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ABSTRACT

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Effects of arsenic (As) on seed germination, root and shoot length and biomass of rice varieties were investigated to elucidate the toxicity of As. Nine rice varieties *viz*. BR 11, BR 22, BRRI dhan29, BRRI dhan33, BRRI dhan39, BRRI dhan40, BRRI dhan47, BRRI dhan49 and BRRI dhan53 were grown with different levels of As (0, 15, 40, 70, 100 and 150 ppm). The experiment was laid out in a complete block design with three replications. Results showed that the germination percentage, root and shoot length and biomass of all rice varieties were gradually decreased with the increasing concentration of As solution. Among the rice varieties, BR 11, BR 22 and BRRI dhan49 showed highest degree of tolerance while BRRI dhan29 was the most susceptible to As toxicity.

Key words: arsenic, seed germination, root length, shoot length, biomass, rice varieties

INTRODUCTION

Environmental As may be derived from various natural sources *viz*. weathered volcanic, marine sedimentary rocks, fossil fuels, minerals, water, air, living organisms and anthropogenic activities including mining, agricultural chemicals, wood preservatives, medicinal products and industrial activities (Dowdle *et al.* 1996; Stolz and Oremland, 1999; Francisco *et al.* 2002; Mandal and Suzuki, 2002). Arsenic contamination of soil, streams, and underground water causes a major environmental and human health risk due to their long-term persistence in the environment (Mercedes *et al.* 2002). Groundwater contaminations with arsenic are also serious problems in Bangladesh. Oxidation of pyrite and arsenopyrite add As in the Bengal Delta Sediments due to excessive withdrawal and lowering of groundwater (Das *et al.* 1995; Chowdhury *et al.* 1999). These contaminated groundwater is used for irrigation as well as for drinking purpose in Bangladesh. Continuous irrigation with As contaminated groundwater increase the As concentration in the surface soil. The form of As present in soils depends on the types and amounts of sorbing components of the soils, the pH and the redox potential (Nriogo 1994).

Most studies on As in plants concentrate on the transformation of arsenical pesticides in crops such as rice, tomato, wheat or carrot. All plants growing on both arsenic-contaminated and uncontaminated sites have more than one As species in their tissues. A range of As compounds are found in plant tissues, for example, inorganic As(III) and As(V), methylated arsenic species, arsenobetaine and arseno-sugars. Plant species, which are not resistant to As suffer considerable stress upon exposure, with symptoms ranging from inhibition of root growth to death (Meharg and Hartley-Whitaker, 2002). Many studies indicated that low concentration of As stimulated the growth of plants; but excessive As did harm to plants (Han *et al.* 2002). Rice is one of the main grains in Bangladesh. Its quality directly affects people's life and food security. It is of practical significance to study the effects of arsenic stress on rice. The aim of this research was to determine the effect arsenic on germination and seedling growth of rice varieties.

MATERIALS AND METHODS

The experiment was conducted in the graduate laboratory of the department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur (24.000⁰N and 90.425⁰E), Bangladesh, during July to August 2011. Nine varieties of rice such as BR 11 (Mukta), BR 22 (Kiron), BRRI dhan29, BRRI dhan33, BRRI dhan39, BRRI dhan40, BRRI dhan47, BRRI dhan49, and BRRI dhan53 were used as test crop and collected from Bangladesh Rice Research Institute (BRRI). Germination test of different varieties of rice seeds were conducted under different levels of As. Six (6) levels of As were used *viz.* 0, 15, 40, 70, 100, 150 mg L⁻¹ As. Twenty five seeds of each test variety were placed in petridish filled with tissue paper. Desired concentrations of As were calculated from sodium arsenite (NaAsO₂). 10 mL of test solution was placed in each petridish. The tissue paper was kept moist constantly with distilled water. The experiment was arranged in a completely randomized design (CRD) with three replications at room temperature ($28\pm1^{\circ}$ C). Data were recorded after seven days of sowing.

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Parameters studied

Seed germination: For each treatment data were counted daily and sum of the data after 7 days were calculated using the formula given below:

Germination percentage = $\frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$

Seedling shoot and root length: The shoot and root length of seedlings were measured after 7 days of the respective germination setting. Ten seedlings were carefully uprooted randomly out of all the seedlings grown from all petridishes of each treatment with the help of forceps. The excess water was soaked with tissue paper. Shoot and root portions were separated with the help of scissor. Shoot and root length were measured in cm.

Seedling shoot and root fresh weight: Ten shoot and root samples of the above samples were packed separately in paper bags and shoot and root fresh weights were recorded by an electronic balance. Seedlings fresh weights were measured in gram (g).

Seedling shoot and root dry weight: After taking fresh weight those ten shoot and root sample packages were dried in an electric oven maintaining 72°C temperatures for 48 hours. After drying, the shoot and root dry weights were weighed by an electronic balance and they were recorded accordingly. Seedlings dry weights were also measured in gram (g).

Statistical analysis: All experimental data were subjected to analysis of variance (ANOVA) using statistical computer package MSTAT-C. Mean values were compared by the LSD or DMRT at the 5% level.

RESULTS AND DISCUSSION

Effect of different levels of arsenic on seed germination of rice varieties

Results revealed that seed germination of rice varieties were significantly affected by the As levels applied (Fig. 1).

BR 11: The highest germination (100%) was found for control treatment where only distilled water was added while the lowest (8%) was observed in 150 ppm As treatment. When As applied at concentration of 15, 40, 70 and 100 ppm then 22, 22, 11 and 7 seeds out of 25 seeds were geminated, respectively. BR 11 showed the best germination performance in 0 ppm As level followed by 15 ppm and 40 ppm As level. Increased levels of As significantly decreased the percentage of germination of seeds. Kaziul (2010) worked with the seeds of jute varieties and observed similar effect on germination under different concentration of As.

BR 22: In control (0 ppm As), 25 seeds were germinated (100%) upto seven days after seed sowing, where only distilled water was added. When the seeds received 15 ppm and 40 ppm As, the percentage of germination were 88 and 80, respectively, which were not significantly different from each other. In the treatments of 70, 100 and 150 ppm As, the percent germination were 9, 7 and 3, respectively.

BRRI dhan29: In case of BRRI dhan29, 25 seeds were germinated (100%) in control treatment (0 ppm As). But in 15 ppm and 40 ppm As, only 18 and 6 seeds were germinated, respectively. In rest of the treatments (70, 100 and 150 ppm of As) no seed was germinated.

BRRI dhan33: The highest germination percentage (100%) was observed in control treatment (0 ppm As) and lowest germination (8%) was observed in 150 ppm As level. In the treatments 15, 40, 70 and 100 ppm As, the germination percentage were 72, 60, 28 and 12%, respectively (Table 1).

BRRI dhan39: In control treatment of arsenic 24 seeds were germinated upto seven days after seed sowing out of 25 seeds, where the germination was 96%. But in 15, 40, 70 and 100 ppm of As treatments it was 84, 72, 32 and 20%, respectively. In rest of treatment not a single seed was germinated upto seven days of sowing and even no symptom of germination was observed later.

BRRI dhan40: Twenty five seeds were germinated within seven days after seed sowing, where germination percentage was 100 in control treatment (0 ppm As). In 15 and 40 ppm of As treatments, 19 and 15 seeds were germinated and it was 76 and 60%, respectively. But in 70 and 100 ppm of As treatments, only three and two seeds were germinated, respectively. No seeds were germinated in the rest of the treatment (150 ppm).

BRRI dhan47: In case of BRRI dhan47, 22 seeds were germinated in control treatment, where the percentage of germination was 88. In 15 and 40 ppm of As treatments, 18 and 13 seeds were germinated where the percentage of germination 72 and 52, respectively. But in 70 and 100 ppm of As treatments only three and two seeds were germinated, respectively. In 150 ppm As treated plates no seed was germinated.

BRRI dhan49: In control (0 ppm As) treatment twenty four seeds were germinated and germination percentage was 96. When the seeds received 15 ppm and 40 ppm As, the percentage of germination were 92 and 88, respectively. But in 70 and 100 ppm of As treatments only nine and five seeds were germinated, respectively. In 150 ppm of As treatment no seed was germinated.

BRRI dhan53: In control treatment of arsenic, 25 seeds were germinated upto seven days after seed sowing where the percentage of germination was 100. Out of twenty five seeds, twelve seeds were germinated within seven days after seed sowing in 15 ppm of As, where the germination percentage was 48. But in 40, 70 and 100 ppm of As treatments, 3, 3 and 1 seeds were germinated, respectively. In rest of the treatment (150 ppm As) not a single seed was germinated up to seven days of sowing and even no symptom of germination was observed later. The effect of As at different levels on germination in nine different varieties of seeds are shown in Fig. 1.

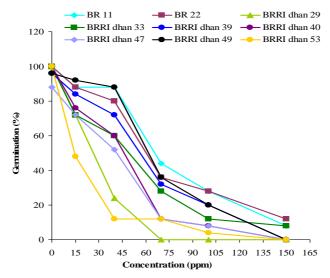


Fig. 1. Effects of different arsenic levels on seed germination of rice varieties

The results of this study revealed that increasing level of arsenic decreased the germination of rice varieties and BR 11, BR 22 and BRRI dhan49 showed highest resistance and BRRI dhan29 showed the susceptibility to arsenic levels. The reduction in germination percentage of rice varieties at higher concentrations may be attributed to the interference of arsenic ions during germination metabolism (Sankar Ganesh, 2008). Similar result was also reported in wheat (Panda and Patra, 1997), Cowpea (Lalitha *et al.* 1999), Pea (Chugh and Sawhney, 1996) and cotton (Shrivastava *et al.* 1997).

Effect of different levels of arsenic on shoot length of rice varieties

Shoot length of rice seedling was affected significantly by different levels of arsenic applied (Table 1). The shoot length of rice seedling for different treatments ranged from 0.30 cm to 5.0 cm. The highest shoot length was found with control treatment (0 ppm As) in all rice varieties compared to other levels (i.e., 15, 40, 70, 100 and 150 ppm of As). It's evident that the shoot length was gradually decreased with the increased As concentration. Therefore, the results of the present study clearly showed that different treatments of As has toxic effects on shoot length of rice seedling.

Effect of different levels of arsenic on root length of rice varieties

Root length also significantly decreased with the increased levels of arsenic concentration (Table 1). In without As plates the highest root length (6.6 cm) was found in BRRI dhan33 while the lowest root length (0.05 cm) was found in BRRI dhan47 at 150 ppm As treatment. The reason behind the decreasing trend of root length might be due to the toxic effect of arsenic in seedling physiology and possibly due to the accumulation of heavy metals in the plant tissues and its interaction with the minerals (Banu *et al.* 1997). Similar result was also reported in wheat (Panda and Patra, 1997), Cowpea (Lalitha *et al.* 1999), Pea (Chugh and Sawhney, 1996) and cotton (Shrivastava *et al.* 1997).

Effect of different levels of arsenic on seedling biomass of rice varieties

Fresh and dry weight of rice seedling was affected significantly due to the effect of arsenic treatments (Table 2). The highest fresh and dry weight of rice seedling was found in seedlings grown without As and the lowest seedling biomass was observed in the higher level of As concentration, in case of all the test rice varieties. These results of the present study showed that seedling biomass was decreased with increased As concentration. When uptake of nutrition was inhibited through roots, the growth of the whole plant was constrained leading to decreased plant biomass (Mitchell and Barr, 1995). The reason is that plant roots were the first organ come in contact with these toxic levels of As species (Abedin and Meharg, 2002).

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Conc. of As	Shoot length (cm)									Root length (cm)								
(ppm)	BR 11	BR 22	BRRI dhan29	BRRI dhan33	BRRI dhan39	BRRI dhan40	BRRI dhan47	BRRI dhan49	BRRI dhan53	BR 11	BR 22	BRRI dhan29	BRRI dhan33	BRRI dhan39	BRRI dhan40	BRRI dhan47	BRRI dhan49	BRRI dhan53
0	4.2 a	4.5 a	3.8 a	4.5 a	4.1 a	4.1 a	3.9 a	5.0 a	3.1 a	6.3 a	5.3 a	5.4 a	6.6 a	6.2 a	6.4 a	5.6 a	5.5 a	4.9 a
15	2.5 ab	1.3 b	1.3 b	2.2 b	2.6 ab	2.0 b	2.3 ab	2.5 b	1.6 ab	0.7 b	2.2 b	0.5 b	1.7 b	1.1 b	0.6 b	1.1 b	0.5 b	0.5 b
40	1.7 b	1.1 b	0.7 b	1.9 b	2.1 ab	1.5 b	1.4 b	2.0 b	1.3 ab	0.2 b	0.8 b	0.1 b	0.8 b	0.8 b	0.4 b	0.2 b	0.3 b	0.3 b
70	1.3 b	0.9 b	0.0	1.7 b	1.6 b	1.4 b	0.6 b	1.3 b	0.9 b	0.1 b	0.0	0.0	0.0	0.0	0	0.1 b	0	0.1 b
100	1.0 b	0.7 b	0.0	1.2 b	1.3 b	1.1 b	0.3 b	0.8 b	0	0.0	0.0	0.0	0.0	0.0	0	0.1 b	0	0
150	0.6 b	0.5 b	0.0	0.6 b	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0	0	0	0
LSD	1.9	1.1	2.0	1.9	2.0	2.0	2.0	2.2	1.8	2.6	2.4	2.5	2.7	2.6	2.3	2.5	2.5	2.3
CV (%)	6.0	8.1	12.4	5.7	6.0	6.9	8.5	6.8	9.3	13.0	10.5	14.6	10.5	11.5	12.93	12.70	14.09	14.50
Significance	**	**	*	**	**	**	**	**	**	*	*	*	*	*	*	**	*	*

Table 1. Effects of different levels of As on shoot and root length of rice varieties in plate culture

In a column figures with same letters do not differ significantly as per DMRT at 0.5% level of significance. ** = Significant at 1% level of probability

*= Significant at 5% level of probability

CV(%) = Coefficient of variation

Conc. of As (ppm)	Fresh weight (g)									Dry weight (g)								
	BR 11	BR 22	BRRI dhan29	BRRI dhan33	BRRI dhan39	BRRI dhan40	BRRI dhan47	BRRI dhan49	BRRI dhan53	BR 11	BR 22	BRRI dhan29	BRRI dhan33	BRRI dhan39	BRRI dhan40	BRRI dhan47	BRRI dhan49	BRRI dhan53
0	0.22 a	1.29 a	0.14 a	0.27 a	0.19 a	0.18 a	0.20 a	0.20 a	0.13 a	0.06 a	0.32 a	0.03 a	0.07 a	0.05 a	0.05 a	0.05 a	0.05 a	0.03 a
15	0.13 b	0.06 b	0.05 b	0.09 b	0.09 b	0.10 b	0.10 b	0.06 b	0.06 b	0.03 b	0.02 b	0.01 b	0.02 b	0.03 ab	0.02 b	0.03 b	0.01 b	0.01 b
40	0.08 b	0.05 b	0.01 c	0.06 b	0.08 b	0.05 c	0.08 b	-	-	0.02 c	0.01 b	0.01 b	0.02 b	0.02 b	0.01 b	0.02 b	-	-
70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD	0.06	0.02	0.01	0.04	0.02	0.02	0.03	0.05	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01
CV (%)	18.12	22.58	13.58	12.24	15.29	16.54	10.24	15.52	21.29	12.35	18.54	21.13	17.16	12.65	15.69	10.68	16.87	22.85
Significance	**	**	*	**	**	**	**	*	*	*	*	*	*	*	*	*	*	*

Table 2. Effects of different levels of As on seedling biomass of rice varieties in plate culture (for 10 seedling)

In a column figures with same letters do not differ significantly as per DMRT at 0.5% level of significance.

** = Significant at 1% level of probability

*= Significant at 5% level of probability

CV(%) = Coefficient of variation

Blank cell (-) indicates trace amount or no seed was germinated

CONCLUSION

From the aforesaid results, it is evident that increasing concentration of As significantly inhibited the seed germination and seedling growth parameters of rice varieties. The inhibition was stronger in root growth than in shoot growth. It also revealed that BR 11, BR 22 and BRRI dhan49 showed highest tolerance and BRRI dhan29 showed the susceptibility to As toxicity.

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