BR-14 had the highest biomass for the arsenate 12ppm (T_5) treatment and BRRI-29 had the lowest. In this experiment, rice plants subjected to higher arsenate treatment produced lower biomass. There are number of reports of reduced shoot biomass/growth (Milam *et al*., 1988 and Marin *et al*.1993a) and root biomass (Abedin *et al*., 2002) in rice. However the result of the present study agrees with the results of those researchers.

Table 2. Effect of different levels of arsenate on tolerant percentage of seven rice cultivars

Cultivars	Parameters	Treatments						Cultivar mean
		T_0	T_1	T_2	T_3	T_4	T_5	
BRRI -29	Tolerance Percentage	100	94.12	97.47	89.41	88.24	87.06	92.55
	STDEV	2.357	7.071	0.00	2.357	2.357	2.357	
	SE	±1.667	±5.00	±0.00	±1.667	±1.667	±1.667	
BR-26	Tolerance Percentage	100	83.53	76.65	78.00	72.95	72.95	81.18
	STDEV	2.36	4.71	2.36	2.36	4.71	7.07	
	SE	±1.67	±3.33	±1.67	±1.67	±3.33	±5.00	
BR-3	Tolerance Percentage	100	95.13	95.13	90.24	86.59	63.42	88.42
	STDEV	2.36	5.05	0.00	7.07	0.00	4.71	
	SE	±1.67	±5.00	±0.00	±5.00	±0.00	±3.33	
BRRI -28	Tolerance Percentage	100	97.56	93.91	86.59	86.59	78.05	90.45
	STDEV	2.36	4.71	2.36	4.71	0.00	2.36	
	SE	±1.67	±3.33	±1.67	±3.33	±0.00	±1.67	
BRRI -45	Tolerance Percentage	100	91.02	94.87	89.74	87.18	85.89	91.45
	STDEV	2.36	2.36	7.07	4.71	2.36	2.36	
	SE	±1.67	±1.67	±5.00	±3.33	±1.67	±1.67	
BRRI -50	Tolerance Percentage	100	98.77	96.30	92.59	88.89	85.19	93.62
	STDEV	2.36	4.71	2.36	2.36	4.71	7.07	
	SE	±1.67	±3.33	±1.67	±1.67	±3.33	±5.00	
BR-14	Tolerance Percentage	100	92.68	89.01	84.15	87.81	84.15	89.63
	STDEV	2.36	5.05	0.00	7.07	0.00	4.71	
	SE	±1.67	±5.00	±0.00	±5.00	±0.00	±3.33	

Note: STDEV means Standard Deviation and SE means Standard Error

Therefore, it can be concluded from the results of the present study that arsenic is highly toxic and very sensitive at germination and seedling growth stage of rice when they are exposed to arsenic. But the severity of arsenic toxicity depends on rice genotypes or cultivars and in various concentrations of arsenic. All the rice cultivars used in this study showed decreasing germination percentage, tolerance percentage, root and shoot length and dry weight with increasing concentration of arsenic level. Therefore BRRI-29, BR-26 and BR-14 rice cultivars may be selected as arsenic tolerant rice regarding germination and tolerant percentage, root and shoot length and biomass of seedlings respectively.

Table 3. Effect of different labels of arsenate on root length (mm) of seven rice cultivars

Treatm-ents	Cultivars							Treat ment mean
	BRRI -29	BR-26	BR-3	BRRI -28	BRRI -45	BRRI -50	BR-14	
T0	114.6± 3.09c	120.9± 2.66c	98.75± 4.52c	107.15± 2.15d	124.8± 3.45d	83.40± 4.42d	104.55± 3.06c	107.74
T1	110.8± 3.60c	118.65± 3.25c	82.10± 4.11b	104.25± 3.39d	101.90± 5.09c	79.05± 3.20bc	97.75± 4.68bc	99.21
T2	104.65± 2.0c	117.05± 2.58c	80.45± 5.07b	99.55± 3.52cd	98.45± 4.96c	69.65± 4.35ab	95.95± 4.35bc	95.11
T3	86.3± 5.22b	108.3± 4.36bc	78.7± 6.96b	94.55± 2.32bc	94.50± 2.70bc	67.45± 4.50a	89.9± 4.85b	88.53
T4	82.5± 4.32b	102.65± 5.09b	77.00± 3.49b	86.05± 2.43ab	84.60± 3.99b	62.40± 3.05a	86.9± 5.59ab	83.56
T5	60.7± 5.22a	90.30± 6.01a	61.60± 3.69a	83.00± 4.31a	61.80± 5.03a	60.10± 2.30a	75.7± 4.19a	70.46
Mean	93.26	109.64	79.77	95.76	94.34	70.34	91.79	
LSD(p< 0.05)	5.35	5.54	6.27	4.09	5.64	4.90	5.92	
CV%	27.8	19.5	29.6	17.05	28.4	26.2	23.8	

In a column followed by common small letters do not differ significantly at 5% level.

Table 4. Effect of different levels of arsenate on Shoot length (mm) of seven rice cultivars

Treat ments	Cultivars							Treatme nt mean
	BRRI - 29	BR-26	BR-3	BRRI - 28	BRRI - 45	BRRI -50	BR-14	
T0	87.90 ± 4.74c	111.90± 5.40 b	91.55 ± 6.80 c	124.40 ± 4.86d	95.55 ± 4.27c	83.40 ± 4.42d	98.90 ± 5.07c	98.64
T1	84.80 ± 3.73c	98.35± 6.61ab	72.25± 7.10b	100.00 ± 3.99c	90.15 ± 6.50bc	79.05 ± 3.20bc	84.60 ± 7.64bc	86.41
T2	69.85 ± 3.83b	96.40 ± 6.50ab	68.55± 5.54ab	96.55± 4.26bc	88.35 ± 4.10bc	69.65 ± 4.35ab	83.00 ± 3.93bc	80.84
T3	59.20± 2.46bc	92.15± 3.88ab	67.65 ± 6.33ab	94.75 ± 4.10bc	82.80 ± 2.53bc	67.45 ± 4.50a	82.15 ± 8.89bc	76.72
T4	56.80 ± 4.87a	89.85 ± 7.37a	63.85 ± 4.62ab	84.05 ± 4.28 b	77.35 ± 5.03 b	62.40± 3.05a	71.90 ± 4.48ab	71.62
T5	53.00 ± 5.61a	80.75 ± 10.00a	51.90 ± 4.73a	66.70 ± 6.10 a	59.75 ± 4.43 a	60.10 ± 2.30 a	64.35 ± 5.25a	61.85
Cultivar Mean	68.60	94.90	69.29	94.40	82.33	70.34	80.82	
LSD (p<0. 05)	5.67	9.03	7.78	6.11	6.07	4.90	8.05	
CV%	34.02	33.3	41.2	28.4	28.4	26.2	35.9	

In a column followed by common small letters do not differ significantly at 5% level.

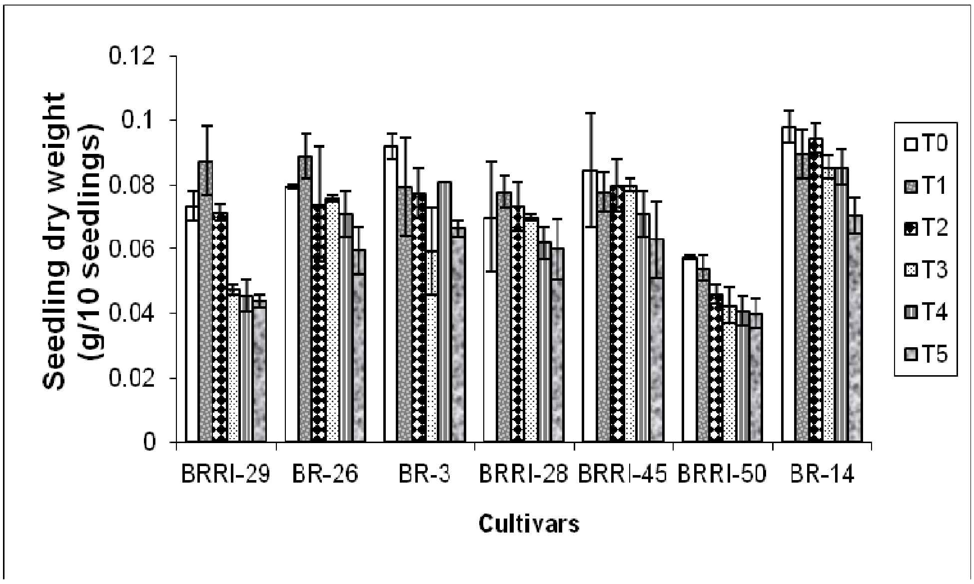

Fig. 1. Effect of different levels of arsenate on biomass or dry weight (g)/ ten seedlings of seven rice cultivars

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