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ARSENIC TOXICITY IN AKITAKOMACHI RICE IN PRESENCE OF FE^{3+}-EDTA

MOLLA RAHMAN SHAIBUR[1] AND SHIGENAO KAWAI[2]

Abstract

An experiment was carried out hydroponically to investigate the arsenic (As) toxicity in rice (*Oryza sativa* L. cv. Akitakomachi) seedlings in presence of Fe^{3+}-EDTA. The As treatments ($NaAsO_2$) were 0, 6.7, 13.4, 26.8, and 33.5 µM for 14 days. The whitish chlorotic symptom was pronounced in the fully expanded young leaves at 13.4 µM As treatment, suggesting that As-induced chlorosis might be Fe (iron)-chlorosis. Chlorophyll indices and Fe concentrations were reduced much in the shoots of As-treated chlorotic seedlings as compared to the control seedlings. Our result showed that As-toxicity largely depended on the concentrations of inorganic As in the medium and the higher was the concentration of As the higher was the As-toxicity.

Keywords: Arsenic, concentration, Fe-chlorosis, rice, young leaves.

Introduction

Arsenic (As) is considered as the king of poison for its severe toxic effect on living beings at very low concentration and widely distribution in nature. High concentration of As shows acute toxicity in plants (Abedin *et al.*, 2002) and animals, but sometimes low concentration of As (6.7 µM) in the growth medium shows slight stimulating effect on the growth of hydroponic sorghum (Shaibur *et al.*, 2008) and spinach (Shaibur and Kawai, 2009). Arsenic is phytotoxic when it is taken up by the plants in excessive amounts from the rooting medium (Shaibur *et al.*, 2008; Shaibur *et al.*, 2009). Elevated concentrations of As in plants have been shown to be associated with necrosis in the leaf tip, chlorosis in the fully developed young leaves of sorghum (Shaibur *et al.*, 2008) and the toxicity symptoms increased with the increasing inorganic As concentrations (Shaibur *et al.*, 2008). Total nitrogen content, total chlorophyll content, root nodulation and DW (dry weight) of the whole plants have been found to be reduced by As in the garden pea, *Pisum sativum* (Päivöke, 1983).

Groundwater of Bangladesh contains high concentration of As and mostly exceeds WHO's guidelines, 0.01 mg As l^{-1} (Tanabe *et al.*, 2001; Imamul Huq *et al.*, 2003). Ingestion of As can occur through As-contaminated food material especially green leafy vegetable spinach (Ahmad *et al.*, 1999; Farid *et al.*, 2003; Anawer *et al.*, 2002; Shaibur and Kawai, 2009; Shaibur and Kawai, 2010) and

[1]Department of Environmental Science & Health Management, Jessore Science & Technology University, Jessore 7408, Bangladesh, [2]Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan.

water spinach (kalmi; Farid *et al.*, 2003; Shaibur *et al.*, 2009). Recently, we showed that sodium meta-arsenite ($NaAsO_2$) in the rooting medium could induce whitish chlorotic symptom in the young leaves of Akihikari rice variety (Shaibur *et al.*, 2006) where Fe was used as Fe^{3+}-citrate. Akitakomachi is a very popular rice in Japan and Fe^{3+}-EDTA is mostly used in hydroponic experiments. Therefore, As-toxicity response of Akitakomachi rice in presence of Fe^{3+}-EDTA in hydroponic culture might be interesting in the field of plant physiology. So far we know there is little or no information about As-toxicity in Akitakomachi rice in presence of Fe^{3+}-EDTA and we did this experiment in hydroponic system.

Materials and Method

Rice (*Oryza sativa* L. cv. Akitakomachi) seeds were surface sterilized with 2% $Ca(OCl)_2$ for 45 minutes and washed with tap water for 1 hour. Seeds were wrapped between moistened towels covered with colorless vinyl wrapping paper and were kept in an electric incubator for 60 hour at 25 ± 2˚C. Germinated seeds were transferred on a plastic net in a yellow plastic box containing 2% $CaCl_2$ for 7 days in the greenhouse under natural conditions. Seedlings were raised in half-strength nutrient solution for another 14 days and were hand-transplanted in bucket (5 seedlings/bunch) at the 4-5th leaf stage. Each bucket (5 l) containing 8 bunches. Treatments were started with full-strength solution containing 1 mM NH_4NO_3; 1 mM K_2SO_4; 0.8 mM $MgSO_4$; 0.5 mM $CaCl_2$; 0.5 mM NaH_2PO_4; 10 µM $MnSO_4$; 1 µM $CuSO_4$; 1 µM $ZnSO_4$; 3 µM H_3BO_3; 0.05 µM H_2MoO_4 and 10 µM Fe^{3+}-EDTA (Hoagland and Arnon, 1938). The As treatments were 0, 6.7, 13.4, 26.8 and 33.5 µM (equal to 0, 0.5, 1.0, 2.0, and 2.5 mg As l^{-1}, respectively) for 14 days. Arsenic was added as sodium meta-arsenite ($NaAsO_2$). The pH of the nutrient solution was adjusted daily at 5.5 with a digital pH meter (Horiba Korea, Seoul, Korea) and with 1 M HCl and/or 1 M NaOH at around 4 p.m. during the experiment (June-July, 2007). Solution was renewed every week and was not aerated. The detailed methodology has been described earlier (Shaibur *et al.*, 2006) with some exception. The experiment was carried out in the greenhouse of Iwate University, Japan, with ambient light (roughly 14 hours day/10 hours night). Temperature fluctuation was between 25ºC to 12ºC in day and at night, respectively. The volume of the roots was determined by the traditional method. The root was placed in a 100 ml measuring cylinder filled with an appropriate volume of water. The total volume of the roots was measured by the apparent change in the water volume caused by the roots.

The seedlings were collected and washed with deionized water properly. Shoots were separated from the roots with a stainless steel scissor. Additionally, the young leaves were separated from the old leaves to determine the nutrient concentrations, especially Fe, as the chlorosis was found in the young leaves. Samples were dried at 55-60˚C for 48 hours in an electric oven and were digested with a nitric acid-perchloric acid mixture. Amounts of K, Ca, Mg, Fe, Mn, Zn and Cu were determined with atomic absorption spectroscopy (AAS). Phosphorus was determined colorimetrically using a UV-visible Spectrophotometer at 420 nm wavelengths after developing the yellow color with vanadomolybdate. Arsenic was measured by using Hydride Generation Atomic Absorption Flame Emission Spectrophotometer. Determination of chlorophyll index, calculation for the parameters and statistical analysis were described elsewhere (Shaibur *et al.*, 2006; Shaibur and Kawai, 2010).

Determination of Critical Toxicity Level (CTL) of As

A two-order polynomial growth curves between DW and As concentration was set up and the following equation was used to calculate the CTL of As in this experiment.

$$ax^2 - bx + c = 0 \text{ --------------- } (1)$$

$$\therefore\ x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

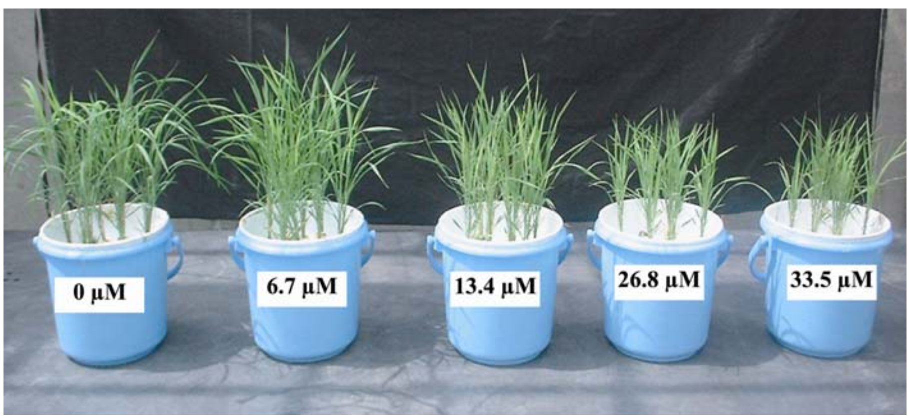
Number (1) equation is the equation that can intercept Y axis. Here “c” (constant; value of y) is the intercept in every equation in this report. The reduction of 10% DW was calculated from the intercept point.

Results and Discussion

Visible Symptom: Dew like water drops appeared in the leaf tip of 0, 6.7, and 13.4 µM As treated plants in the evening to early morning, however, it was absent in 26.8 and 33.5 µM As treated plants. The 6.7 µM As treated plants were healthy as compared to others (Fig. 1) and looked like green for the first week. Plants showed whitish chlorotic symptom at 6.7 and 13.4 µM As levels at 10 DAT (days after treatments), in which the symptom was more pronounced at 13.4 µM As exposure (Fig. 1). The chlorotic symptoms appeared this time (June-July, 2007) were not as conspicuous as it was in the previous time (August-September; Shaibur *et al.*, 2006). Chlorotic symptoms seem to be clearer in warmer season at 13.4 µM As exposure (August-September; Shaibur *et al.*, 2006) because the growth was higher and Fe concentration might be diluted in the young leaves. At sunlight, seedlings were curled at 26.8 and 33.5 µM As levels and the turgidity decreased with the increasing As and sunlight intensity. Arsenic-treated root was white up to 72 hours and after that the reddish color appeared in As-treated plants. Iron plaques were visible clearly as the reddish coating on the root surface of As-treated rice seedlings. The effect of Fe plaque on As toxicity has been studied recently in Bangladesh rice (Hossain *et al.*, 2009). The roots contained algae like green plaque on their surface. It may be due to the fact that the pots were little bit light transparent and facilitated to produce algae plaque. The green color in the old leaves was not as pronounced in As-treated plants as it was in control plants. It seemed that the deep green color of the old leaves turned to mild green due to As-toxicity. It is well known that As first attacks the older leaves as compared to the younger leaves.

Chlorophyll Index: The initial chlorophyll index value was 22.0, but it increased with time and at the harvesting stage, the value was 29.0 in the control plants (Table 1). Chlorophyll index decreased with increasing As (Table 1). At 26.8 and 33.5 µM As levels, we could not see the fully expanded 5^{th} leaf to take chlorophyll data. For these two levels, the 5^{th} leaf was just about to be appeared. Determination of chlorophyll content is often accomplished to assess the impact of most environmental stresses, as pigment content is linked to the visual symptoms and photosynthetic plant productivity (Jain and Gadre, 1997).

Fig. 1. Physiological response of Akitakomachi rice variety at elevated concentrations of As.



Shoot Height, Root Length, Width of Leaf Blade, Leaf Number and Tiller Number**:** The initial shoot height was 22.0 cm and the root length was 18.0 cm (Table 1). Our result showed that after exposure to As at 6.7 µM As level, the root length increased a little. The height and the length decreased with the increasing As in the medium (Table 1). Similar trends were also found for the width of leaf blade and leaf number. In this experiment we did not see any new tiller (Shaibur *et al.,* 2006).

Dry Weight: The initial DW was 21.5 mg/plant shoot and 8.29 mg/plant root (Table 1). At harvest, the DW was 111.8 mg/plant shoot and 39.3 mg/plant root in the control plants (Table 1), indicating that the growth increased by 420% for shoots and 374% for roots in 14 days. The increased growths were 330, 250, 144, and 98% in shoots as compared to initial stage at 6.7, 13.4, 26.8, and 33.5 µM As treatments, respectively, indicating that As-toxicity depressed DW. Similarly, root DW increased by 429, 357, 259, and 162 % for the same treatments, respectively (Table 1). In the present experiment, the fully developed young leaves with the 0, 6.7 and 13.4 µM As treatments were separated and the DW was measured. The result showed that As-toxicity decreased the DW of young leaves also with the increasing As in the media (data not shown). Arsenic toxicity decreased the DW in pot cultured rice (Hossain *et al.*, 2009).

Table 1. Physiological parameters as affected by the different levels of As in the nutrient solution.

Treat-ments (µM As)	Chlorophyll **Index	Length (cm)		Leaf		DW (mg/plant)		Volume
		Shoot	Root	**Width (mm)	No.	Shoot	Root	cc root $^{-1}$
*Initial	22.0b	22.0d	18.0b	4.00c	20c	21.5f	8.29e	0.13e
0	29.0a	38.0a	21.3a	6.30a	30a	111.8a	39.3ab	3.00a
6.7	23.3b	41.0a	23.0a	6.70a	30a	92.6b	43.9a	3.55a
13.4	12.8c	31.0b	22.7a	5.00b	25b	75.1c	37.9b	2.11b
26.8	nd	25.0c	19.7ab	nd	20c	52.5d	29.8c	1.44c
33.5	nd	20.7d	16.7b	nd	20c	42.6e	21.7d	1.00d

Means followed by the different letters in each column are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. *Indicated the primary data at the beginning of As treatments. At *initial stage, the widths of leaf blade and chlorophyll index were taken from fully developed 3rd leaf. **Reading was taken from fully developed 5th leaf at the harvesting stage. The 5th leaf did not appear at the harvesting stage at 26.8 and 33.5 µM As treatments. DW = dry weight, nd = not detected.

Root volume**:** The initial root volume was 0.13 cc per plant root (Table 1). Root volume increased with time in every treatment (Table 1) as compared to the initial stage. However, decreased in As-treated plants as compared to control with the increasing As, though it showed slight stimulating effect at 6.7 µM As treatment (Table 1).

Macronutrients: In most of the cases, As decreased the concentrations of K, Ca, and Mg in shoots (Table 2). Phosphorus and K concentrations increased in some cases (Table 2). Arsenic increased P concentrations in the young leaves though the K concentrations were not much affected (Table 3). In this experiment, As decreased Ca and Mg concentrations in the fully developed young leaves at 6.7 and 13.4 µM As treatments (Table 3). At harvest, As decreased the uptake of macronutrients as compared to the control (Table 4).

Table 2. Concentrations of nutrients in shoots and roots of rice seedlings grown in nutrient solution with different levels of As.

Treatments (µM As)	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	--------------mg/g DW--------------				----------------µg/g DW-----------------			
	Concentrations in shoots							
*Initial	11.8a	40.9b	2.60a	2.95a	186.5a	491b	106.2a	19.8b
0	9.29b	45.7ab	2.21ab	2.96a	94.0b	773a	72.2b	23.4a
6.7	12.3a	51.0a	2.08ab	3.25a	82.9c	700a	57.9c	20.4b
13.4	13.1a	48.3a	1.64c	2.39b	64.9d	365c	44.6d	15.7c
26.8	12.1a	35.2c	1.56c	1.27c	69.2d	202e	40.2d	11.2d
33.5	11.2a	35.0c	1.86b	1.41c	82.1c	272d	48.0d	15.2c
	Concentrations in roots							
*Initial	5.98a	27.7c	0.63b	1.49a	1341b	142.0bc	187.6a	97.5b
0	5.92a	47.6a	0.59b	1.37a	545d	133.0c	67.2c	167.0a
6.7	5.49a	42.6a	0.66b	1.19b	565d	169.0b	116.8b	84.3b
13.4	4.86b	34.6b	0.84a	0.61c	870c	334.0a	71.5c	41.0c
26.8	4.09b	20.4d	0.83a	0.69c	1506ab	75.0d	56.5d	34.1d
33.5	3.77c	20.6d	0.79a	0.66c	1753a	58.5e	68.0c	46.1c

Means followed by the different letters in each column of individual group are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. *Indicated the primary data at the beginning of As treatments. DW = dry weight.

Micronutrients: Iron concentrations were 186.5 and 1341 µg/g DW in shoots and in roots at the initial stage of As treatments (Table 2). At harvest, Fe concentrations were lower in the shoots of the control plants as compared to the initial concentrations (Table 2), indicating that Fe concentration was higher in shoots at the early stage of the seedlings. Arsenic decreased Fe concentrations in shoots but increased in roots (Table 2).

Table 3. Concentrations of nutrients in fully developed young leaves of rice seedlings grown in nutrient solution with different levels of As.

Treatments (µM As)	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	-------------mg/g DW--------------				-------------µg/g DW--------------			
0	5.89c	40.1a	1.94a	3.31a	80.7a	527a	38.0a	25.0a
6.7	7.72b	43.0a	1.25b	2.69b	50.6b	354b	24.2b	21.0b
13.4	10.5a	45.9a	1.14b	2.30b	43.9b	181c	29.7b	14.8c
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Means followed by the different letters in each column are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. ***Indicated that the reading of 26.8 and 33.5 µM As treatments could not be incorporated as the young leaves in these treatments did not develop fully. DW = dry weight.

Iron concentration was 64.9 µg/g DW in shoots at 13.4 µM As level (Table 2) and this lower concentration of Fe was mostly responsible for the induction of whitish chlorotic symptom in the fully developed young leaves. In this case, the stems, old leaves and young leaves were digested together and the Fe concentration was measured. Arsenic decreased Fe concentration in shoots and was within the critical deficient level (CDL; 65 µg Fe g^{-1} DW), resulting in the whitish chlorotic symptom in the fully expanded young leaves. The CDL of Fe is almost similar for almost all the plant species and ranging from 66-72 µg/g DW (Marschner, 1998). In As-treated plants, Fe uptake (Table 4) decreased in As-treated plants as compared to control plants. Not only Fe concentration but also Mn, Zn and Cu concentrations were decreased in shoots by the applied As-treatments (Table 2).

Table 4. Uptake of nutrients in shoots and roots of rice seedlings grown in nutrient solution with different levels of As.

Treatments (µM As)	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	-------------mg/plant-----------------				--------------µg/plant -------------------			
				Uptake in shoots				
*Initial	0.26d	0.88 e	0.06 d	0.06c	3.14d	10.6d	2.28d	0.43e
0	1.04a	5.11 a	0.25 a	0.33a	10.50a	86.4a	8.09a	2.61a
6.7	1.14a	4.72 a	0.19 b	0.30a	7.68b	64.8b	5.37b	1.89b
13.4	0.96a	3.53 b	0.12 c	0.17b	4.75c	26.8c	3.26c	1.15c
26.8	0.64b	1.85 c	0.08 d	0.07c	3.64d	10.6d	2.11d	0.59d
33.5	0.48c	1.48 d	0.08 d	0.06c	3.48d	11.7d	2.04d	0.64d
				Uptake in roots				
*Initial	0.05d	0.22e	0.005d	0.012c	8.81d	1.13d	1.49c	0.78e
0	0.23a	1.87a	0.023b	0.054a	21.4c	5.25b	2.65b	6.56a
6.7	0.24a	1.87a	0.029ab	0.051a	24.8c	7.41b	5.12a	3.71b
13.4	0.18b	1.31b	0.032a	0.023b	33.0b	12.7a	2.72b	1.55c
26.8	0.12c	0.61c	0.025b	0.021b	44.9a	2.23c	1.68c	1.01d
33.5	0.08d	0.44d	0.017c	0.014c	38.4ab	1.27d	1.47c	0.99d

Means followed by the different letters in each column of individual group are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. *Indicated the primary data at the beginning of As treatments.

Table 5. Concentrations and uptake of As in hydroponic rice seedlings as affected by the different treatments.

Treatments (µM As)	Concentrations -------µg As g^{-1} DW -------		Uptake --------µg As $plant^{-1}$ --------	
	Shoot	Root	Shoot	Root
0	nd	nd	nd	nd
6.7	44.7 b	414.1 d	4.14 a	18.1 b
13.4	36.8 b	685.2 c	2.70 b	25.9 a
26.8	43.8 b	762.1 b	2.30 b	22.6 a
33.5	58.7 a	866.1 a	2.47 b	18.6 b

Means followed by the different letters in each column of individual group are significantly different (p= 0.05) according to Ryan-Einot-Gabriel-Welsch multiple range test. DW = dry weight, nd = not detected.

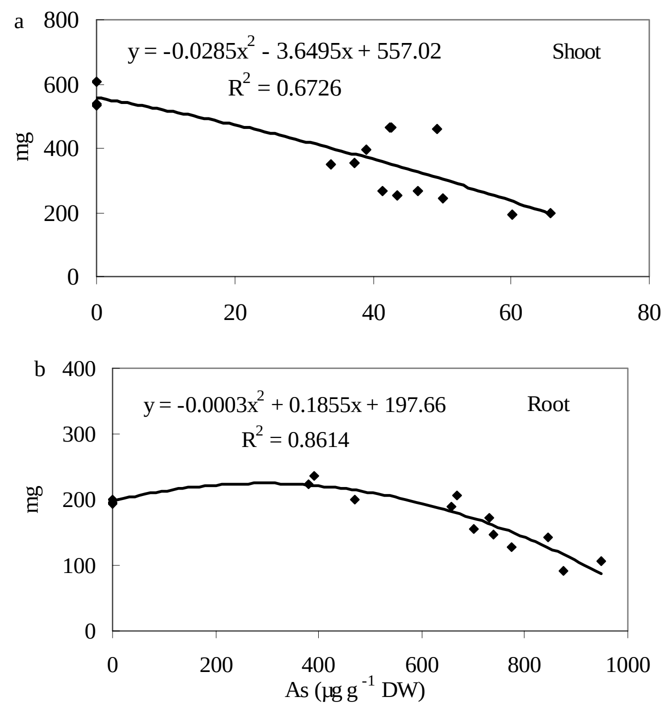

Fig. 2. Polynomial two order growth curve (a) shoot and (b) root of rice seedlings with different concentrations of As in plant tissues. Considering 10% DW (dry weight) reduction, the critical toxicity levels (CTL) of As in shoots and roots were determined from these curves.

Further, we separated the young chlorotic leaves from the old green leaves and the Fe concentrations were measured. Iron concentrations were 50.6 and 43.9 in the chlorotic young leaves of 6.7 and 13.4 µM As treated plants (Table 3). We believe, these lower concentrations of Fe were mostly responsible for the induction of whitish chlorosis. The other elements like Mn, Zn, and Cu also decreased in young leaves with increasing As in the solution (Table 3). The lower concentrations of these elements may also be responsible for the induction of whitish chlorosis in the young leaves (Marschner, 1998), however, this supposition demands further study to prove or disprove it.

Arsenic: Arsenic concentrations increased both in shoots and in roots at the 33.5 µM As treatment though the concentrations in the plant parts were not proportional to the As concentrations in the medium (Table 5), indicating that the bioavailability of As depended on the inorganic As concentrations in the medium. Root concentrated almost 9.26, 18.6, 17.4, and 14.8 times higher As concentrations as compared to shoots (Shaibur *et al.,* 2006) in 6.7, 13.4, 26.8, and 33.5 µM As treatments, respectively. Uptake of As decreased in shoots in the 13.4, 26.8 and 33.5 µM As treatments as compared to the 6.7 µM As treatment (Table 5). This was because the DW decreased in those treatments as compared to the 6.7 µM As treatment (Table 1). Arsenic concentrations in the young leaves of 0, 6.7 and 13.4 µM treatments were 0 (not detected), 40.6 and 30.6 µg/g DW (data were not presented here).

The CTL of arsenic in this rice variety were calculated as 13.3 µg As g^{-1} DW in shoot and 714.6 µg As g^{-1} DW in root which could reduce 10% DW. The CTL was calculated from the polynomial two order growth curves (Figs. 2ab). The CTL of As is little bit different than the previously reported data (Shaibur *et al.,* 2008). These differences were most probably due to the varietal, environmental, experimental methods, source of Fe and together with the other factors. It was suggested that the CTL might be very much dependent on some factors e.g.- content of other mineral nutrients, species of the experimental plants and also the species of As used.

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