

**ORIGINAL RESEARCH**

Arsenic-induced morphological variations and the role of phosphorus in alleviating arsenic toxicity in rice (*Oryza sativa* L.)

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ABSTRACT

Primarily, this study was conducted to screen for As tolerant rice genotypes based on morpho-physiological features. Root length and root fresh weight significantly reduced in BRRI-39, BRRI-41 and BRRI-51 due to As treatment compared to controls excluding BRRI-33. Further, BRRI-33 did not show significant decrease in shoot height and shoot fresh weight due to As treatment; whereas these parameters were severely affected by same conditions in other genotypes. It does suggest that BRRI-33 is able to tolerate As and its growth parameters are not affected by As treatment. In contrast, BRRI-39, BRRI-41 and BRRI-51 are sensitive to As. Secondly, As and P supplementation significantly increased chlorophyll concentrations in BRRI-33, BRRI-44 and BRRI-51 but not in BRRI-39 compared to controls and As treated plants. Again, As and P treatment significantly increased the root and shoot dry weight in BRRI-33 and BRRI-44 compared to As treated plants. Finally, dual treatment of As and P significantly decreased the As concentration in leaves of BRRI-33, BRRI-44 and BRRI-51 but not in BRRI-39. It suggests that P is involved in alleviating As toxicity and inhibiting As uptake in leaves of rice. Among them, BRRI-33 is most efficient in utilizing P in alleviating As toxicity.

Keywords: Hydroponic culture, arsenic toxicity, rice, phosphorus, screening.

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Introduction

Arsenic (As) is a metalloid element naturally occurring in the environment (Duan et al. 2005). Contamination of food with arsenic is a potential health risk for both humans and animals in many regions of the world, especially in Asia. Arsenic can be accumulated in humans, animals and plants for a longer period and a long-term exposure of humans to arsenic results in severe damage of kidney, liver, heart etc. and many other vascular diseases. Rice grains produced in the south-west regions of Bangladesh have shown to contain 1.8mg of arsenic/kg dried rice (Guo et al. 2007). In non-hyper-accumulating plants, such as rice, the accumulation of arsenic seems to follow the trend from root to straw to husk to grain, i.e. highest concentration in root tissue. Hyper-accumulating plants, e.g. *Pteris vittata*; Chinese brake fern, have higher concentrations of arsenic in their above ground parts (Cesaro et al. 2015).

Among the barriers to the breeding of heavy metal tolerant plants is the proper selection methodology of these individuals. The main problems are related to the difficulty of evaluating a large number of plants under uniform conditions while keeping the amount of environmental residues low. As an alternative to soil, sand or substrate, plants can be grown in nutrient solution. In these so-called hydroponic systems, different crops are being successfully produced on a commercial scale, under highly homogeneous and controlled cultivation conditions. Furthermore, cultivation in a hydroponic system has the advantage of generating less waste, since the contaminated nutrient solution can be evaporated, reducing the final metal residue to few milligrams. For these reasons, some authors have proposed

different methods for selecting Al-tolerant barley (Tamás et al. 2006) and maize plants (Giaveno et al. 2000), grown in nutrient solution. However, screening of As tolerant rice had never been extensively performed.

As uptake depends on environmental factors such as soil type, nutrient supply and medium pH. Thus, elucidation of the relationships between As and plant nutrients is essential for developing an efficient production technique to grow plants for phytoremediation purposes. Of these all factors, P and pH are the most important ones influencing plant growth and As uptake (Tu and Ma 2003). Moreover, the interaction between P and As needs careful attention as they are chemical analogues. It has been demonstrated that arsenate uptake into the root cytoplasm is mediated by phosphate carriers in the plasma membrane (Begum et al. 2016, Tu and Ma 2003). Therefore, most studies dealing with As have coupled the two while investigating their interactions in both higher and lower plants.

Phosphate has long been reported to suppress plant uptake of arsenate. In a hydroponic solution containing 50 μM arsenate, sufficient phosphate will alleviate arsenate toxicity and improve plant growth. Plant arsenate uptake rate is reduced by 75% at 0.5 mM phosphate (Meharg et al. 2009). A molar P/As ratio of at least 12 is needed to protect plants against arsenate toxicity. Nonetheless, plant arsenate uptake and toxicity depends on both the P/As ratio and phosphate nutrition levels. A hydroponic study has shown that at the same P/As ratio arsenate is much less toxic at high phosphate levels since more arsenate is taken up by the plants at low phosphate levels (Sneller

et al. 1999).

Knowledge of arsenate and phosphate interactions is important for a better understanding of their uptake and accumulation by plants due to the similarities in chemical behaviors of the two ions. Little is known about the interactive effects of As and P on plant growth and uptake of As and P, especially in Bangladeshi rice cultivars. Therefore, the objectives of this study were to (i) investigate the tolerance of Bangladeshi rice cultivars in response to As in hydroponic culture, and (ii) examine the effect of phosphate in alleviating As toxicity and As uptake in rice. The results will provide critical information for better understanding arsenate tolerance by rice and optimizing soil conditions for arsenate phytoextraction.

Materials and methods

Plant materials

Four genotypes of *Oryza sativa* L. (BRRI-33, BRRI-39, BRRI-44 and BRRI-51), with different tolerance to As, were used in this study.

Germination and growth conditions

Before growing, seeds were surface sterilised in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min, respectively. Seeds were then rinsed five times in deionised water. Seeds were germinated on moist filter paper wetted with deionised water for 3–4 days in the dark at room temperature. Only healthy and uniform seedlings were transplanted to solution culture. A basal nutrient solution (Hoagland and Arnon 1950) was used with the following nutrient concentrations (μM): KNO_3 (16000), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (6000), $\text{NH}_4\text{H}_2\text{PO}_4$ (4000), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2000), KCl (50), H_3BO_3 (25), Fe-

EDTA (25), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2), ZnSO_4 (2), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5). Plants were grown in 600ml of aerated solution and the environment was strictly maintained under 10 h light and 14 h dark ($550\text{--}560 \mu\text{mol s}^{-1}$ per μA). The pH was adjusted to 6.3 by using NaOH or HCl. As was exposed by adding KH_2AsO_4 (20 μM) to the treatment solutions. The seedlings kept in Hoagland not containing KH_2AsO_4 served as control.

For phosphorus experiment, seeds of surface-sterilized seeds were grown in small pots containing soil under polythene shade. The soil contained all plant essential nutrients (N, K, P, Fe, P, S, etc.). Plants were grown three different conditions: Condition A: Soil pot without As; Condition B: Soil pot containing 15 mg As/kg; Condition C: Soil pot containing 15 mg As + P.

Measurement of morphological characters

Shoot height, root length, shoot fresh weight and root fresh weight were measured on 2-week old plants grown on solution culture. Total roots developed by each plant sample were washed in distilled water to remove nutrient and then quickly blotted in tissue paper.

Measurement of chlorophyll concentrations

A chlorophyll content of leaves was determined spectrophotometrically as described previously (Mandal and Suzuki 2002). Firstly, 100 mg leaf was weighted and placed in 95% acetone in a 5 ml falcon tube. The leaf sample was then grinded using mortar-pestle. The homogenate was filtered through whatman filter and was centrifuged at 2500 rpm for 10 min. The supernatant was separated and the absorbance were read at 662 (chlorophyll a) and 645 (chlorophyll b) on spectrophotometer. The

amount of these pigments was calculated according to the formula (Mandal and Suzuki 2002).

Determination of As content in leaves

Firstly, the dried leaf samples (1g) were digested and HNO₃ mix is heated at 75°C for 10 min, followed by 109°C for 15 min. After cooling for 10 min, 1 ml of H₂O₂ was added to each vessel through the ventilation hole and the sample mix is heated at 109°C for a further 15 min. The samples were then analysed for As concentration by Flame Atomic Absorption Spectroscopy (AAS) outfitted with ASC-6100 auto sampler and air-acetylene atomization gas mixture system (Model No. AA-6800, Shimadzu). Standard solutions of As were prepared from their respective concentration of 1000 ppm stock solutions (Shimadzu), from which further serial dilutions (0.1-4 ppm) were made to cover the optimum absorbance range for the standard calibration curve. For the determination, two solutions were prepared for

each sample. Reagent blank determinations were used to correct the instrument readings.

Statistical analysis

Statistical analyses (t-test) were performed using Genstat software (14th Edition). Significance was set at P≤0.05. Three replications of each sample have been used for all experiments.

Results

Root morphological features

Root length and root fresh weight were measured in all rice genotypes grown in both As deficient and As treated solution culture. Root length was not significant decreased due to As treatment in nutrient media compared to the plants grown without As only in BRRI-33 (Table 3.1, Fig. 1). However, BRRI-39, BRRI-41 and BRRI-51 significantly decreased in root length under As treatment compared to the As less nutrient media (Table 1, Fig. 1).

Table 1. Root length (cm) in different genotypes of rice grown on As deficient (As -) and As treated (As +) hydroponic culture. There were three replications for each sample. Data were taken on two weeks old plants.

Genotypes	As -	As +	t-test
BRRI-33	4.83±0.76	4.33±0.57	NS
BRRI-39	4.83±0.56	3.66±0.76	*
BRRI-44	6.03±0.55	4.50±0.86	*
BRRI-51	8.00±3.96	5.33±0.57	*

statistically significant

^{NS}statistically non-significant.

Similarly, BRRI-33 did not show significant decrease in root fresh weight under As treatment compared to the plants grown on As free nutrient solution (Table 2). In contrast, root fresh weight was significantly reduced in BRRI-39, BRRI-41 and BRRI-51 due As treatment

compared to controls.

Shoot morphological features

Shoot height and shoot fresh weight were measured in all rice genotypes grown in both As deficient and As treated solution culture.

Table 2. Root fresh weight (g) in different genotypes of rice grown on As deficient (As -) and As treated (As +) hydroponic culture. There were three replications for each sample. Data were taken on two weeks old plants.

Genotypes	As -	As +	t-test
BRR1-33	0.058±0.017	0.049±0.011	NS
BRR1-39	0.074±0.040	0.038±0.022	*
BRR1-44	0.109±0.045	0.024±0.004	*
BRR1-51	0.082±0.010	0.091±0.039	*

*statistically significant

^{NS}statistically non-significant.

Table 3. Shoot height (cm) in different genotypes of rice grown on As deficient (As -) and As treated (As +) hydroponic culture. There were three replications for each sample. Data were taken on two weeks old plants.

Genotypes	As -	As +	t-test
BRR1-33	13.50±0.50	12.33±0.57	NS
BRR1-39	8.93±0.40	8.50±0.50	*
BRR1-44	11.00±1.80	10.33±1.52	*
BRR1-51	13.66±1.52	9.33±1.15	*

*statistically significant

^{NS}statistically non-significant.

Shoot height was not significantly decreased due to As treatment in nutrient media compared to the plants grown without As only in BRR1-33 (Table 3).

However, BRR1-39, BRR1-41 and BRR1-51 significantly decreased in shoot height under As treatment compared to the As less nutrient media (Table 3).

Similarly, BRR1-33 did not show significant decrease in shoot fresh weight under As treatment compared to the plants grown on As free nutrient solution (Table 4).

In contrast, shoot fresh weight was significantly reduced in BRR1-39, BRR1-41 and BRR1-51 due to As treatment compared to controls (Table 4).

Table 4. Shoot fresh weight (g) in different genotypes of rice grown on As deficient (As -) and As treated (As +) hydroponic culture. There were three replications for each sample. Data were taken on two weeks old plants.

Genotypes	As -	As +	t-test
BRR1-33	0.075±0.005	0.066±0.009	NS
BRR1-39	0.033±0.010	0.031±0.011	*
BRR1-44	0.055±0.005	0.059±0.014	*
BRR1-51	0.037±0.016	0.040±0.021	*

*statistically significant

^{NS}statistically non-significant.

Effect of phosphorus on chlorophyll (a and b) concentrations

Chlorophyll concentrations (a and b) were measured on all the four genotypes grown under three different conditions (without As, As treated and As + P treated). Chlorophyll concentrations (a and b) were similar in all genotypes except for BRRI-44 under control. When plants were treated with As in soil, chlorophyll concentrations (a and b) in leaves were significantly reduced in BRRI-39, BRRI-44 and BRRI-51 (Fig. 2). However, no significant difference was found in chlorophyll concentrations (a and b) in leaves of BRRI-33 between plants grown in control (without As) and As treated soil. In addition, when plants were grown in As and P together, significant

increase of chlorophyll concentrations were observed in BRRI-33, BRRI-44 and BRRI-51. However, P treatment was not able to enhance chlorophyll concentrations in BRRI-39 compared to control and As treated plants (Fig. 2).

Effect of phosphorus on in root and shoot biomass

Root and shoot dry weights were measured on all the four genotypes grown under three different conditions (without As, As treated and As + P treated). As treatment significantly reduced root dry weight in all genotypes except for BRRI-33 compared to controls. When plants were grown with As and P treated soil, root dry weight was significantly increased in BRRI-33 and BRRI-44 (Table 5).

Table 5. Effect of phosphorus on root dry weight.

Genotypes	Control (without As)	15 mg As	15 mg As + P
BRRI-33	0.042±0.0035	0.040±0.0007	0.050±0.0007*
BRRI-39	0.050±0.0007	0.040±0.0007	0.041±0.0014
BRRI-44	0.051±0.0021	0.037±0.0035	0.046±0.0014*
BRRI-51	0.012±0.0021	0.011±0.0014	0.010±0.0007

*statistically significant.

Increase was also observed in BRRI-39 and BRRI-51 in similar conditions though it was not statistically significant. Similarly, As treatments reduced the shoot dry weight in all four genotypes compared to As deficient controls.

But As and P treatments significantly increased shoot dry weight in BRRI-33, BRRI-44 and BRRI-51 compared to the plants grown only in As treated soil pots (Table 6).

Table 6. Effect of phosphorus on shoot dry weight.

Genotypes	Control (without As)	15 mg As	15 mg As + P
BRRI-33	0.213±0.0049	0.192±0.0028	0.246±*0.0049
BRRI-39	0.171±0.0014	0.150±0.0007	0.166±0.0056
BRRI-44	0.133±0.0049	0.124±0.0056	0.176±*0.0084
BRRI-51	0.210±0.0141	0.153±0.0042	0.211±*0.0155

*statistically significant

Effect of phosphorus on As uptake in leaves
Concentration of As in leaves of rice plants were

determined grown under control, As treated and As+P treated soil pots. It was observed that dual

treatment of As and P significantly decreased the As concentration in leaves of BRRI-33, BRRI-44 and BRRI-51 compared to As treated plants (Fig. 3). However, As+P treatment did not affect the As uptake in leaves of BRRI-39 (Fig. 3).

Discussion

Arsenic (As) is a metalloid ubiquitously present in soils, normally at trace quantities. Worldwide, natural soil concentrations are around 5mg kg^{-1} , and this varies depending on the origin of the soil (Mandal and Suzuki 2002).

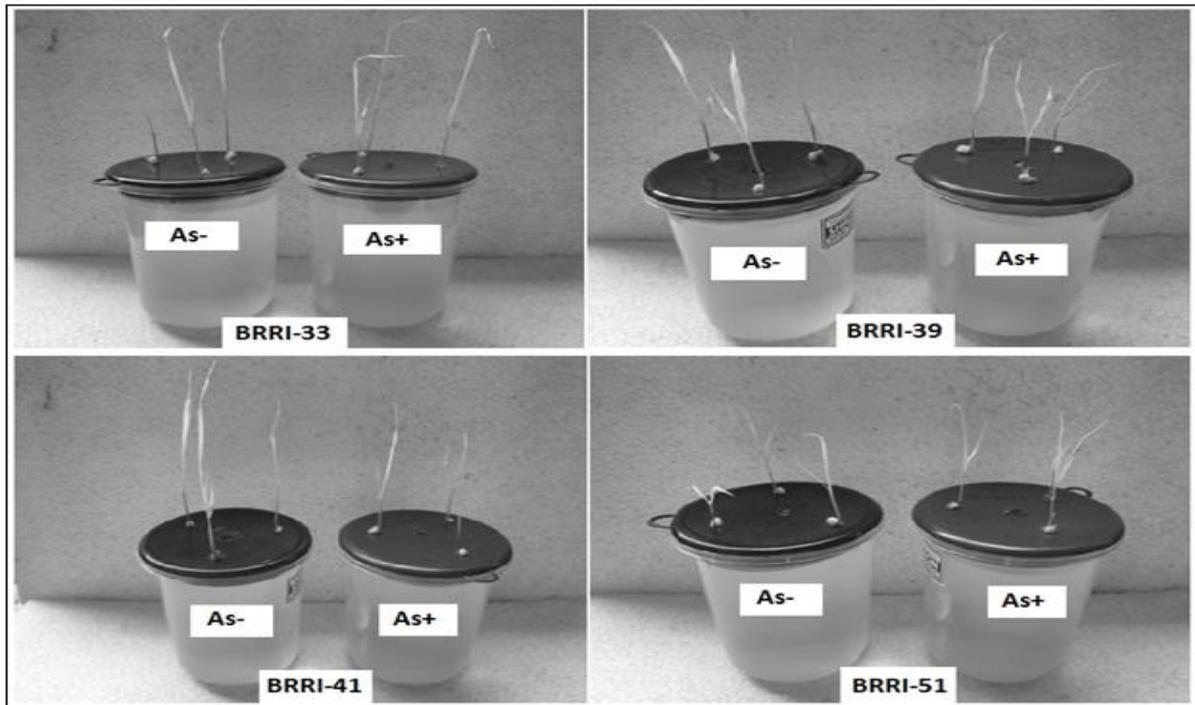


Fig. 1. Morphological view of BRRI-33, BRRI-39, BRRI-41 and BRRI-51 grown in As deficient (As -) and As treated (As +) hydroponic culture.

The arsenic content of agricultural soils has increased during recent decades. Its levels were highly increased by industrial activities such as metal smelting, coal combustion and glass manufacture and the present use of As compounds as fertilizers, pesticides, desiccants and growth promoters for poultry and pigs (Mahimairaja et al. 2005, Christen 2001). High levels of arsenic in soils have been phytotoxic in plants: decreases in plant growth and fruit yields; discolored and stunted roots; withered and yellow leaves; reductions in chlorophyll and protein contents, and in photosynthetic capacity (Marin et al. 1993). Rice (*Oryza sativa*) is the

principal cereal crop in Bangladesh that plays the most important role in the national economy. Rice contributes roughly 73% of the calorific and 66% of the protein intake (Alam et al. 2002). Bangladesh is a delta of high arsenic (As) contamination in groundwater and the water being widely used for irrigation.

Comparative growth performance of control and treated experimental plants were evaluated during the study period of two weeks and presented in this thesis. Although varieties tested in this study differed greatly in their response to As addition in soil, they all followed

the same pattern: when As was added, a negative response by plant part biomass. However, BRRI-33 showed some sort of tolerance to As treatment in hydroponic conditions. As treatment did not significantly reduce the morphological features in BRRI-33

compared to the plants grown without As treatment. It does suggest that BRRI-33 is able to tolerate As and its morphological characteristics is not effected by As treatment in roots.

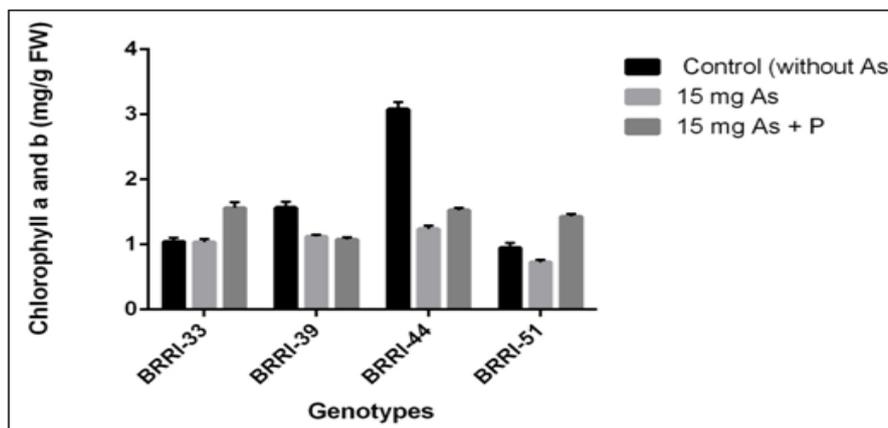


Fig. 2. Effect of phosphorus on chlorophyll (a and b) concentrations.

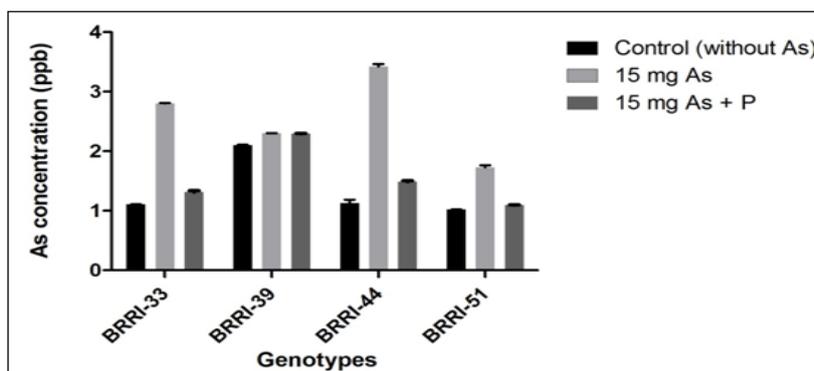


Fig. 3. Effect of phosphorus on As uptake in leaves in soil.

In contrast, morphological features were severely affected by As treatment and showed significant decrease in BRRI-39, BRRI-41 and BRRI-51. These results suggest that these three genotypes are highly sensitive to As and are not efficient in operation As tolerance mechanisms to cope with this As treated conditions. Generally, roots are involved in mineral acquisition by plants, and function at the interface with the rhizosphere. Alterations of root architecture and inhibition of root elongation are considered primary symptoms of As-toxicity

(Norton *et al.* 2008). In many circumstances, it is the As-sensitivity of the root that limits the productivity of the entire plant (Srivastava *et al.* 2009). Hence, plants exposed to As show inhibited root growth and reduced photosynthetic rate (Tripathi *et al.* 2007). These reports are consistent with findings of this study in rice. The results clearly indicate that in As treatment BRRI-33 showed better As tolerance in comparison to the other three rice genotypes. Arsenic tolerance by a plant system depends on

its inherent ability for avoidance of metal entry to the plant body as well as biochemical adaptation to tolerate intracellular As stress.

P fertilization is the common agriculture strategy to improve crop yield, especially for rice. In this study, rice genotypes showed As toxicity symptoms and chlorophyll reduction exposed to As treatment. However, P supplementation in soil pot remarkably improves the root dry weight, shoot dry weight and chlorophyll concentrations in BRRI-33, BRRI-44 and BRRI-51. Furthermore, P treatment did inhibit As uptake in these three genotypes compared to the plants grown under As treatment. These results indicate that external phosphate may eliminate As toxicity symptoms and affect As uptake in rice plants. In a previous report, it was suggested that P possibly is preferentially taken up by P/As transporters compared to its toxic analogue (Meharg and Macnair 1994). It was also reported that phosphate had a higher affinity to the uptake system in *Pteris vittata* root than arsenate (Wang et al. 2002).

The results of this study confirm the important role of P supplementation in minimizing the negative effects due to the As toxicity in rice. The addition of this important nutrient has also determined a limited translocation of the toxic element from roots to aboveground plant tissues.

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