

INTERACTION EFFECTS OF ARSENIC AND PHOSPHORUS ON SEEDLING GROWTH, LEAF PIGMENTS AND LEAF PROTEIN OF RICE CULTIVARS UNDER HYDROPONIC CULTURE

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Abstract

Present study was conducted to evaluate the effect of arsenic and its interaction with phosphorus (P) on root and shoot growth, chlorophyll contents and leaf protein at seedling stage under hydroponic culture. Three cultivars of rice BR-26, BR-3 and BRRI-45 were used in this study. Arsenate significantly reduced root and shoot growth and chlorophyll content in all the cultivars. However leaf protein increased in BR-26, BR-3 and reduced in BRRI-45. Combined treatment of As and P significantly increased root and shoot length. Significant reduction of As toxicity was observed in hydroponic rice cultivars treated with As and P together. It may be said that P plays a vital role in the reduction of As contamination of rice and the results show that BR-26 is relatively tolerant to arsenate with respect to the root-shoot growth and leaf protein.

Key words: Arsenic, Phosphorus, Rice, Root length, Shoot length, Leaf pigment, Leaf protein

Introduction

Arsenic (As) contamination of groundwater is a severe problem in Bangladesh and it has affected at least 25 million people (Ravenscroft *et al.* 2005). Next to drinking water, rice could be a potential source of As exposure to the people living in the As affected areas of Bangladesh (Hossain *et al.* 2008 and Panaullah *et al.* 2009). Roberts *et al.* (2007) estimated that over 1000 tons of As might be transferred to arable land each year through As contaminated groundwater irrigation, creating a potential risk for future agricultural sustainability and food security of the country. People of Bangladesh not only drink the As contaminated groundwater, they also irrigate their crops (mainly rice) with it mostly in the Boro season. Long term use of As contaminated water for irrigation may result in the elevation of As concentration in soils and plants (Ullah 1998 and Haq *et al.* 2003). Normal irrigated soils in Bangladesh contain 4-8 mg As kg⁻¹ while soils irrigated with As contaminated water contain up to 83 mg As kg⁻¹ (Ullah 1998).

Arsenate uptake and toxicity/tolerance have been well characterized in plants (Hartley-Whitaker *et al.* 2001). Plants can develop toxicity symptoms such as: inhibition of seed

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germination (Abedin and Meharg 2002); decrease in plant height (Marin *et al.* 1992, Carbonel-Barrachina *et al.* 1995, Abedin *et al.* 2002b and Jahan *et al.* 2003a); depressed tillering (Kang *et al.* 1996 and Rahman *et al.* 2004); reduction in root growth (Carbonel-Barrachina *et al.* 1998 and Abedin *et al.* 2002); decrease in shoot growth (Cox *et al.* 1996 and Carbonel-Barrachina *et al.* 1998); lower fruit and grain yield (Carbonel-Barrachina *et al.* 1995, Kang *et al.* 1996 and Abedin *et al.* 2002b) and reduction in photosynthesis rate (Miteva and Merakehiyska, 2002) while they are exposed to excess As either in soil or in solution culture. Arsenate the dominant form of As in aerobic conditions is taken up by plants via the phosphate transport systems because of the chemical similarity between arsenate and phosphate (Dixon 1997). Phosphorus is a chemical analogue of As (Adriano 2001) which competes with As in plant uptake (Meharg and Macnair 1992), is one of essential elements for plant growth (Raghothama 1999). The effect of P on the absorption of As in soil environments has received great attention, especially when P is used as a crop fertilizer (Peryea 1998). Phosphorus fertilizer is common in rice cultivation which might interact with As uptake (Qafoku *et al.* 1999). In rice seedlings, cultivars found to be susceptible to arsenate and become more resistant by raising level of intracellular P (Geng *et al.* 2006 and Wang and Duan 2009).

Arsenic contamination in rice has become a serious problem, and it is therefore, urgent to develop strategies to minimize As accumulation or toxicity in rice plants. With this in view hydroponic culture of some rice cultivars was used to study the effect of arsenate-phosphorus interaction on seedling growth, chlorophyll content and protein content in leaf at early seedling stage of rice.

Materials and Methods

Rice cultivars: Based on the previous knowledge (Ghosh 2011) on germination and seedling growth three cultivars of rice were chosen for the present study. The cultivars collected from Bangladesh Rice Research Institute (BRRI) were BR-26, BR-3 and BRRI-45.

Treatments: Following Islam and Jahiruddin (2010) a total of nine treatments of which two concentrations were sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) solution, such as $T_1=6\text{ppm}$ and $T_2=12\text{ppm}$ As, six were mixed sodium arsenate and phosphate (source KH_2PO_4) solutions eg., $T_0P_1=0$ ppm (As) and 31 ppm (P), $T_0P_2=0$ ppm (As) and 62 ppm (P), $T_1P_1=6$ ppm (As) and 31 ppm (P), $T_1P_2=6\text{ppm}$ (As) and 62 ppm (P), $T_2P_1=12\text{ppm}$ (As) and 31 ppm (P), $T_2P_2=12$ ppm (As) and 62 ppm (P) and one of distilled water was used as the control.

Seedling culture: The seedling growth experiment was conducted by hydroponic cultures under semi laboratory condition. On the emergence of the radicals, 5 germinated seeds were placed in the hole of plastic nets attached with cork sheets all around the net. These materials are round in shapes which were then placed on the mouth of a 250 ml plastic pot. The outside of the plastic pots were painted with black colour. The pots were filled with 250 ml of nutrient solution composed of 5 mM $\text{Ca}(\text{NO}_3)_2$, 5 mM KNO_3 , 5mM

K_2SO_4 and 5 mM KH_2PO_4 in distilled water (Abbas and Meharg 2008). The plants were grown hydroponically in these pots. Deep colour plastic beads were added at the upper portion of each pot to prevent the light from penetrating the solution. The nutrient solutions were contaminated with the treatments mentioned in section treatments separately. 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 (30°C) for 15 days. The solutions of the growth media were renewed every week. The seedlings were collected and washed with distilled water on 15 DAT (Days after treatment). Shoots and roots were separated and root length and shoot length of growing seedlings were measured.

Quantitative determination of leaf pigment: Chlorophyll a and chlorophyll b content of leaves were determined from the samples of the control, arsenate treatments and arsenate-phosphate interaction treatments. The leaf pigments were determined from 10 day old plants. The analysis of pigments was determined spectrophotometrically. Leaf materials were cut into small pieces and mixed thoroughly. One gm of fresh leaf was smashed finely in a mortar and made into paste with 100 ml of cold 80% acetone. The homogenate was filtered through Whatman No. 1 filter paper. The final volume of the mixture was made 100 ml by adding extra acetone. The optical density (OD) for each solution was measured at 663 and 645 nm against 80% acetone as blank. Specific absorption coefficient method of Mckinney (1940) and the formula of Machlachalan and Zalik (1963) were used to determine the amount of chlorophyll-a and chlorophyll-b as mg/g fresh leaf.

Quantitative determination of leaf protein: Using the method of Lowry *et al.* (1951) soluble protein content of fresh leaves of seedlings was determined for each treatment at the growth stage. The water soluble leaf protein was extracted by smashing 0.1 gm of coarsely chopped leaf tissue with 10 ml of distilled water in a mortar with a pestle. The homogenate was rapidly filtered in Whatman No.1 filter paper and the filtrate was used for protein assay. The protein solution was measured at 650 nm by using UV spectrophotometer (Unicum) and the amount of protein was determined by comparing with a standard curve prepared from known amounts of bovine albumin (BDH, England).

Statistical analysis: Completely randomized design (CRD) was used for the experiment. Recorded data were analyzed with using SPSS 16.0 program and results are compared by least significance difference test (LSD Test) at 5% probability level (Gomez and Gomez 1984). Increases or decreases of leaf protein percentage at different treatments were calculated by taking the protein content in control plant as 100%.

Results and Discussion

Root length: Root growth of three cultivars of rice is presented in Table 1. Significant differences in results (at 5% probability level) were observed among the treatments. The longest root length was found for the control plants in all cultivars, e.g., 121.7 mm for BR-26, 106.5 mm for BR-3 and 88 mm for BRRI-45, whereas for the highest As treatment ($T_2=12$ ppm As), shortest root length was found in all cultivars, e.g., 38 mm for BR-26, 29.5 mm for BR-3 and 28.2 mm for BRRI-45 (Table 1). The significant effects of combined arsenate-phosphorus were found for T_1P_1 and T_2P_2 . At T_2P_2 (highest

concentration of As and P) treatments such that the root lengths 60.3 mm for BR-26, 43.3 mm for BR-3 and 40.90 mm for BRRI-45, which are longer than that of the root length for T₂ (highest concentration of As) treatment. The mean root length for T₂ treatment was 31.9 mm, whereas that for T₂P₂ was 48.17 mm, which is 51% higher than T₂ treatment. The mean root length decreased from 105.4 mm for the control to 31.9 mm for T₂ (12ppm of As) and increased to 77.43 mm for T₂P₁ (12ppm As and 31ppm P). Among all cultivars for all the treatments it was observed that BR-26 produced relatively the longest root followed by BR-3, while BRRI-45 produced the shortest root. From the CV (%), it can be said that the root length of BRRI-45 was more scattered and BR-3 was more homogeneous (Table 1). The results also show that the control plants produced the longest root length, whereas only As treated plants formed shortest root length and when the plants were treated with both the As and P, root growth was increased over the arsenic treated plants for all cultivars. This result indicates that the P inhibits the toxicity of As in rice cultivars and also induces the root growth in the presence of As. The result of the present study agrees well with those of Shaibur *et al.* (2013) and Sneller *et al.* (1999).

Table 1. Effect of sodium arsenate and phosphate interaction on root length (mm) of three rice cultivars.

Treatments			Cultivars			Treatment means
	Arsenate (ppm)	Phosphate (ppm)	BR26	BR 3	BRRI 45	
T ₀	0	0	121.70	106.50	88.00	104.40
T ₁	6	0	54.20	38.9	36.30	43.13
T ₂	12	0	38.00	29.50	28.20	31.90
T ₀ P ₁	0	31	123.60	73.10	101.20	99.30
T ₀ P ₂	0	62	109.60	63.90	72.00	81.83
T ₁ P ₁	6	31	82.50	61.20	46.40	63.37
T ₁ P ₂	6	62	92.10	76.70	63.50	77.43
T ₂ P ₁	12	31	52.20	37.90	26.80	38.97
T ₂ P ₂	12	62	60.30	43.30	40.90	48
Cultivars mean			81.00	59.00	55.92	
LSD at 5%			5.63	5.02	5.79	
CV %			42.01	45.89	53.79	

Shoot Length: Shoot length was recorded 15 days after the hydroponic culture. The longest shoot length was found for the control plants in all cultivars, e.g., 128.70 mm for BR 26, 162.20 mm for BR 3 and 171.00 mm for BRRI 45, whereas for the highest sodium arsenate treatment (T₂), the shortest shoot length was found in all the cultivars, e.g., 74.40 mm for BR 26, 66.00 mm for BR 3 and 58.20 mm for BRRI 45 (Table 2). The

significant effect of arsenate-phosphorus was found for the treatment T_1P_2 , where the shoot lengths have 103.4 mm for BR 26, 115.8 mm for BR 3 and 77.9 mm for BRRI 45, which were 38.98, 75.45 and 34.31% increase respectively over that of T_2 treatment (highest As level). Treatment mean of shoot length was decreased gradually from 153.97 for the control treatment to 66.2 mm for T_2 (12ppm As), whereas shoot length increased from 66.2mm for Control treatment to 84.17mm for T_2P_2 (12ppm As and 62ppm P), which was 27.15% higher than the T_2 treatment. From the CV (%) it is found that the shoot lengths of BRRI 45 are more scattered and the shoot lengths of BR 26 are more homogeneous (Table 2). P has a significant effect on the shoot growth. The combined effects of As and P on the growth of shoot length were found significant at 5% probability level. The results of the present study showed that the cultivar BR 26 produced the longest shoot for the combined treatment 12 ppm As and 62 ppm P except for the control. It can then be said that 62 ppm P treatment can reduce the toxicity of As better when rice grown in As contaminated media. Sharma and Travlos (2012) suggested that increased supply of phosphate provided protection to the plant system and the effect of phosphate appears in the form of a reduced amount of growth inhibition during arsenic toxicity.

Table 2. Effect of sodium arsenate and phosphate interaction on shoot length (mm) of three rice cultivars.

Treatments	Cultivars			Treatment means		
	Arsenate (ppm)	Phosphate (ppm)	BR 26		BR 3	BRRI 45
T_0	0	0	128.70	162.20	171.00	153.97
T_1	6	0	94.50	71.10	74.20	79.93
T_2	12	0	74.40	66.00	58.20	66.2
T_0P_1	0	31	115.90	148.40	130.00	131.43
T_0P_2	0	62	110.40	102.00	128.70	113.7
T_1P_1	6	31	101.50	104.60	61.00	89.03
T_1P_2	6	62	103.40	115.80	77.90	99.03
T_2P_1	12	31	87.00	79.50	73.30	79.33
T_2P_2	12	62	93.30	82.30	76.90	84.17
Cultivars mean			101.01	103.54	94.58	
LSD at 5%			7.07	9.99	6.54	
CV %			24.80	41.04	43.63	

Chlorophyll content: Chlorophyll content in leaves of early seedling was measured after 11 days of growth. Chlorophyll-a, chlorophyll-b and total chlorophyll of three cultivars of rice are presented in Table 3. Highest chlorophyll-a and chlorophyll-b contents were found in rice cultivar BR 45 for the control plants. The mean value of chlorophyll-a and chlorophyll-b contents decreased for both As and combined As and P treated seedlings of

BR 26 and BR 3. The chlorophyll content decreased significantly with the increase of arsenate concentration in all the cultivars. Beside these, the chlorophyll-a, chlorophyll-b and total chlorophyll increased in seedlings of BRRI 45 for the combined treatments of T₁P₁ and T₁P₂ compared with the As treatments T₁ and T₂ (Table 3). However the chlorophyll content and increasing or decreasing tendencies of chlorophyll content varied within the cultivars and also for the different treatments. The chlorophyll content was found to increase in BRRI 45 for the combined As and P treatments T₁P₁ and T₁P₂ compared to T₂ treatment. Van and Clijsters (1990) suggested that the protochlorophyllide reductase activity was inhibited by heavy metal. Chlorophyll content was decreased by As treatment, because heavy metal entered in leaves and accumulated excessively in some parts and combined with –SH base of protein or substituted for Fe²⁺, Zn²⁺, Mg²⁺ and so on and then destroyed the structure and function of chloroplast. Thus the high concentration of heavy metals, chlorophyll content in plants decreases. The decreasing tendency of chlorophyll found in present study for As treated seedlings agrees well with the findings of Van and Clijsters (1990).

Table 3. Arsenate-phosphorus interaction effect on chlorophyll content of rice seedlings at hydroponic culture.

Cultivars	Treatments	Chlorophyll- a content		Chlorophyll- b content		Total Chlorophyll	
		mg/g	Relative % (over control)	mg/g	Relative % (over control)	mg/g	Relative % (over control)
BR 26	T ₀	0.791	100	0.421	100	1.212	100
	T ₁	0.579	73.23	0.289	68.51	0.868	71.59
	T ₂	0.285	36.05	0.144	34.26	0.429	35.42
	T ₀ P ₁	0.186	23.44	0.097	23.02	0.282	23.29
	T ₀ P ₂	0.185	19.98	0.064	15.19	0.222	18.31
	T ₁ P ₁	0.199	25.17	0.077	18.20	0.276	22.74
	T ₁ P ₂	0.208	26.26	0.087	20.81	0.295	24.37
	T ₂ P ₁	0.165	20.87	0.072	17.17	0.237	19.58
	T ₂ P ₂	0.175	22.13	0.096	22.86	0.271	22.39
BR 3	T ₀	0.992	100	0.379	100	1.371	100
	T ₁	0.874	88.10	0.167	44.06	1.041	75.93
	T ₂	0.654	65.89	0.154	40.81	0.808	58.96
	T ₀ P ₁	0.861	86.79	0.374	98.68	1.235	90.08
	T ₀ P ₂	0.53	53.43	0.263	69.31	0.793	57.82
	T ₁ P ₁	0.291	29.33	0.143	37.73	0.434	31.66
	T ₁ P ₂	0.317	31.96	0.176	46.44	0.493	35.96
	T ₂ P ₁	0.175	17.64	0.153	40.37	0.328	23.92
	T ₂ P ₂	0.298	30.04	0.168	44.42	0.466	34.01
BRRI 45	T ₀	1.136	100	0.742	100	1.878	100
	T ₁	0.548	48.23	0.355	47.87	0.903	48.08
	T ₂	0.214	18.83	0.144	19.37	0.358	19.01
	T ₀ P ₁	1.244	109.47	0.665	89.76	1.910	101.69
	T ₀ P ₂	0.992	87.30	0.505	68.18	1.498	79.75
	T ₁ P ₁	0.782	68.82	0.420	56.63	1.202	64.00
	T ₁ P ₂	0.879	77.35	0.492	66.34	1.371	73.00
	T ₂ P ₁	0.287	25.29	0.089	11.96	0.376	20.02
	T ₂ P ₂	0.542	47.73	0.263	35.46	0.805	42.88

Note: Data are the average value of three replicates. Relative percentage of chlorophyll a, b and total chlorophyll was calculated by taking the chlorophyll content in control plant as 100%.

Protein content: The results of protein content in fresh leaves of early seedlings (11 days after growth) at different concentrations of As and As-P treatments of three cultivars are presented in the (Figs.1 and 2). An interesting result is found that the protein contents in leaves increased in BR 26 and BR 3, but slightly decreased in BRRI 45 for the As treatment T₂. For the combined treatment T₂P₂ the total protein content 22.187 mg/g were found for BR 26, 24.201 mg/g for BR 3 and 28.567 mg/g fresh leaf for BRRI 45 which were lower than that of T₂ treatment. It was also observed that the protein content of BRRI 45 was decreased by both sodium arsenate and As-P treatments (Fig. 2). In the present study it was found that the protein content of rice cultivar BR 26 and BR 3 has an increasing tendency with the increase of As concentration but it turned to a decreasing tendency for BRRI 45. The soluble protein content in plant cells is an indicator for their physiological state. The growth of plants was inhibited by arsenate treatment and the protein content that was not used, was accumulated in cells and this caused the increased of soluble protein (Yu *et al.* 1995).

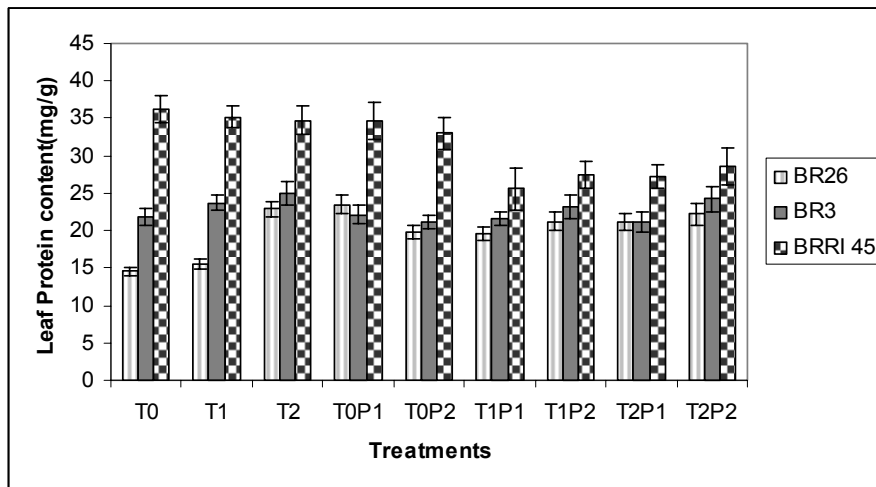


Fig.1. Effect of sodium arsenate and arsenic-phosphorus interaction on seedling leaf protein of three rice cultivars grown in hydroponic culture.

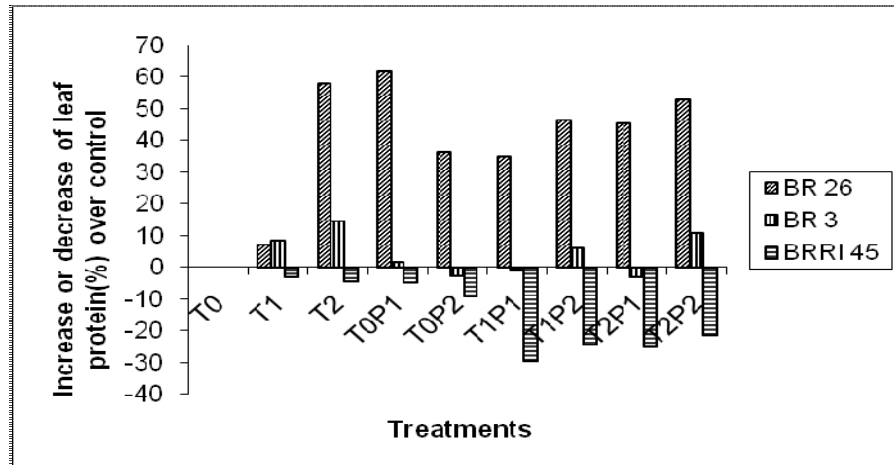


Fig. 2. Leaf protein percentage increases or decreases over control for different levels of arsenic and phosphorus of three rice cultivar.

In conclusion it may be said that heavy metal As inhibits root, shoot growth and chlorophyll synthesis of rice cultivars severely but leaf protein content increased in As treated rice cultivars, BR 26 and BR 3. Macro nutrient P plays an important role for reducing the toxicity of As and it provokes to increase the root and shoot growth of As treated rice cultivars. In the present study it is found that rice cultivar BR 26 seems to be As tolerant considering the root-shoot growth and leaf protein.

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(Received revised manuscript on 20 March 2014)