Research Article

Regulation of sugar metabolism in rice (*Oryza sativa* L.) seedlings under arsenate toxicity and its improvement by phosphate

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ABSTRACT

The effect of arsenate with or without phosphate on the growth and sugar metabolism in rice seedlings cv. MTU 1010 was studied. Arsenate was found to be more toxic for root growth than shoot growth and water content of the seedlings gradually decreased with increasing concentrations. Arsenate exposure at 20 μM and 100 μM resulted in an increase in reducing sugar content and decrease in non-reducing sugar content. There was a small increase in starch content, the activity of starch phosphorylase was increased but α-amylase activity was found to be decreased. Arsenate toxicity also affected the activities of different carbohydrate metabolizing enzymes. The activities of sucrose degrading enzymes viz., acid invertase and sucrose synthase were increased whereas, the activity of sucrose synthesizing enzyme, viz. sucrose phosphate synthase declined. The combined application of arsenate with phosphate exhibited significant alterations of all the parameters tested under the purview of arsenate treatment alone which was congenial to better growth and efficient sugar metabolism in rice seedlings. Thus, the use of phosphorus enriched fertilizers may serve to ensure the production of healthy rice plants in arsenic contaminated soils. **[Physiol. Mol. Biol. Plants 2010; 16(1) : 59-68]** *E-mail : asokbiswas@ymail.com*

Keywords : *Amelioration, Arsenic, Phosphate, Rice, Sugar metabolism*

Abbreviations : *As- arsenic, cv.- cultivar, DNSA- 3,5-dinitrosalicylic acid, DTT- dithiothreitol, EDTA- ethylenediamine tetraacetic acid, fw- fresh weight, HEPES- N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid, KI- potassium iodide, PFD- photon flux density, PMSF- phenyl methyl sulphonyl fluoride, SE- standard error, SPS- sucrose phosphate synthase, SS- sucrose synthase, TCA- trichloroacetic acid, UDP- uridine-di-phosphate*

INTRODUCTION

Arsenic is a naturally occurring pollutant and arsenic pollution has gained at present a burning global importance. Arsenic contaminated ground water is extensively used for irrigating crops. The impact of this contaminated irrigation on the arsenic content of rice (*Oryza sativa* L.) is important as it is the major staple food crop of the world population. It is grown in the flooded soil where chances of arsenic availability is very high and arsenic toxicity can lead to reduced yield.

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Arsenic is found as inorganic form in ground water. Two forms of inorganic arsenic viz., arsenite (Na_2HAsO_3)[As(III)] and arsenate (Na_3AsO_4)[As(V)] are interconvertable depending on the redox condition of the soil (Rochette *et al*.,1998) with arsenite dominating in flooded paddy soil.

Arsenate, the dominant form of arsenic in aerobic conditions, is taken up by the plants via the phosphate transport system because of the chemical similarity between arsenate and phosphate which compete for the same site in the soil and is taken up via phosphate transporters on the plasma membrane of the epidermal cells (Ullrich and Eberius *et al*.,1989). Arsenate inhibits the phosphate uptake in yeast (Rothstein and Donovan, 1963), phytoplanktons (Blum, 1966), *Arabidopsis thalliana* (Clark *et al*., 2003), wheat (Geng *et al*., 2006) and in the arsenate hyperaccumulators like *Pteris vittata*.

Similarly, phosphate suppresses arsenate uptake in rice (Abedin *et al*., 2002) and arsenic tolerant plants like *Holcus lanatus, Cytisus striatus.*

Arsenate, after accumulation in plants, is converted to arsenite in presence of arsenate reductase (Rosen, 2002; Duan *et al*., 2005; Ellis *et al*., 2006; Dhankher *et al*., 2006). Arsenite reacts with sulphydryl groups (-SH) of enzymes and tissue proteins, leading to the inhibition of cellular function and death of the plant (Ullrich-Eberius *et al*., 1989).

Many environmental stressful conditions affect the process of sugar metabolism in growing plants leading to the accumulation of soluble sugar in the tissues. Accumulation of sugars under stress appears to contribute to osmoregulation and provides protection of biomolecules. Starch and sucrose are principal end products of photosynthesis. Starch accumulates in the leaves as a temporary reserve form of carbon and then stored in the cereal grains. Sucrose is the major form of translocated carbon (Zhou *et al*., 2002).

Major starch hydrolyzing enzymes present in plants are α-amylase and starch phosphorylase (Yang *et al*., 2001). α-amylase produces D-glucose and oligosaccharide units from starch which provide the energy for the growth of shoots and roots. Starch phosphorylase degrades starch beginning at a non-reducing end by incorporating phosphate (Salisbury and Ross, 1991).

Sucrose phosphate synthase (SPS) catalyses the synthesis of sucrose in photosynthetic and non-photosynthetic plant tissues (Geigenberger and Stitt, 1993). Sucrose synthase (SS) and acid invertase are involved in sucrose breakdown and play an important role in energy metabolism by metabolizing sucrose into diverse pathways relating to metabolic function of storage cells (Ranwala and Miller, 1998). Sucrose synthase is a cytosolic enzyme catalyzing sucrose breakdown *in vivo* (Geigenberger and Stitt, 1993). Invertase is the ubiquitous enzyme with different pH optima, acid invertase is located in the vacuoles and catalyzes hydrolysis of sucrose to glucose and fructose (Van den Ende *et al*., 2002). In cases of anoxia of rice plants, an increase in the activities of SS and SPS and in cold hardened winter oat plant an increase in invertase activity have been noticed (Livingston and Henson, 1998).

Therefore, the present investigation was undertaken to examine the effect of arsenate on the metabolic status of starch, sugars (both reducing and non-reducing) and different sugar metabolizing enzymes in germinating rice seedlings. A combined application of arsenate with phosphate was tried to combat the serious problem of arsenic toxicity and to provide a possible method of arsenic tolerance.

MATERIALS AND METHODS

Plant materials and arsenic treatments

Rice (*Oryza sativa* L.) seeds cv. MTU 1010 obtained from the State Rice Research Station, Chinsurah, Hooghly, West Bengal were surface sterilized with $HgCl_2$ (0.1 %, w/v) and washed thoroughly. About 50 seeds for each treatment were spread over in petridishes (ϕ 10cm) lined with filter papers containing either different concentrations of sodium arsenate ($Na_2HAsO_4.7H_2O$) only, purchased from *Loba-Chemie*, India or arsenate combined with potassium dihydrogen orthophosphate (KH_2PO_4) from *Merck*, India. The seeds were kept in dark and humid conditions for 48 h in a germinator at 30 ± 2 °C, then exposed to 16 h photoperiod (260 μmol m^{-2} s^{-1} PFD) for five days. The seedlings were harvested after ten days for the following studies. The above mentioned arsenate concentrations are comparable to soil conditions and environmentally relevant.

Morphological studies

After ten days, arsenic induced damaging effects were observed and the relevant root lengths and shoot lengths of growing rice seedlings were measured. The seedlings were harvested, weighed in equal amount for each set and stored at -40 °C for further biochemical studies.

Biomass measurement

From each treatment, 10 seedlings were harvested and weighed. The roots and shoots of each set were separated and were allowed to dry in an oven at 70 °C for 3 days and then at 100 °C for 2 more days. After the stipulated period, dry weights were taken and the differences of fresh and dry weight (water content) between the treatment sets were observed.

Estimation of total soluble sugar

The amount of total soluble sugar was estimated by phenol sulphuric acid reagent method (Dubois *et al.,* 1956). The shoot and root samples were homogenized with 80 % ethanol. The alcoholic extracts were centrifuged at 2000 rpm for 20 minutes. To 1.0 ml of supernatant, 0.05 ml of 5 % phenol solution and 98 % sulphuric acid were added. The mixtures were incubated

in water bath at 30 °C for 20 minutes. The OD values of the yellow orange colour were measured at 490 nm in Hitachi-2000 spectrophotometer. By using standard curve of glucose, the quantity of the total soluble sugar was calculated and expressed as mg g^{-1} fw.

Estimation of reducing and non-reducing sugar

Estimation of reducing sugar was done by the method of Miller (1972). 1 gm of plant material was crushed with 80 % ethanol and centrifuged at 2000 rpm for 20 minutes. To 1.0 ml of alcoholic extract, DNSA reagent was added and boiled in water bath for 5 min. The absorbance was measured at 515 nm using Hitachi-2000 spectrophotometer. From a standard curve of glucose, the quantity of reducing sugar was calculated and expressed as mg g^{-1} fw. The amount of non-reducing sugar was measured by substracting the value of reducing sugar from the value of total soluble sugar.

Estimation of starch

Estimation of starch was done by the method of McCready *et al.* (1950). The residual mass, obtained after centrifugation for the extraction of total soluble sugar was suspended in distilled water followed by the addition of perchloric acid. After stirring, the mixture was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected and poured in conical flasks and the total volume was made upto 100 ml by adding distilled water for each set. Then 1.0 ml of filtrate from each set was taken and starch content measured following the same procedure as the total soluble sugar. Quantity of starch was calculated in terms of glucose and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed in mg g^{-1} fw.

Assay of α-amylase activity

α-amylase activity was assayed followed by the method of Bush *et al.* (1989). The plant materials were homogenized in 0.1 M sodium acetate buffer (pH 4.8) containing 5μM cysteine and centrifuged at 10000 rpm and 4 ^{0}C for 15 minutes. Supernatants were heated at 70 ^{0}C for 5 minutes in presence of 3mM $CaCl_2$, which destroy the activity of β-amylase. The reaction mixture contained 0.1 M sodium acetate buffer (pH 4.8), 1 % soluble starch in 0.15 M NaCl and enzyme extract to make the total volume of 4.0 ml. After incubation at 30 ^{0}C for 5 minutes, the reaction was terminated with the addition of 6 M HCl. 1.0 ml aliquots were transferred to a conical flask followed by the addition of 0.5 ml of IKI solution (0.2 % I_2 in 2 % KI). Volume was finally made upto 25 ml with distilled water and absorbance was measured at 660 nm. Enzyme activity is expressed as μg of starch hydrolyzed g^{-1} fw. min^{-1}.

Assay of acid invertase activity

Acid invertase was assayed according to Borkowska and Szczerba (1991). Root and shoot samples were homogenized in 10mM sodium acetate buffer (pH 4.6) containing 3.3mM $MgCl_2$, 1mM EDTA, and 1mM PMSF. The homogenates were centrifuged at 10000 rpm and 4 ^{0}C for 20 minutes. The assay mixture contained 10 mM sodium acetate buffer (pH 4.6), 0.4 M sucrose, and enzyme extract to make the total volume upto 1.0 ml. After incubation at 30 ^{0}C for 30 minutes, the reaction was terminated with the addition of 0.5 M Na_2HPO_4. The resulting reducing sugars were estimated by Nelson-Somogyi method (Nelson, 1944; Somogyi, 1945). Invertase activity was expressed as nmol sucrose hydrolysed g^{-1} fw. min^{-1}.

Assay of starch phosphorylase activity

For determination of starch phosphorylase activity (Dubey and Singh, 1999), plant materials from each treatment were homogenized in 50mM citrate buffer (pH 6.0) containing 1mM EDTA, 5mM β-mercaptoethanol and 1mM PMSF and centrifuged at 10000 rpm for 20 minutes at 4 ^{0}C. The assay mixture contained 50 mM citrate buffer (pH 6.0), 5 % soluble starch (w/v), 0.1mM glucose-1-phosphate and enzyme extract to make the total volume upto 4.0 ml. The reaction was stopped after 10 minutes by adding 5 % TCA. The mixture was centrifuged and phosphorus content in the supernatant was estimated following the method of Fiske and Subbarow (1925). The enzyme activity was calculated as nmol of P_i liberated g^{-1} fw. min^{-1}.

Assay of sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity

For the assay of sucrose phosphate synthase (SPS) and sucrose synthase (SS), the respective tissues were extracted following the method of Hubbard *et al.* (1989) and assayed according to Miron and Schaffer (1991). Plant samples were extracted in 50 mM HEPES-NaOH buffer (pH 7.5) containing 5mM $MgCl_2$, 1mM EDTA, 2.5mM DTT and 0.05 % (v/v) Triton X-100, at 4 ^{0}C, centrifuged at 10000 rpm and 4 ^{0}C for 10 minutes. Assay mixture for SPS contained 50 mM HEPES-NaOH buffer (pH 7.5), 15mM $MgCl_2$, 25mM fructose-6-phosphate, 25mM glucose-6-phosphate, 25 mM UDP-glucose and enzyme extract. Mixtures were incubated for 30 min at 37 °C and reaction was terminated with the addition of

30 % KOH. The reaction mixture for SS assay was similar to SPS assay but it contained 25 mM fructose instead of fructose-6-phosphate and was devoid of glucose-6-phosphate. The sucrose hydrolysed during SS catalyzed reaction and sucrose formed during SPS catalyzed reaction were estimated according to Vassey *et al.* (1991). The enzyme activities were expressed as nmol sucrose hydrolysed or formed g^{-1} fw. min^{-1} respectively.

Statistical analysis

The experiments were carried out in a completely randomized design (CRD) with five replicates; each replica comprising a single petridish containing an average of 50 seeds. The data and significant differences among the mean values were compared by descriptive statistics (± SE).

RESULTS

Effect of arsenate and phosphate on the water content of rice seedlings

Arsenic toxicity adversely affected the water content of ten days old rice seedlings. The water content in both roots and shoots decreased significantly with increasing arsenate treatment (Table 1). The water content of roots was markedly decreased by 7 % and 42 % at 20 μM and 100 μM arsenate treatment respectively compared to water control whereas, phosphate application along with same level of arsenate was found to exhibit increased water content of root by 13 % and 82 % with respect to seedlings having only arsenic treatment (Table 1).

Shoot water content (stem plus leaf) decreased with increasing arsenic treatment especially in 100μM concentration. There was about 5 % and 19 % decreased water content at 20 μM and 100 μM arsenate treatment respectively in comparison with water control. Contrarily, by joint application of phosphate and arsenate, the water of shoot was increased, on an average, by about 20 % (Table 1).

Effect of arsenate and phosphate on the starch and sugar content

Starch

The 10 days old arsenate treated rice seedlings showed a little higher level of starch contents as compared with the water control. In shoot, 20 μM and 100 μM arsenate treatments led to about 4 % and 6 % increment of starch content respectively, while in root, the rate of increase of starch contents were about 2 % and 7 % respectively. When 20 μM and 100 μM arsenic treatment sets were treated with 2 mM phosphate, the starch content decreased in shoot, the amount of decrease being 7 %, on an average, whereas, root starch content changed but little (Table 2).

Table 1. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on the growth and water contents in root and shoot of rice (cv. MTU 1010) seedlings. The data were recorded from ten days old rice seedlings.

Treatments	Seedling Length (cm)		Water Content (mg water g^{-1} fresh tissue)	
	Root	Shoot	Root	Shoot
Control	5.54 ± 1.01	7.32 ± 0.41	843.0 ± 0.21	804.5 ± 0.45
Arsenate (μM)				
20.0	5.36 ± 0.94	7.16 ± 0.22	785.0 ± 0.16	761.5 ± 0.31
100.0	0.98 ± 0.06	3.44 ± 0.25	488.0 ± 0.14	654.0 ± 0.21
Phosphate (2mM)	8.00 ± 0.16	9.56 ± 0.89	928.0 ± 0.18	905.0 ± 0.22
+Arsenate (μM)				
20.0	7.60 ± 0.17	8.50 ± 0.26	886.0 ± 0.09	830.0 ± 0.16
100.0	5.00 ± 0.88	7.80 ± 0.38	891.0 ± 0.14	825.0 ± 0.24

The values are means of 5 replicates ± SE

Reducing sugar

The amount of reducing sugar in root was found to be decreased by arsenate treatment from water control. In 20μM and 100μM arsenate treated sets about 66 % and 74 % decrease in reducing sugar contents of roots were noticed respectively but 2 mM phosphate treatment along with 20 μM and 100 μM arsenate led to dramatic increase by about 5 to 6 times. This trend was opposite in case of shoots, where 20 μM and 100 μM arsenate treatment alone could induce 120 % and 132 % increase in reducing sugar contents which were also maintained at higher levels in the phosphate plus arsenate treated seedlings (Table 2).

Non-reducing sugar

In both shoot and root, the amount of non-reducing sugar content was found to be decreased with higher concentrations of arsenate. In root, at 20 μM and 100 μM arsenate treated seedlings, about 30 % and 40 % decrease in non-reducing sugar contents were observed from water control whereas, phosphate treatment along with arsenate led to 15 % and 33 % increase in non-reducing sugar contents than seedlings receiving only arsenate. Likewise, in shoots, the non-reducing sugar content was found to be decreased by 55 % and 57 % at 20 μM and 100 μM arsenate treatment respectively. By joint application of phosphate along with arsenate the amount of non-reducing sugar could be raised to an elevated level (Table 2).

Effect of arsenate and phosphate on sucrose degrading enzyme activities

Sucrose synthase

The toxic effect of arsenate on ten days old rice seedlings showed increased activity of sucrose synthase in both root and shoot. The activity was a little increased by 5 % and 10 % in roots at 20 μM and 100 μM arsenate treatments respectively while in shoots, about 14 % and 28 % increase in enzyme activity was recorded under same concentrations of arsenate (Fig. 2A). When the

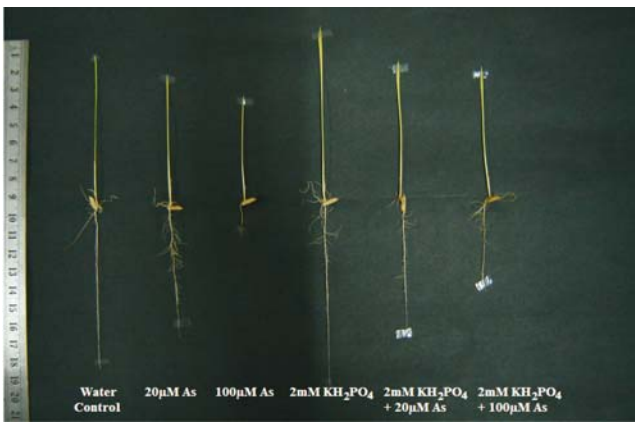

Fig. 1. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on relative root and shoot length of ten days old rice (cv. MTU 1010) seedlings.

Table 2. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on the sugar and starch contents in root and shoot of rice (cv. MTU 1010) seedlings. The data were recorded from ten days old rice seedlings.

Treatments	Sugar and starch content (mg g^{-1} fresh tissue)					
	Reducing		Non-Reducing		Starch	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	5.4 ± 0.04	5.0 ± 0.01	8.6 ± 0.06	9.4 ± 0.04	108.0 ± 1.3	122.4 ± 1.0
Arsenate (μM)						
20.0	1.8 ± 0.05	11.0 ± 0.03	6.0 ± 0.01	4.2 ± 0.02	109.8 ± 1.8	127.8 ± 1.4
100.0	1.4 ± 0.01	11.6 ± 0.04	5.2 ± 0.03	4.0 ± 0.02	115.2 ± 1.0	129.6 ± 1.8
Phosphate (2mM)	11.4 ± 0.02	7.0 ± 0.01	6.0 ± 0.02	7.8 ± 0.01	100.8 ± 2.1	126.0 ± 2.3
+Arsenate (μM)						
20.0	8.7 ± 0.01	8.8 ± 0.01	6.9 ± 0.01	5.6 ± 0.02	111.6 ± 2.5	118.8 ± 1.9
100.0	8.1 ± 0.02	10.2 ± 0.03	6.9 ± 0.01	4.2 ± 0.01	115.2 ± 2.0	120.6 ± 2.0

The values are means of 5 replicates ± SE

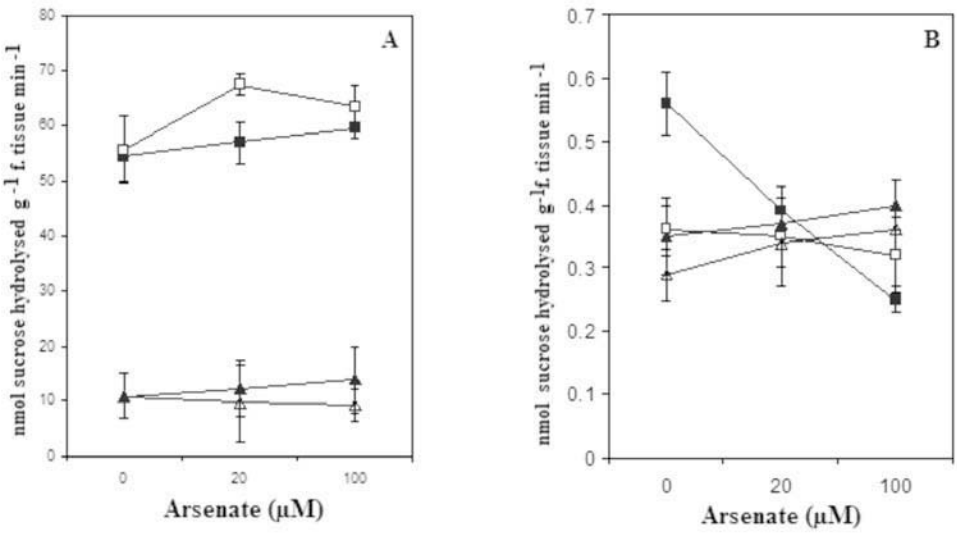

Fig. 2. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on the activities of sucrose synthase (SS) [A] and acid invertase [B] of ten days old rice (cv. MTU 1010) seedlings. Each data point expressed as the mean ± SE (n=5). ■- As (Root); ▲– As (Shoot); □- As+PO_4 (Root); Δ- As+PO_4 (Shoot).

seedlings were treated with 2 mM phosphate along with same doses of arsenate, the sucrose synthase activity was found to be decreased in shoots by about 21 % and 34 % whereas in roots, the SS activity was increased upto 18 % and 7 % in 20 µM and 100 µM arsenate treatments along with phosphate respectively (Fig. 2A).

Acid invertase

In this rice cultivar, a little increase in acid invertase activity was observed in response to 20 µM and 100 µM arsenate in shoots, which were 6 % and 14 % respectively over water control. But in root, acid invertase activity was decreased by about 30 % and 55 % at 20 µM and 100 µM arsenate treatments respectively (Fig. 2B). By phosphate application along with 20 µM and 100 µM arsenate treatments, the amount of inhibition caused by arsenate alone was reduced to about 8 % and 10 % respectively in shoots whereas, in roots there was 28 % increment in enzyme activity when 100 µM arsenate was combined with phosphate (Fig. 2B).

Effect of arsenate and phosphate on sucrose synthesizing enzyme activity

Sucrose phosphate synthase

The activity of sucrose phosphate synthase was found to be decreased in both roots and shoots on arsenate exposure in ten days old rice seedlings. In roots, about 19 % and 26 % decrease in enzyme activity was recorded at 20 µM and 100 µM arsenate treatments respectively while in shoots, the enzyme activity was decreased by about 17 % and 31 % from water control at the same levels of arsenate treatments (Fig. 3). On the contrary, the phosphate application along with 20 µM and 100 µM arsenate treatments led to decrease in

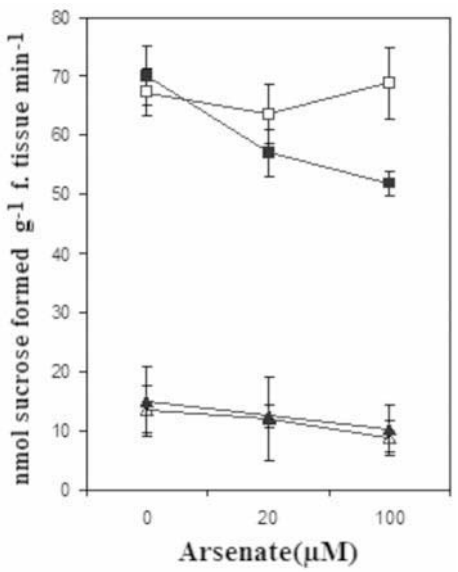

Fig. 3. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on the activity of sucrose phosphates synthase (SPS) of ten days old rice (cv. MTU 1010) seedlings. Each data point expressed as the mean ± SE (n=5). ■- As (Root); ▲- As (Shoot); □- As+PO_4 (Root); Δ- As+PO_4 (Shoot).

enzyme activity to about 3 % and 15 % respectively in shoots. In roots, the SPS activity was found to be increased by about 12 % and 33 % when 20 μM and 100 μM arsenate were combined with phosphate (Fig.3).

Effect of arsenate and phosphate on starch hydrolyzing enzymes activities

Starch phosphorylase

Rice seedlings showed increased activity of starch phosphorylase in both roots and shoots due to arsenate toxicity after ten days. In roots, about 8 % and 33 % increase in starch phosphorylase activity was observed in 20 μM and 100 μM treatments respectively, while in shoots, about 12 % and 20 % increase in enzyme activity was found in respect of water control (Fig. 4A). When the seedlings were treated with 2 mM phosphate along with the same concentrations of arsenate, variable results in enzyme activity were found. In roots, phosphate with 20 μM arsenate treatment exhibit no change in enzyme activity but the activity was increased up to 13 % with 100 μM arsenate alone. In shoots, phosphate application combined with arsenate also caused changes in enzyme activity and that was about 9 % increase in 20 μM arsenate and about 8 % decrease in 100 μM arsenate treatments along with phosphate respectively (Fig. 4A).

α-amylase

In both shoot and root, the activity of α-amylase was found to be decreased with higher concentrations of arsenate. In root of 20 μM and 100 μM arsenate treated seedlings, about 38 % and 50 % decrease in enzyme activity were observed from water control (Fig. 4B) whereas, in phosphate treatment along with arsenate increased level in α-amylase activity and variable amounts of relief of inhibition caused by arsenate alone were obtained. In shoots, the enzyme activity was found to be decreased by 44 % and 72 % at 20 μM and 100 μM arsenate treatments respectively. By joint application of arsenate along with phosphate, the arsenic induced inhibition was partially relieved (Fig. 4B).

DISCUSSION

Effect of arsenate and phosphate on the growth of seedlings

Arsenate exposure significantly altered the normal growth and development of rice seedlings. The reduction of both root and shoot lengths was observed with increasing concentrations of arsenate. The rate of reduction in root length was much more prominent than the shoot length. Under 100 μM of arsenate treatment, the root was found to be completely damaged with browning of the tissues. Several workers reported the reduction of root length in different plant materials due to arsenate toxicity (Sneller *et al*., 1999; Hartley-Whitaker *et al*., 2001). In our study, the effect was found to be ameliorated by the application of phosphate along

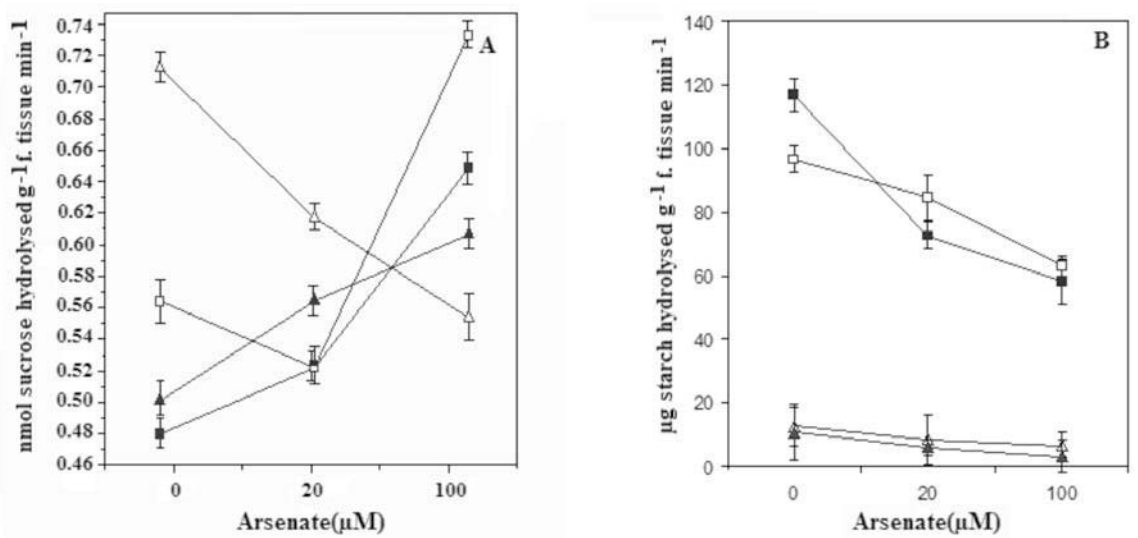

Fig. 4. Effect of arsenate ($Na_2HAsO_4.7H_2O$) and phosphate (KH_2PO_4) applied either singly or in combination on the activities of starch phosphorylase [A] and alpha- amylase [B] of ten days old rice (cv. MTU 1010) seedlings. Each data point expressed as the mean ± SE (n=5). ■- As (Root); ▲- As (Shoot); □- As+PO_4 (Root); Δ- As+PO_4 (Shoot).

with arsenate. Arsenate exposure also affected water contents of root and shoot, with a large scale reduction by increasing arsenate concentrations. The loss of water contents can be recovered both in roots and shoots with the addition of phosphate along with arsenate.

Effect of arsenate and phosphate on carbohydrate metabolism

The rate of transport of photosynthates into individual sink organ is an important component of growth and development of plant organs and it is a criterion deciding crop productivity as they contribute to major part of the dry matter of plant. Photosynthetically fixed carbon is ultimately converted to sucrose and starch in the photosynthetic cells. During photosynthesis, starch is formed as a temporary storage form of fixed carbon and is deposited as starch granules in the chloroplast and finally stored in the cereal grains. Sucrose is transported to different organs and is used for various metabolic activities of the seedlings.

The present results indicate the increase of soluble reducing sugar and decrease of non-reducing sugar with increasing concentrations of arsenate. The accumulation of higher level of sugars in arsenate stressed rice plant (mainly in shoots) may provide an adaptive mechanism in maintaining the favourable osmotic potential and in protecting the biomolecules and membranes from dehydration (Hayashi *et al*., 1997; Sinniah *et al*., 1998). Increased levels of soluble sugar have been reported to be increased under salinity (Dubey, 1997), water-stress (Foyer *et al*., 1998) and chilling effect (Hurry *et al*., 1995).

Sucrose phosphate synthase (SPS) is the major enzyme which catalyses the synthesis of sucrose phosphate during the last step of dark CO_2 fixation in photosynthetic and non-photosynthetic plant tissues (Geigenberger and Stitt, 1993) and is an important control point in biosynthesis of sucrose (Stitt *et al.,* 1987; Krause *et al.,*1998). Prolonged water stress limits the SPS activity and photosynthesis in leaves of mungbean, whereas in spinach leaves stimulation of SPS activity was observed (Quick *et al.,* 1989). The activity of SPS is also induced under low temperature and osmotic stress (Krause *et al.,* 1998). The present study, however, reveals the decreased activity of SPS with increasing arsenate concentration that finds support in the decrease in non-reducing sugar like sucrose as reported earlier. But phosphate treatment is found to ameliorate the deleterious effect of arsenic on SPS activity along with the increase of sucrose content in rice seedlings.

Sucrose synthase (SS) and acid invertase are involved in the breakdown of sucrose and play an important role in energy metabolism by metabolizing sucrose into diverse pathways relating to metabolic function of storage cells (Pfeiffer and Kutschera, 1996; Ranwala and Miller, 1998). Sucrose synthase is a cytosolic enzyme that catalyzes sucrose breakdown *in vivo* (Geigenberger and Stitt, 1993). Acid invertase is located in the vacuoles and catalyzes hydrolysis of sucrose to glucose and fructose (Van den Ende *et al.*, 2002). These enzymes play an important role in phloem loading and unloading by maintaining sucrose concentration gradient (Lohaus *et al.,* 1995). Young leaves of growing plants are sinks for sucrose and possess high activities of both the enzymes (Witt, 1989). During anoxia of rice plants, there is an increase in the activities of SS and in cold hardened winter oat plant an increase in invertase activity was noticed (Livingston and Henson, 1998). The results of the present study of increase in acid invertase activity (mainly in shoots) and SS activity is found to be parallel with the increased level of reducing sugar and decreasing level of non-reducing sugar content. But due to phosphate treatment along with arsenate, the activity was found to be reversed which may cause an increase in sucrose contents in stressed condition. It has been noticed in the present study that among the sucrose hydrolyzing enzymes, the activity of sucrose synthase was more pronounced than the acid invertase activity.

Major starch hydrolyzing enzymes present in plants are α-amylase and starch phosphorylase (Yang *et al.,* 2001). α-amylase catalyzes breakdown of starch producing D-glucose and oligosaccharide units which provide the energy for the growth of shoots and roots. Starch phosphorylase is another starch degrading enzyme which catalyzes reversible phosphorylation of α**-**glucans producing glucose-1-phosphate and degrades starch beginning at a non-reducing end by incorporating phosphate (Salisbury and Ross, 1991). The present result shows the increase in starch phosphorylase activity and decrease in α-amylase activity under increased concentrations of arsenate treatment. The result also indicates that increase in starch phosphorylase activity does not affect the starch content but it is mainly controlled by α-amylase activity. But the activities of these enzymes may be reversed with the application of phosphate along with arsenate. This causes increase in α-amylase activity but the level of starch contents was found to be almost constant or below the highest value caused to arsenate treatment. Although there was decrease in α-amylase and sucrose phosphate synthase

activity which may cause impairment in carbohydrate metabolism, increased activity of sucrose degrading enzymes may have the beneficial role in adaptation under arsenate stressed environment.

From the present study, it is evident that there is an increase of soluble reducing sugar and decrease of non-reducing sugar content in arsenic stressed rice seedlings. The alteration of carbohydrate level and inhibition in activities of some major enzymes involved in carbohydrate metabolism under arsenate stress condition may impair the growth and metabolism of the rice seedlings which was relevant from the morphological data as well as from the measurement of water contents of seedlings. Previously, contents of soluble sugar have been reported to be increased under salinity (Dubey, 1997), water-stress (Foyer *et al.,* 1998) and chilling effect (Hurry *et al*., 1995).

Increase in reducing sugar contents due to arsenate treatment is supported by the increased activity of reducing sugar forming enzymes, acid invertase and sucrose synthase. Decreased level of non-reducing sugar content is also accompanied by lower activity of sucrose phosphate synthase. Increase in starch phosphorylase activity is relevant to the result of little increment of starch content. Increase in reducing sugar and starch content may provide an adaptive mechanism to maintain a favourable osmotic condition and to protect the membrane and biomolecules. Soluble sugar serves to allow the plants to maximize sufficient carbohydrate storage reserve to support basal metabolism specially as respiratory substrate under stressed environment (Hurry *et al.,* 1995; Dubey and Singh, 1999). Thus, increase in the activities of sucrose degrading enzymes may be beneficial to combat the effects of arsenic stress.

Arsenate is analogous to phosphate, therefore, external phosphate treatment may overcome the detrimental effect of arsenic which is relevant from the morphological and biochemical study. The application of phosphate simultaneously with arsenate ameliorate to some extent the damaging effect caused by arsenic toxicity. Therefore, the use of phosphate enriched fertilizers in arsenic contaminated soils may improve the health with better sugar metabolizing activity as well as production of rice plants, but the concentration of phosphorus in fertilizers should be carefully considered (Gunes *et al*., 2008).

ACKNOWLEDGMENTS

This study was supported financially by a research grant from the University Grants Commission, New Delhi. The authors are grateful to Dr. S. Mukherji, Professor of Botany (Retd.), University of Calcutta for his valuable comments during preparation of the manuscript.

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