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Arsenic uptake and phytotoxicity of T-aman rice (*Oryza sativa* L.) grown in the As-amended soil of Bangladesh

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Abstract A pot-culture experiment was conducted in open-field conditions with highly cultivated locally transplanted (T) aman rice (*Oryza sativa* L.) named BR-22 in arsenic (As)-amended soil (0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg kg^{-1} As) of Bangladesh to see the effect of As on the growth, yield and metal uptake of rice. Arsenic was applied to soil in the form of sodium arsenate (Na_2HAsO_4). Arsenic affected the plant height, tiller and panicle numbers, grain and straw yield of T-aman rice significantly ($P \leq 0.05$). The grain As uptake of T-aman rice was found to increase with increase of As in soil and a high grain As uptake was observed in the treatments of 30–50 mg kg^{-1} As-containing soil. These levels exceed the food hygiene concentration limit of 1.0 mg kg^{-1} As. However, the straw As uptake varied significantly ($P \leq 0.05$) from a low concentration of As in soil (5 mg kg^{-1}) and the highest uptake was noticed in 20 mg kg^{-1} As treatment.

Keywords Arsenic · Rice · Growth · Grain · Straw · Soil

1 Introduction

Arsenic (As) is found in inorganic form in soil (Johnson and Hiltbold 1969) and groundwater (Samanta et al. 1999). Under aerobic soil conditions, arsenate form is dominate; however, in submerged soil conditions, the predominant species is arsenite (Marin et al. 1993; Onken and Hossner 1996). The chemical form of As is found to be more important than As concentration in growing media in determining the phytotoxic effect of As on rice. Among the four arsenicals used, arsenite and monomethylarsonic acid have been found to be phytotoxic to rice (Marin et al. 1992). Upon uptake, most of the arsenite, arsenate and monomethylarsonic acid are accumulated in the roots, while dimethylarsinic acid is readily translocated to the shoot (Odanaka et al. 1987; Marin et al. 1992).

Groundwater As contamination is a severe problem in Bangladesh. Sixty-one of 64 districts and about 60% of groundwater sources of this country are affected by As contamination (Joarder et al. 2002). About 75% of the total cropped areas are used for rice (*Oryza sativa*) cultivation, depending on the groundwater irrigation system (Dey et al. 1996). In Bangladesh, the background As concentration in soil ranges from 4 to 8 mg kg^{-1}; however, in areas where irrigation is performed with As-contaminated groundwater, the soil As level can reach up to 83 mg kg^{-1} (Ullah 1998). There has been a considerable amount of investigation carried out on drinking water contamination as well as As mitigation options (Adeel 2002). However, there is no systematic study yet performed on the phytotoxicity, plant growth, grain yield and As uptake of any local T-aman rice in the context of the Bangladeshi environment. This study examined the As effect on this rice in a pot-culture system using a highly cultivated T-aman rice variety (BR-22) in Bangladesh. This research was carried out considering its significant importance in respect to the agriculture, environment and public health of Bangladesh. The findings of this study would certainly help the agriculturists, environmentalists and economists to find out the impacts of

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naturally contaminated As on rice is which cultivated throughout the rural areas of Bangladesh.

2 Materials and methods

2.1 Chemicals

Sodium arsenate (Na_2HAsO_4) and nitric acid were purchased from Merck, Germany. Fertilisers collected from local markets are urea [$CO(NH_2)_2$], potassium biphosphate [KH_2PO_4], muriate of potash [KCl] and gypsum [$CaSO_4.2H_2O$] for the nutrient sources of nitrogen, phosphorus, potassium and sulphur, respectively.

2.2 Soil collection and pot preparation

The soil was collected from a garden of sericulture of Rajshahi University Campus at a depth of 0–15 cm. The soil was dried in air and the clods were broken. This soil was mixed up thoroughly and a portion of soil was used for physical and chemical analysis. The characteristics of these soils were pH 6.4 ± 0.20, organic matter 1.59%, total N 0.11%, available P 10.23 ± 0.15 mg kg^{-1}, available S 13.21 ± 0.31 mg kg^{-1} and total As 2.1 ± 0.06 mg kg^{-1}.

Large earthen pots (43 × 40 cm) were used for rice cultivation. About 10 kg of soil was taken in a series of earthen pots. There were, in total, 24 pots comprising eight different arsenic treatments with three replications. The pots were sequentially placed in an open net-house. There were eight arsenic treatments, 0.0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg kg^{-1} As (on a soil weight basis), used in this experiment. Then, 100 mg l^{-1} N, 25.0 mg l^{-1} P, 40 mg l^{-1} K and 25 mg l^{-1} S were added to the soil in each pot and mixed thoroughly. The arsenic was applied in the form of arsenate, which can easily convert into arsenite form under the reducing and submerged condition of paddy soil.

2.3 Rice plantation and harvesting

Thirty-five-day old T-aman rice (BR-22) seedlings were uprooted carefully from the seedbed in the morning from the Regional Rice Research Institute, Rajshahi Station, and transplanted on the same day in experimental pots on 4th August 2005. Each pot had one hill and each hill contained three seedlings. After transplanting, 5–6 cm of water was maintained in each pot throughout the growth period of this rice. The irrigation water was tap water of Rajshahi University and was arsenic-free. The second split of urea was applied after 25 days of transplantation, i.e. at maximum tillering stage, and the third final split 50 days after transplanting, i.e. at the panicle initiation stage of T-aman rice.

The rice was harvested at full maturity on 4th December 2005. All of the data, including the plant growth, tiller and panicle numbers, and yield were collected from each pot during this experiment.

2.4 Description of experimental site

The pot culture experiment of rice was conducted in an open net-house at Rajshahi University Campus, Bangladesh, during August to December 2005. This experiment site had good sunshine throughout the day. The climate of this area is sub-tropical and humid, characterised by high temperatures during June–September, high rainfall during the monsoon season and low temperatures during winter (December–February).

2.5 Total arsenic measurement

The total arsenic from soil, rice grain and straw was measured in the Chemistry Lab of the Bangladesh Atomic Energy Commission (BAEC), Savar, Dhaka, Bangladesh. According to the BAEC Chemical Manual, 0.5 g of dried plant materials (grain and straw) were placed into clean digestion tubes. Then, 5.0 ml of concentrated HNO_3 was added and kept overnight under a fume hood. Later, the tubes were placed on a digester and the temperature was raised to 80°C for 1 h, and, finally, the temperature was maintained between 120 and 130°C for 20 h. After finishing the digestion, the sample was cooled down and 25 ml of distilled deionised water was added. The solution was filtered by Whatman No. 1 and preserved in plastic bottles. The total As was determined by hydride generation atomic absorption spectrophotometry using matrix-matched standards (Welsch et al. 1990). For analytical quality control, each sample was analysed thrice and the variation of each measurement was checked by a statistical program.

2.6 Statistical analysis

The analysis of variance (ANOVA) for various crop characters of T-aman rice was done following the *F*-statistics. Mean comparisons of the treatments were made by Duncan's multiple range test (DMRT) using SPSS version 12.01 software.

3 Results and discussion

There is no evidence that As is essential for plant growth, but it has phytotoxic effects on different crops. Arsenic is translocated to many parts of the plants; mostly, it is found in old leaves and roots. In rice, the critical level in tops ranges from 20 to 100 mg kg^{-1} As, and in roots 1,000 mg kg^{-1} As

(Odanaka et al. 1987). The tillers of rice are reported to be severely depressed with high concentrations of As (Chino 1981). From our experiment on T-aman rice, it was found that As had a significant ($P \leq 0.05$) effect on the plant height and tiller formation, panicle numbers and yield of T-aman rice (Tables 1, 2 and 3, respectively). The plant height of T-aman rice varied from 92.67 ± 3.91 to 61.65 ± 2.05 cm. The tallest plant was recorded in the As-free control pot and the smallest in 50 mg kg^{-1} As treatment (Table 1). We applied Na-arsenate as As in soil. This arsenate is found to affect the root development and reduce the plant height of one variety of rice (Abedin et al. 2002a). Some studies have reported that plant roots are unable to accumulate the essential nutrients from soil in the presence of excess arsenic because As(III) reacts with the sulphydryl groups of proteins (Speer 1973), causing disruption of root functions of plants (Orwick et al. 1976).

In T-aman rice, the number of tillers per pot was not significantly affected up to 20 mg kg^{-1} of As in soil (Table 1). The same type of trend was also reported by another study. A severely depressed tillering of rice is noticed when a high concentration of As is applied to soil (Chino 1981). Just like the tiller numbers, the panicle numbers of T-aman were also not affected at low doses of As in soil (Table 2). However, both of these parameters were affected significantly ($P \leq 0.05$) at 40 and 50 mg kg^{-1} As treatments. The panicle length also followed the same kind of trend. The highest panicle length recorded was 23.15 ± 1.10 cm in the control and the lowest was 16.72 ± 0.21 cm in 50 mg kg^{-1} As treatment (Table 2). Similarly, a significant effect on plant height and effective tiller numbers has been observed when a different variety of rice is cultivated in soil artificially enriched with arsanilic acid (Wang et al. 2006).

The grain weight of individual rice grains of T-aman was significantly ($P \leq 0.05$) affected by the added As in soil. The highest weight of 1,000 grains was 22.62 ± 1.79 g, recorded in 1.0 mg kg^{-1} As treatment, whereas the lowest weight of 1,000 grains, 14.41 ± 0.27 g, was noted in 50 mg kg^{-1} As treatment (Table 2). Like grain weight, the total yield of T-aman rice per pot was also significantly affected by the As in soil. The grain yield variation between the control and 50 mg kg^{-1} As treatment was very high. In the control, the grain yield was 66.30 ± 2.37 g per pot, whereas this yield was only 4.31 ± 0.83 g in 50 mg kg^{-1} As treatment (Table 3). However, there were no significant yield differences observed for 0, 1.0 and 5 mg kg^{-1} As doses. The yield markedly declined at 20 mg kg^{-1} As treatment and above. The grain yield was reduced to 93.1% for 50 mg kg^{-1} As treatment, whereas its reduction was only 1.8% in 1.0 mg kg^{-1} As treatment compared to the control. The reduced grain weight and yield was reported in another study when rice is grown in As-enriched irrigation water for up to 170 days during the post-transplantation growing period (Abedin et al. 2002b).

Table 1 Effect of added As on plant height and tiller numbers of T-aman rice

Arsenic added to soil (mg kg^{-1})	Plant height (cm)	Tillers pot^{-1} (no.)
0.0	92.67 ± 3.91^{a}	24.32 ± 2.31^{a}
1.0	89.66 ± 1.94^{ab}	23.23 ± 1.32^{a}
5.0	88.33 ± 2.50^{bc}	23.35 ± 2.42^{a}
10.0	82.66 ± 0.27^{b}	22.33 ± 3.54^{a}
20.0	81.11 ± 2.83^{c}	21.41 ± 1.25^{a}
30.0	72.66 ± 1.10^{e}	20.80 ± 0.42^{ab}
40.0	68.22 ± 2.22^{f}	17.66 ± 0.15^{b}
50.0	61.65 ± 2.05^{g}	13.11 ± 2.16^{c}

Figures having the same letters do not differ significantly at the 5% level by DMRT

Table 2 Effect of added As on the panicles and grain weight of T-aman rice

Arsenic added to soil (mg kg^{-1})	Panicles pot^{-1} (no.)	Panicle length (cm)	1,000 grain weight (g)
0.0	23.22 ± 0.62^{a}	23.15 ± 1.10^{a}	22.12 ± 1.53^{a}
1.0	21.33 ± 1.65^{a}	22.54 ± 1.78^{a}	22.62 ± 1.79^{a}
5.0	21.55 ± 0.99^{a}	22.19 ± 0.10^{a}	22.22 ± 0.42^{a}
10.0	21.12 ± 1.00^{a}	21.44 ± 2.45^{a}	22.11 ± 1.96^{a}
20.0	19.22 ± 1.22^{a}	21.12 ± 0.39^{a}	22.09 ± 0.43^{a}
30.0	19.51 ± 1.06^{a}	20.62 ± 0.97^{a}	21.65 ± 0.33^{ab}
40.0	13.11 ± 0.86^{b}	19.16 ± 1.25^{ab}	18.11 ± 0.87^{bc}
50.0	9.33 ± 0.91^{c}	16.72 ± 0.21^{b}	14.41 ± 0.27^{c}

Figures having the same letters do not differ significantly at the 5% level by DMRT

Table 3 Effect of added As on the grain and straw yields of T-aman rice

Arsenic added to soil (mg kg^{-1})	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})
0.0	66.30 ± 2.37^{a}	79.45 ± 1.18^{a}
1.0	65.15 ± 2.66^{a}	77.11 ± 2.80^{b}
5.0	64.31 ± 2.17^{a}	78.32 ± 0.92^{ab}
10.0	55.21 ± 2.00^{b}	69.74 ± 2.24^{c}
20.0	45.66 ± 0.61^{c}	57.23 ± 1.84^{d}
30.0	25.41 ± 0.31^{d}	41.46 ± 1.50^{e}
40.0	11.32 ± 0.37^{e}	28.14 ± 0.71^{f}
50.0	4.31 ± 0.83^{f}	18.11 ± 0.80^{g}

Figures having the same letters do not differ significantly at the 5% level by DMRT

The straw yield of T-aman rice was significantly affected by a low level of As in soil (1.0 mg kg^{-1}), although the grain yield was not reduced up to 5 mg kg^{-1} As level. The straw yield varied between 18.11 ± 0.80 and 79.45 ± 1.18 g pot $^{-1}$, with the lowest level in 50 mg kg^{-1} As treatment and the highest in the control. The straw yield of T-aman rice was significantly ($P \leq 0.05$) reduced due to the decrease of plant height, leaf number and leaf width. Arsenic toxicity induced chlorosis symptoms in the youngest leaf of rice by decreasing the chlorophyll content (Shaibur et al. 2006). In the present experiment on T-aman rice, sodium arsenate was applied in soil as the As source. This arsenate is known to be a decoupler of phosphorylation in mitochondria, which can inhibit the leaf uptake of other chemicals (ter Welle and Slater 1967; Sargent and Blackman 1969). This phenomenon may be responsible for the reduction of plant height, as well as the straw production of T-aman rice.

Table 4 shows the grain and straw uptake of As of T-aman rice. From the As uptake study of T-aman rice, it was found that the arsenic in rice grain increases with increase of As in soil, but did not vary significantly up to 20 mg kg^{-1} As treatment. It is found that As uptake and accumulation is greatly affected by arsenic contamination in soil and increased greatly with increasing levels of As (Marin et al. 1992; Abedin et al. 2002c; Meharg and Rahman 2002). In the case of T-aman rice, a high grain As uptake (1.29 ± 0.04 to 1.62 ± 0.05 mg kg^{-1} As dry weight) was found in the treatments of 30–50 mg kg^{-1} As-containing soil (Table 4). This level exceeds the food hygiene concentration limit of 1.0 mg kg^{-1} As on a dry weight basis (Abedin et al. 2002a). The straw As uptake of T-aman rice was found to differ significantly from a low concentration of As in soil (5 mg kg^{-1}) and the highest uptake, 2.26 ± 0.10 mg kg^{-1} As, was observed for the treatment containing 20 mg kg^{-1} As in soil; for higher concentrations, the uptakes decline slowly (Table 4). The reduction level of As uptake in straw above 20 mg kg^{-1} As treatment may be for dwarfism of plant height (Table 1) or due to enzyme saturation for the active transport of As in tissues. The same type of declining As uptake pattern for straw is found for one variety of rice grown in a glasshouse (Azizur et al. 2008). However, in T-aman rice, the straw accumulated twice as much As than the grain (Table 4). Tsutsumi (1980) found an elevated As concentration in rice straw (up to 149 mg kg^{-1} As by dry weight) when rice was grown in soil amended with sodium arsenate at different levels (0–312.5 mg kg^{-1} As). Rice grain generally has lower As concentration and the concentration remains much below the maximum permissible limit of 1 mg kg^{-1} As (Schoof et al. 1998). Some studies also noticed that As uptake and accumulation is greatly affected by As concentration in soil or nutrient media, and increased greatly with increasing levels (Marin et al. 1992, 1993). The arsenic concentration pattern in rice plant parts generally follow the order: root > straw > husk > whole grain > -husked rice. Odanaka et al. (1987) and Marin et al. (1992) have reported a higher accumulation of As in roots than in any other plant parts. Unfortunately, we did not analyse the roots of T-aman rice.

Table 4 Arsenic uptake by grain and straw of T-aman rice

Arsenic added to soil (mg kg^{-1})	Arsenic uptake by rice grain (mg kg^{-1})	Arsenic uptake by rice straw (mg kg^{-1})
0.0	0.69 ± 0.05[b]	1.49 ± 0.04[b]
1.0	0.94 ± 0.02[b]	1.83 ± 0.02[b]
5.0	1.07 ± 0.02[b]	1.91 ± 0.03[ab]
10.0	1.08 ± 0.01[b]	1.98 ± 0.01[ab]
20.0	1.09 ± 0.01[b]	2.26 ± 0.10[a]
30.0	1.29 ± 0.04[ab]	2.11 ± 0.05[a]
40.0	1.41 ± 0.02[ab]	2.07 ± 0.03[a]
50.0	1.62 ± 0.05[a]	1.87 ± 0.04[ab]

Figures having the same letters do not differ significantly at the 5% level by DMRT

4 Conclusions

From this experiment, it was found that addition of arsenic (As) to soil significantly affects the plant height, effective tiller numbers and panicle length of local T-aman rice (BR-22). The grain and straw yields of this rice were also markedly reduced by the artificial introduction of As into soil. A high As uptake in grain and rice straw was observed in this experiment. Rice cultivation in the naturally As-contaminated soil of Bangladesh may also reduce the yield of rice grain and straw. The transfer of As from soil into the food chain may have catastrophic effects on the human health of the people of Bangladesh due to arsenic exposure via the water–soil–plant–human pathway.

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