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ORIGINAL ARTICLE

Physiological and mineralogical properties of arsenic-induced chlorosis in rice seedlings grown hydroponically

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Abstract

A hydroponic experiment was conducted to observe the effect of arsenic (As) on a number of physiological and mineralogical properties of rice (*Oryza sativa* L. cv. Akihikari) seedlings. Seedlings were treated with 0, 6.7, 13.4 and 26.8 μmol L^{-1} As (0, 0.5, 1.0 and 2.0 mg As L^{-1}) for 14 days in a greenhouse. Shoot dry matter yield decreased by 23, 56 and 64%; however, the values for roots were 15, 35 and 42% for the 6.7, 13.4 and 26.8 μmol L^{-1} As treatments, respectively. Shoot height decreased by 11, 35 and 43%, while that of the roots decreased by 6, 11 and 33%, respectively. These results indicated that the shoot was more sensitive to As than the root in rice. Leaf number and width of leaf blade also decreased with As toxicity. Arsenic toxicity induced chlorosis symptoms in the youngest leaves of rice seedlings by decreasing chlorophyll content. Concentrations and accumulations of K, Mg, Fe, Mn, Zn and Cu decreased significantly in shoots in the 26.8 μmol L^{-1} As treatment. However, the concentration of P increased in shoots at 6.7 and 13.4 μmol L^{-1} As levels, indicating a cooperative rather than antagonistic relationship. Arsenic and Fe concentration increased in roots at higher As treatments. Arsenic translocation (%) decreased in the 13.4 and 26.8 μmol L^{-1} As treatments compared with the 6.7 μmol L^{-1} As treatment. Arsenic and Fe were mostly concentrated in the roots of rice seedlings, assuming co-existence of these two elements. Roots contained an almost 8–16-fold higher As concentration than shoots in plants in the As treatments. Considering the concentration of Mn, Zn and Cu, it was suggested that chlorosis resulted from Fe deficiency induced by As and not heavy-metal-induced Fe deficiency.

Key words: arsenic, chlorophyll, chlorosis, concentration, iron, rice.

INTRODUCTION

Arsenic (As) contamination in groundwater in Bangladesh was first discovered in 1993 by the Chemistry Division of the Atomic Energy Centre, Dhaka (AECD), at the village of Baroghari, Chapainawabganj District, Rajshahi Division (Ali 1995). The As-contaminated groundwater is the main source of drinking and home use for most village people of the country. It is also being used for agricultural purpose and this could be one of the major exposure routes to As toxicity in humans and animals. It was only in 1994 that the first groups of arsenicosis patients were found in Bangladesh; however, As contamination of groundwater had already been reported in 1978 in the state of West Bengal in India, the neighboring country on the western border of Bangladesh.

Around that time (1978), rice (*Oryza sativa* L.) growing during the dry season was started in Bangladesh under the "green revolution" to increase food production. Rice is the staple food in Bangladesh and covers approximately 75% of the total cropped area and 83% of the irrigated area (Dey *et al.* 1996). The average background concentration of As in Bangladesh is well below 10 mg kg^{-1} soil. However, in some areas where soils receive As-contaminated groundwater irrigation, the concentration has been found to be as high as 80 mg kg^{-1} soil (Huq *et al.* 2003). The maximum As

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concentration in irrigation water was found to be 0.55 mg L^{-1}. If a rice field is irrigated with this water when the water requirement is 1000 mm $year^{-1}$, it is calculated that the As load will be 5 kg ha^{-1} $year^{-1}$ (Huq *et al.* 2003). Many crops receiving As-contaminated water as irrigation have been found to accumulate As at levels that exceed the minimum allowable daily limit (MADL) of 0.2 mg kg^{-1} dry weight (dw). Rice and wheat (*Triticum aestivum* L.) receiving As-contaminated irrigation water have been found to sequester the toxic metalloid into roots and stems (Huq *et al.* 2003). However, the amount of As obtained through rice grains per person per day may, in many instances, surpass the MADL in Bangladesh.

More than 80% of the people in Bangladesh are involved in agriculture. Approximately 90% of men and 80% of women in rural areas of the country are engaged in agriculture (Bangladesh Bureau of Statistics 1998). Most groundwater used for irrigation in Bangladesh is contaminated with As (Khan *et al.* 1998). If the groundwater contaminated with As is applied as irrigation water, it may reduce the growth as well as the production of the crops. Davis and Coker (1979) reported that a 20% loss of cereal resulted from high concentrations of As (30 mg kg^{-1}). Although the groundwater contained a high level of As, to date the source is obscure. There are a number of assumptions regarding the source of As in groundwater in Bangladesh. Deep tubewells were dug and a large volume of groundwater has been withdrawn for irrigation, particularly in the dry season. It is considered that withdrawal of groundwater may change the geochemical and physical changes of the underground, causing As contamination in turn. In general, groundwater contains 50% arsenate and 50% arsenite (Samanta *et al.* 1999), which may convert from one form to another. Redox potential is generally governing this transformation (Masscheleyn *et al.* 1991; Onken and Hossner 1995). Arsenite is the dominant form in flooded paddy soil (Takamatsu *et al.* 1982), which is considered to be the most toxic form. Arsenic toxicity is responsible for shorter plant height, weaker tillering, thinner leaf coloring, earlier root coloring to yellowish brown or brown, and curled leaves under sunlight in rice plants (Yamane 1989). Some data have already been published regarding plant responses at high As levels in, for example, rice (Abedin *et al.* 2002), Chinese Brake fern (*Pteris vittata* L.) (Ma *et al.* 2001), bush bean (*Phaseolus vulgaris* L.) (Wallace *et al.* 1980), and tomato (*Lycopersicum esculentum* L.) (Carbonell-Barrachina *et al.*1995). However, there is little data on the physiological response of rice under As toxicity. It is, therefore, necessary to observe the effect of As toxicity on the physiological and mineralogical properties of rice.

MATERIALS AND METHODS

Seed germination and plant culture

Rice (*Oryza sativa* L. cv. Akihikari) seeds were surface sterilized with 2% chlorinated lime [$Ca(OCl)_2$] for 45 min and washed with tap water for 1 h. After washing, the seeds were wrapped between moistened towels and kept in a seed growth chamber at 25°C for 48 h. Then, the seeds were transferred on a net in a box containing 2% $CaCl_2$ for 7 days and the seedlings were transferred to half-strength nutrient solution and grown for 14 days. Twenty-one days after germination, seedlings were hand-transplanted in groups of five in one bunch, and each bucket (10 L) contained 16 bunches. When the seedlings were suitable for transplantation (21 days after germination, at the fourth–fifth leaf stage of the seedlings), the treatments were started with full-strength solution containing 1 mmol L^{-1} NH_4NO_3, 1 mmol L^{-1} K_2SO_4, 0.8 mmol L^{-1} $MgSO_4$, 0.5 mmol L^{-1} $CaCl_2$, 0.5 mmol L^{-1} NaH_2PO_4, 10 μmol L^{-1} $MnSO_4$, 1 μmol L^{-1} $CuSO_4$, 1 μmol L^{-1} $ZnSO_4$, 3 μmol L^{-1} H_3BO_3, 0.05 μmol L^{-1} H_2MoO_4 and 10 μmol L^{-1} Fe-citrate. The As treatments were 0, 6.7, 13.4 and 26.8 μmol L^{-1} (0, 0.5, 1.0 and 2.0 mg As L^{-1}) and the duration of the As treatments was 14 days. Arsenic was added as sodium arsenite ($NaAsO_2$). The pH (pH 5.5) was adjusted daily with a digital pH meter (Horiba Korea, Seoul, Korea) and with 1 mol L^{-1} HCl and/or 1 mol L^{-1} NaOH at approximately 16.00 hours during the experiment (August–September 2004). The solution was renewed every week and was not aerated.

SPAD value

The chlorophyll index (SPAD value) of fully developed (fifth leaf) new leaves was measured using a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokyo Japan) 14 days after treatment. In each leaf, the SPAD values of three points were measured and the average was calculated. Means of each bunch were obtained. An average of the data of three bunches was calculated.

Analysis of plant samples

The rice seedlings were collected and washed with deionized water three times. Shoots and roots were separated and dried at 55°C for 48 h. The oven-dried samples were digested with a nitric acid–perchloric acid mixture (Piper 1942). The amounts of K, Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectroscopy (Loeppert and Inskeep 2001). Phosphorus was determined colorimetrically using an ultraviolet (UV)-visible spectrophotometer (model UV mini 1240, Shimadzu Corp., Kyoto Japan) at 420 nm wavelengths after developing the yellow color with vanadomolybdate as described by Barton (1948) and Jackson (1958).

Determination of arsenic

Arsenic was measured using a hydride generation atomic absorption spectrophotometric (HGAAS) technique using a Hitachi HFS-3 instrument. We digested the samples with a nitric–perchloric acid mixture, and the volume of the solution was approximately 5 mL. After that, the volume of the digested solution was made up to 50 mL with MQ water. For As determination, the samples were further diluted up to 100–2000 times. As a result, the interference of nitrate on As determination could be minimized. Reduced nitrogen oxides (resulting from HNO_3 digestion) and nitrite could suppress the instrumental response for As (Huang and Fujii 2001).

Calculation of the parameters

Concentration in mg or μg of element g^{-1} dw; accumulation in shoot in mg or μg of element $plant^{-1}$ shoot; accumulation in root in mg or μg of element $plant^{-1}$ root; and translocation % in nutrient accumulation in shoot/total accumulation (shoot + root) × 100. Tu *et al.* (2004) defined the translocation factor (TF) as the ratio of the As concentration in fronds of Chinese Brake fern to that in the roots of the plant.

Reagents

All chemicals used were of analytical reagent grade. All solutions were prepared previously with pure water. The stock solution of As was prepared by dissolving $NaAsO_2$ (Kanto Chemical Company, Tokyo, Japan) in pure water and was kept at room temperature.

Statistical analysis

The experiment was arranged in randomized blocks with three replications. Data on shoot and root dry matter yield, shoot height, root length, leaf number, leaf blade, SPAD value and mineral elements were subjected to ANOVA. Differences between means were evaluated using a Ryan–Einot–Gabriel–Welsch multiple range test ($P = 0.05$) (SAS 1988) using computer origin 5 at Iwate University, Morioka, Japan.

RESULTS AND DISCUSSION

Visible symptoms

Arsenic toxicity produced whitish chlorotic symptoms in the youngest leaf of rice seedlings after 14 days of treatment at 13.4 and 26.8 μmol L^{-1} As levels. At 13.4 μmol L^{-1} As treatment, the chlorotic symptoms were more pronounced than the other As treatments. We assumed that the chlorotic symptoms of rice induced by As were most probably Fe chlorosis because the symptoms were observed in the youngest leaves (Mengel and Kirkby 2001). If the chlorotic symptoms could be found in old leaves, it would be Mg chlorosis (Maynard 1979). We found that the chlorophyll index was also lowest in the chlorotic leaves (Fig. 1c). Not only chlorosis symptoms, but also necrosis (burning of leaf tip) was observed. In the early stage of As treatments, the leaves showed symptoms of curling at day time and the youngest leaves failed to unfold compared with control plants. This symptom may indicate water deficit on rice seedlings under As toxicity (Yamane 1989). The most common visible symptom was growth reduction, particularly in the shoot. A reddish color along the root length was found and felt slippery to touch because of As toxicity. Reduction of root length because of As toxicity could be termed "little root". The depth of reddish color was greater with increasing As supply. The reddish color of the roots may result from the

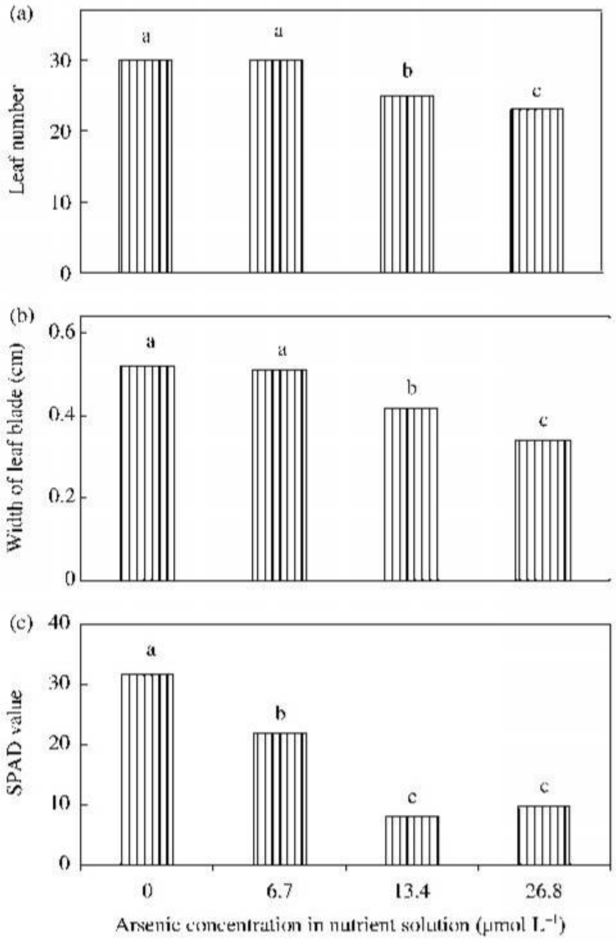

Figure 1 (a) Leaf number, (b) width of leaf blade and (c) SPAD value of fully developed young leaves (fifth) of rice seedlings with different levels of As. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

formation of Fe precipitates or Fe plaque on the root surface of the rice (Batty and Younger 2003).

Dry matter yield

It was clearly indicated that dry matter yield in shoot and root decreased significantly with As treatments in the nutrient solution (Fig. 2a). In the presence of As, the activity of enzyme or protein or plant growth regulators may be decreased, decreasing plant growth. This speculation needs verification. Dry matter yield decrease may also be associated with the reduction of leaf number, width of leaf blade; and shoot height and root length (Figs 1a,b,2b). Shoot dry matter yield decreased by 23, 56 and 64%, while the values of the roots were 15, 35 and 42% for the 6.7, 13.4 and 26.8 μmol L^{-1} As treatments, respectively. This result indicated that As caused greater reduction of shoot dry matter yield than that of the root. Our result suggested that the threshold value of As sensitivity in hydroponic culture was between 0 and 6.7 μmol L^{-1} As (0.5 mg As L^{-1}), considering that elemental concentration inducing > 10% reduction of dry matter yield is a critical toxic level (Ohki 1984). Abedin *et al.* (2002) found considerable reduction in straw and root biomass of rice with an 8 mg As L^{-1} concentration in a greenhouse pot experiment.

Shoot height and root length

Shoot height and root length decreased significantly in the As treatments (Fig. 2b). Shoot height decreased by 11, 35 and 43%, while the values for root length were 6, 11 and 33% for 6.7, 13.4 and 26.8 μmol L^{-1} As treatments, respectively. This result indicated that shoot height was more sensitive than root length. In the case of roots, As might destroy the root structure, resulting in a decreased root length. It is known that arsenite reacts with sulfhydryl groups of proteins of roots (Speer 1973) causing disruption of the root function (Isensee *et al.* 1971; Orwick *et al.* 1976) and even cellular death. Carbonell-Barrachina *et al.* (1995) found that the height of tomato plants decreased at 2, 5 and 10 mg As L^{-1} treatments. Arsenite and arsenate decreased the shoot height and root length of rice seedlings in a greenhouse pot experiment (Abedin and Meharg 2002). Plant height also decreased significantly in an 8 mg As L^{-1} treatment (Abedin *et al.* 2002). Tsutsumi (1980) observed, in rice, no reduction of plant height up to 125 mg As kg^{-1}, but observed 63% reduction at 312.5 mg As kg^{-1}.

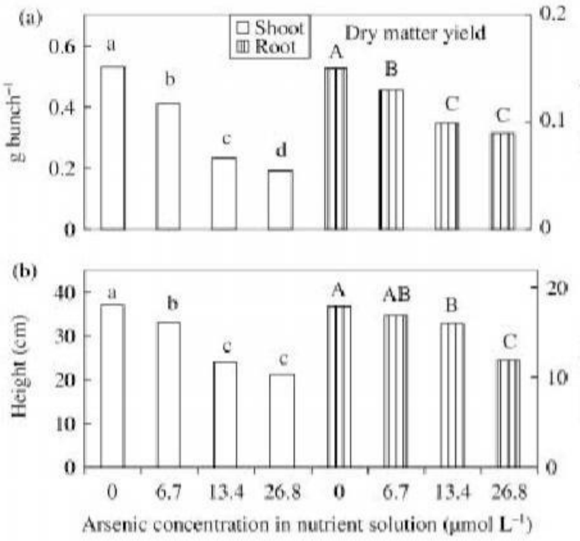

Figure 2 (a) Dry matter yield and (b) shoot height and root length of rice seedlings with different levels of As. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

Tiller number, leaf number and leaf blade

During the duration of this experiment (35 days), we did not find any new tiller even in control plants. However, leaf number and width of leaf blade decreased significantly in the 13.4 and 26.8 μM As treatments (Fig. 1a), and these parameters were considered to be responsible for the reduction in dry matter yield. Abedin *et al.* (2002) found lower numbers of tiller of rice in the 8 mg As L^{-1} treatment in their greenhouse pot experiment. Marin *et al.* (1993) reported that rice leaf area decreased in 0.8 and 1.6 mg As (dimethylarsinic acid, DMAA) L^{-1} treatments.

SPAD value (chlorophyll index)

Chlorophyll content in the fully developed youngest leaf of rice seedlings decreased significantly with As treatment (Fig. 1c). The lowest value was recorded in the 13.4 μmol L^{-1} As treatment. We found that the concentration of Fe decreased with As treatments and the lowest value was recorded in the 13.4 μmol L^{-1} As treatment (Fig. 3a). It is well known that Fe is essential for the formation of chlorophyll. In this experiment, Fe concentration decreased; therefore, the reduction in Fe concentration could be highly responsible for the reduction in chlorophyll content. It is our belief that we are presenting chlorophyll data in As toxic rice plants for the first time. It was considered that As-induced chlorosis resulted from Fe-deficiency because the chlorosis was found in the youngest leaves (Mengel and Kirkby 2001).

Arsenic concentration, accumulation and translocation

Arsenic concentration increased both in shoot and root as a result of the applied As in the nutrient solution (Fig. 4a). Arsenic concentration in the roots was 8, 16 10-fold greater than the concentration in the shoots at 6.7, 13.4 and 26.8 μmol L^{-1} As treatments, respectively, indicating that As was mostly concentrated in the root.

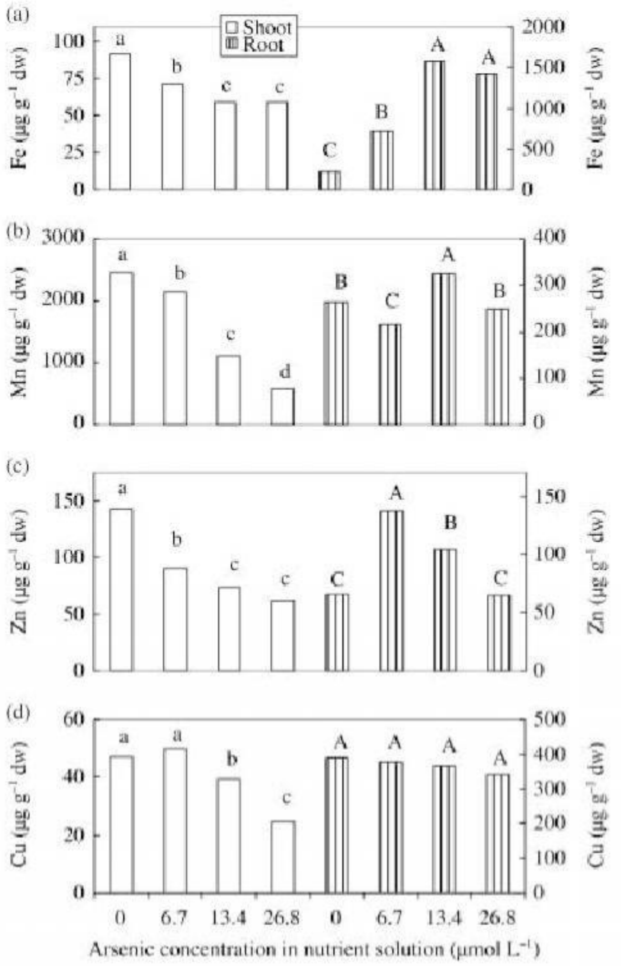

Figure 3 Effect of As on the concentration of (a) Fe, (b) Mn, (c) Zn and (d) Cu in shoots and roots of rice seedlings. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

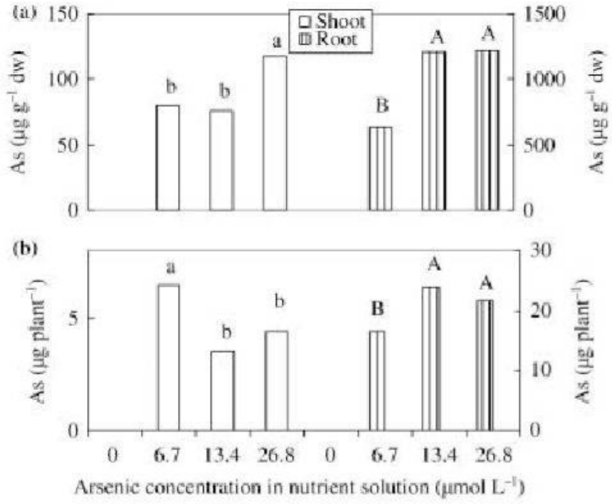

Figure 4 Effect of As on the (a) concentration and (b) accumulation of As in shoots and roots of rice seedlings. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

Yamane (1989) also reported a similar result that the roots of rice (*Oryza sativa* L. cv. Nihonbare) accumulated almost 90% of As. As for the accumulation, the values were 3, 7 and 5-fold greater for the treatments (Fig. 4b). Generally, anions are strongly adsorbed to the membrane surface of the roots. The As anions (arsenite and arsenate) may rapidly adsorb to the root surface, leading to the intense high As concentration, particularly in hydroponic culture (Wauchope 1983). This may be the reason why the highest levels of As are found in roots. In addition, the formation of Fe plaque in roots might be involved. The Fe plaque, coating of Fe hydroxides/oxides is commonly formed on the roots of aquatic plants such as rice (*Oryza sativa* L.). It is the consequence of oxidation of roots by release of oxygen and oxidants into the rhizosphere (Armstrong 1967; Chen *et al.* 1980). There may be two pathways by which arsenite enters into rice roots. In the primary way, part of the arsenite may be oxidized to arsenate in the root rhizosphere, which has a high affinity for iron plaque, co-precipitate with Fe^{3+} and adsorb on the plaque (Otte *et al.* 1991). At the root–plaque interface, siderophores by microbes or phytosiderophores exuded by rice roots may form a complex with Fe^{3+} and mobilize Fe-bound arsenate, and be taken up through phosphate co-transporters (Liu *et al.* 2005). This may stimulate the uptake of Fe and arsenate in/on the root surface and may increase As and Fe concentration in arsenite-treated plants in the present experiment. In the second possible pathway, arsenite may be accumulated on the Fe plaque in the form of H_3 $H_3AsO_3^0$ and then transported into rice roots via aquaporins (Meharg and Jardine 2003). In our experiment we found that in the presence of higher As concentrations, As accumulation was higher in roots and lower in shoots (Fig. 4b) compared to the lower concentration, this may be because of the fact that Fe plaque can act as a barrier to the uptake of toxic metals on the roots (Batty *et al.* 2000; Chen *et al.* 2005).

In a previous experiment, Wallace *et al.* (1980) could not detect As in root, stem and leaves of bush bean plants in 0, 10^{-6} and 10^{-5} mol L^{-1} $H_2AsO_4^-$ treatments in the nutrient solution, but observed 41.7, 18.8 and 3.6 μg As g^{-1} dw in 10^{-4} mol L^{-1} $H_2AsO_4^-$ treatment for root, stem and leaves, respectively. It was also reported that fronds contained an almost 24-fold greater As concentration than the roots in Brake fern (Ma *et al.* 2001). In our experiment, As translocation decreased in 13.4 and 26.8 μmol L^{-1} As treatments compared to the 6.7 μmol L^{-1} As treatment (Fig. 5). This suggested that the toxicity was very severe and reduced translocation

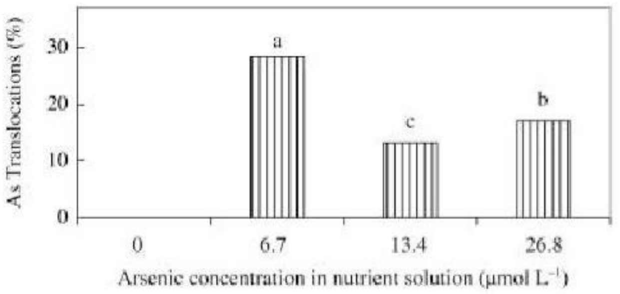

Figure 5 Effect of As on the translocation (%) of As from roots to shoots of rice seedlings. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

(%) in higher As treatments. It has already been reported that As (arsenite) translocation from root to shoot is limited by its high toxicity to root membranes (Sachs and Michael 1971). Liu *et al.* (2005) concluded that the main barrier to uptake and translocation of As, fed in the form of arsenite, might be the root tissue rather than Fe plaque.

Phosphorus concentration, accumulation and translocation

We observed that the concentration of P increased in shoots significantly in the 6.7 and 13.4 µmol L^{-1} As treatments and marginally in the 26.8 µmol L^{-1} As treatment (Fig. 6a) compared to the control plants, which might be a concentration effect because of dry matter yield (Fig. 2a) and the accumulation of P (Table 1) decreased. Marin *et al.* (1993) found that P concentration increased marginally in shoot and root of rice seedlings in 0.8 and 1.6 mg As (DMAA) L^{-1} treatments. It has been well documented that arsenate is taken up by the phosphate uptake system in plants (Asher and Reay 1979; Meharg and Macnair 1990). We found that the accumulation of P in shoots and roots decreased significantly (Table 1), but that translocation (%) increased (Table 2) with As toxicity. The antagonistic relationship is probably dependant on the concentration of P, the species of plant and the composition of the rooting medium. The relationship of the mechanism for P and As needs to be investigated.

Potassium concentration, accumulation and translocation

Concentration of K decreased significantly in shoots in the 26.8 µmol L^{-1} As treatment in the nutrient solution; but K concentration in roots decreased according to the increase of As concentration, indicating an antagonistic relationship between K and As (Fig. 6b). Accumulation was also negatively influenced by As (Table 1), but translocation (%) was not markedly affected (Table 2).

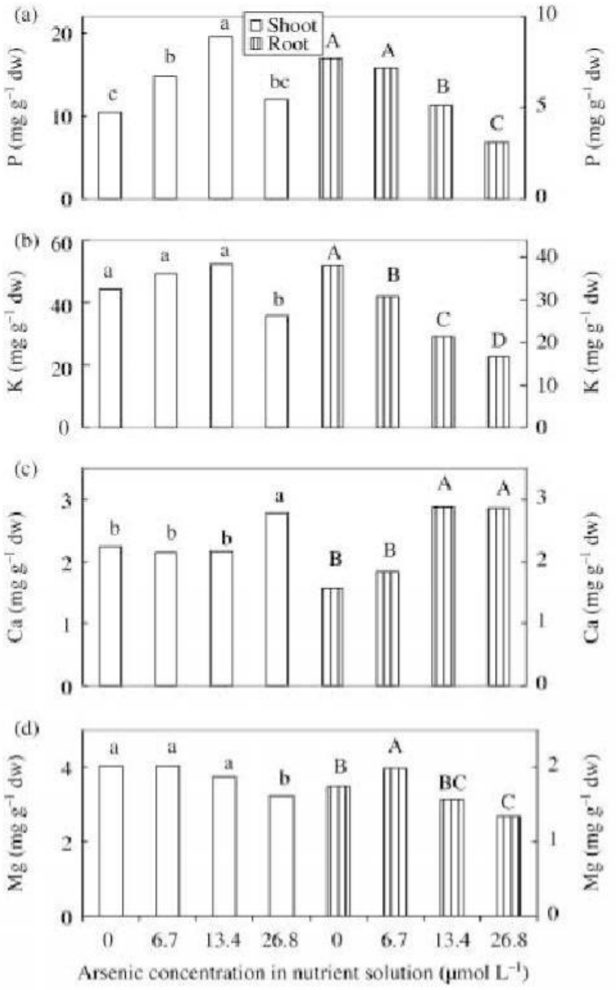

Figure 6 Effect of As on the concentration of (a) P, (b) K, (c) Ca and (d) Mg in shoots and roots of rice seedlings. Bars with different letters are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

It is well established that K is taken up as K^+ cation and As is taken up as anion. There may be no direct interaction between the absorption of As and K, but an antagonistic relationship between them was observed; assuming a toxic effect of As on K absorption. Yamane (1989) reported that the K concentration decreased in rice plants. It was also reported that the concentration of K decreased significantly in shoots, but marginally in roots, of rice seedlings in 0.8 and 1.6 mg As (DMAA) L^{-1} treatments (Marin *et al.* 1993).

Calcium concentration, accumulation and translocation

In shoots, the concentration of Ca was constant in the range between 0 and 13.4 µmol L^{-1} As, but increased significantly in the 26.8 µmol L^{-1} As treatment. Similar results were also found in roots in the 13.4 and

Table 1 Accumulation of nutrients in shoots and roots of rice seedlings grown in nutrient solution with different levels of As

Treatment (μmol L^{-1})	P (mg $plant^{-1}$)	K (mg $plant^{-1}$)	Ca (mg $plant^{-1}$)	Mg (mg $plant^{-1}$)	Fe (μg $plant^{-1}$)	Mn (μg $plant^{-1}$)	Zn (μg $plant^{-1}$)	Cu (μg $plant^{-1}$)
				Accumulation in shoot				
0	1.11 a	4.64 a	0.24 a	0.43 a	9.74 a	258.7 a	15.07 a	4.96 a
6.7	1.17 a	3.93 a	0.17 b	0.32 b	5.73 b	174.8 b	7.25 b	4.03 b
13.4	0.91 b	2.43 b	0.10 c	0.17 c	2.72 c	51.6 c	3.38 c	1.82 c
26.8	0.45 c	1.35 c	0.11 c	0.12 d	2.22 c	22.2 d	2.31 d	0.91 d
				Accumulation in root				
0	0.23 A	1.16 A	0.048 B	0.053 A	6.66 D	8.17 A	2.00 B	11.9 A
6.7	0.19 A	0.81 B	0.047 B	0.052 A	17.9 C	5.73 B	3.52 A	9.77 B
13.4	0.10 B	0.42 C	0.057 A	0.031 B	30.4 A	6.38 B	2.05 B	7.10 C
26.8	0.06 C	0.30 D	0.051 A	0.024 B	25.2 B	4.44 C	1.15 C	6.02 C

Means followed by different letters in each column are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

Table 2 Translocation (%) of elements from roots to shoots in rice seedlings grown in nutrient solution with different levels of As

Treatment (μmol L^{-1})	P	K	Ca	Mg	Fe	Mn	Zn	Cu
0	83 b	80 b	83 a	89 a	59 a	97 a	88 a	29 a
6.7	86 b	83 ab	78 b	86 b	24 b	97 a	67 b	29 a
13.4	90 a	85 a	64 c	85 b	8 c	89 b	62 b	21 b
26.8	89 a	82 b	67 c	84 b	8 c	83 b	67 b	13 c

Means followed by different letters in each column are significantly different ($P < 0.05$) according to a Ryan–Einot–Gabriel–Welsch multiple range test.

26.8 μmol L^{-1} As treatments (Fig. 6c). In the case of shoots, the concentration effect is related to the result because the dry matter yield of shoots decreased significantly. Accumulation of Ca in shoot (Table 1) and translocation (%) from root to shoot (Table 2) decreased significantly with increasing As. This was certainly because of As toxicity. Marin *et al.* (1993) found that the Ca concentration increased significantly in shoots in 1.6 mg As (DMAA) L^{-1} treatment, but increased in roots in the 0.8 mg As (DMAA) L^{-1} treatment. However, it has also been reported that the concentration of Ca decreased in leaves and roots of bush bean plant in 10^{-5} and 10^{-4} mol L^{-1} $H_2AsO_4^-$ treatments (Wallace *et al.* 1980). The different results obtained were probably due to varietal or species differences in the plants examined, the form of As, or the pH or methodology of the experiments.

Magnesium concentration, accumulation and translocation

It was clearly observed that the concentration of Mg in shoots was statistically constant in the range between 0 and 13.4 μmol L^{-1} As; however, it decreased significantly in the 26.8 μmol L^{-1} As treatment (Fig. 6d). A similar result was also obtained for roots at the highest As treatment. This may be the toxic effect of As on the nutritional status of Mg in the plant. Accumulation of Mg in the shoots was also negatively influenced by the increase in As (Table 1). We observed that the translocation (%) of Mg was also negatively influenced by As toxicity (Table 2). It has been reported that Mg concentration in shoots increased in 1.6 mg As (DMAA) L^{-1} treatment, but there was no change in rice roots (Marin *et al.* 1993). This dissimilarity may result from varietal differences in rice plants and/or the form of As. Wallace *et al.* (1980) reported that Mg concentration increased in leaves and stems in 10^{-4} mol L^{-1} $H_2AsO_4^-$ treatment, but decreased in roots at the same condition in bush bean plants. Carbonell-Barrachina *et al.* (1997) reported that Mg concentration in leaves decreased in 2 and 5 mg As L^{-1} treatments at the first fruiting stage, but increased at the harvesting stage.

Iron concentration, accumulation and translocation

Concentration of Fe was negatively influenced by As in shoots, but the opposite results were obtained for roots (Fig. 3a). In shoots, the highest concentration was recorded in control plants and the lowest in the 13.4 and 26.8 μmol L^{-1} As treatments. The highest value was for 13.4 and 26.8 μmol L^{-1} As treatments in roots. These results indicated that As concentrated Fe in roots and blocked Fe translocation from roots to shoots, which was supported by Table 2. It was found that the translocation (%) of Fe was mostly reduced by As among the metal micronutrients (Table 2). The obtained data of our water culture experiment suggested that Fe might be inactivated by As in or on the surface of roots. By using an X-ray micro analyzer, Yamane (1989) suggested that Fe and As accumulated on the surface of rice roots. The author also discussed that Fe might be precipitated with As in the rhizosphere; therefore, application of Fe^{3+} or Fe^{2+} might alleviate As toxicity in the soil. Marin *et al.* (1993) found that the concentration of Fe remained constant statistically in shoots but increased in the roots of rice plants in 1.6 mg As (DMAA) L^{-1} treatment. However, it has also been reported that Fe concentration increased in leaves, stems and roots of bush bean plants (Wallace *et al.* 1980). It is well established that Mg chlorosis is found first in old leaves (Maynard 1979), but that the Fe chlorosis is found in young leaves (Mengel and Kirkby 2001). In our experiment, the chlorotic symptoms were observed in the youngest leaf, indicating Fe chlorosis induced by As toxicity. Generally, leaves containing 30–50 μg Fe g^{-1} dw are considered to be Fe-deficient leaves (Bergmann 1988). We found that plants treated with As contained Fe concentrations slightly higher than the deficient level, this may be because we digested the old leaf together with the new leaf and determined the Fe concentration.

Manganese concentration, accumulation and translocation

The effect of As toxicity on Mn concentration was more pronounced in shoots than roots. Manganese concentration decreased significantly with increasing As concentration and the lowest value was obtained for the highest As concentration (Fig. 3b). Marin *et al.* (1993) also found a similar result in roots of rice seedlings, although the differences were not significant. However, Yamane (1989) reported that the concentration of Mn increased both in shoots and roots of rice with the application of As^{3+} or As^{5+} at a rate of 33.5, 67 and 134 mg kg^{-1} of soil. Manganese concentration decreased in leaves and roots of bush bean plant in 10^{-4} mol L^{-1} $H_2AsO_4^-$ treatment, but increased in stems in the same treatment (Wallace *et al.* 1980). It was suggested that As did not induce Mn toxicity in our experiment. It is known that Mn toxicity induces Fe deficiency (Alam *et al.* 2000). Shoots of rice containing 9,181 μg g^{-1} dw showed symptoms of Mn toxicity (Alam *et al.* 2003). We found that accumulation (Table 1) and translocation (%) of Mn (Table 2) decreased significantly in the highest As treatment. In contrast, the critical deficient level of Mn in plants was similar; varying between 10–20 mg Mn kg^{-1} dw in fully expanded leaves (Marschner 1998). The values of this experiment were much higher than the critical deficient level of Mn. The plants treated with 13.4 and 26.8 μmol L^{-1} As were not Mn deficient or suffering from Mn toxicity. Therefore, it was considered that Mn toxicity was not related to the induction of whitish chlorosis symptoms observed in the youngest leaves of the rice.

Zinc concentration, accumulation and translocation

Concentration of Zn decreased in shoots treated with As, but increased in 6.7 and 13.4 μmol L^{-1} As treatments in roots (Fig. 3c) compared with the control treatment. Not only the concentration, but also the accumulation, of Zn decreased at the highest As treatment. It is well known that P can induce Zn deficiency (Marschner and Schropp 1977) and As could compete with P (Asher and Reay 1979). The facts may explain the result that high quantities of As may reduce Zn concentration. In leaves, the critical deficiency levels are below 15–20 μg Zn g^{-1} dw. Marin *et al.* (1993) found that the Zn concentration increased in shoots of rice in 1.6 mg As (DMAA) L^{-1} treatment but decreased in roots in 0.2 mg As (DMAA) L^{-1} treatment. It has been shown that Zn toxicity leads to chlorosis in young leaves (Marschner 1998). The critical toxicity level of crop plants is between 100 and more than 300 μg Zn g^{-1} dw (Ruano *et al.* 1988), with the latter values more typical (Marschner 1998). Our data was within the normal level (from 144 [in control plants] to 60 μg Zn g^{-1} dw [in 26.8 μmol L^{-1} As treated plants]). Based on our results, it was considered that Zn toxicity was not involved in the production of chlorotic symptoms in the leaves in this experiment.

Copper concentration, accumulation and translocation

Copper concentration in shoots decreased with increasing As. However, the concentration of Cu in the roots was constant (Fig. 3d), indicating that As hindered the translocation of Cu from roots to shoots. Accumulation and translocation (%) of Cu decreased with As toxicity (Tables 1,2). This was considered to be a toxic effect of As. It has been reported that Cu concentration increased in roots of rice in 1.6 mg As L^{-1} treatment (Marin *et al.* 1993). Our experimental rice plants contained Cu concentrations (24.4–50.0 μg g^{-1} dw) over the critical toxic level. For most crop species, the critical toxicity level of Cu in the leaves is above 20–30 μg g^{-1} dw (Robson and Reuter 1981). However, marked differences in Cu tolerance among plant species may also be possible. In certain Cu-tolerant species (metallophytes) of the natural vegetation, the Cu content in leaves can be as high as 1,000 μg g^{-1} dw (Morrison *et al.* 1981). Considering the fact that the Cu concentration in shoots decreased with increasing As, Cu toxicity may not be involved in the production of chlorosis symptoms in leaves.

Conclusion

Arsenic toxicity decreased the dry matter yield, shoot height, root length, leaf number and leaf blade. Shoots were more sensitive to As than roots. The critical toxic level for As in this rice cultivar in hydroponic culture may be between 0 to 6.7 µmol L^{-1} As (0–0.5 mg L^{-1}). Arsenic induced chlorotic symptoms in the youngest leaves. In the plants treated with higher As concentrations, Fe, Mn, Zn and Cu concentrations in shoots were reduced compared with control plants. Therefore, it was suggested that the chlorosis was most probably Fe chlorosis caused by Fe deficiency induced by As, and was not the symptom of heavy-metal-induced Fe deficiency. This chlorosis symptom may be caused by a Fe-translocation problem because Fe-translocation was most affected among the nutrient elements. Arsenic and Fe were mostly concentrated in or at the surface of the rice roots. Our experimental results suggested that Fe may be inactivated by As in or at the surface of the roots.

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