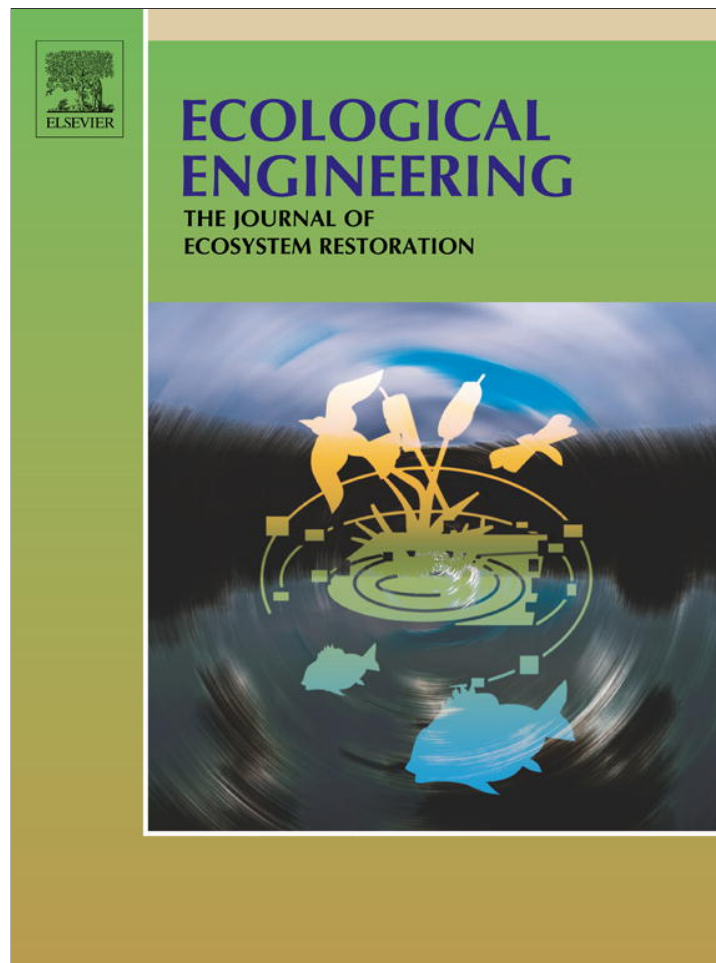




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Silicon mediates arsenic tolerance in rice (*Oryza sativa* L.) through lowering of arsenic uptake and improved antioxidant defence system

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ABSTRACT

Arsenic (As) contamination of paddy rice in South and South-East Asia has raised much concern as rice is the subsistence diet for millions. Two contrasting rice (*Oryza sativa* L.) cultivars i.e. Triguna (As tolerant) and IET-4786 (As sensitive) were grown hydroponically to study the effect of silicon (Si) supplementation on As accumulation, growth, oxidative stress and antioxidative defence system in shoots during arsenite [As(III)] stress. Rice seedlings were exposed to three As(III) levels (0, 10 and 25 μ M) and three silicic acid levels (0, 0.5 and 1 mM Si) in solution culture experiments. Addition of 1 mM Si during As(III) exposure significantly lowers shoot As accumulation in both the cultivar, but more prominently in Triguna ($P \leq 0.01$) than IET-4786 ($P \leq 0.05$). However, addition of Si during As(III) stress had no significant effect on shoot length and dry weight ($P < 0.01$) of both the cultivars, compared to their As(III) treated plants. In contrast to IET-4786 ($P \leq 0.05$), Triguna tolerated As induced oxidative stress through elevated level of cysteine, enhanced antioxidant enzymes activities and their isozymes. Upon Si supplementation lower conglomeration of oxidative stress parameters viz., superoxide and peroxide radicals, lipid peroxidation and electrolyte leakage coincides with increased antioxidants activities and enhanced level of thiols, more effectively in shoots of Triguna than IET-4786 during As(III) stress ($P \leq 0.05$). In conclusion, 1 mM Si addition, significantly ameliorates As induced oxidative stress in Triguna cultivar by lowering the As accumulation and improving antioxidant and thiolic system compared to IET-4786, implying genotypic differences with Triguna being less susceptible to stress dependent membrane lipid peroxidation.

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1. Introduction

Arsenic (As) is a class one carcinogen and toxic to all forms of life. In South and South-East Asia, mining activities, use of arsenical pesticides, and irrigation with As-contaminated groundwater result in substantial As accumulation in soil (Zhao et al., 2010). The presence of As in soils or irrigation with As contaminated water may lead to its accumulation in crop plants especially rice. Recent work on rice-As demonstrated that Asian rice contains more inorganic As (As_i) than US rice and play important role for food chain contamination (Meharg et al., 2009; Zhao et al., 2010). Now it is established that consumption of rice constitutes a large proportion of the dietary intake of As_i for populations whose staple food is rice (Mondal and Polya, 2008; Meharg et al., 2009). Furthermore, rice

straw is widely used as cattle feed, also containing higher levels of As than grain, thus exhibiting another route of As entry into the food chain (Panaullah et al., 2009).

Though As toxicity depends on the presence of exact As species, but generally the inorganic forms viz., arsenite [As(III)] and arsenate [As(V)] are more prevalent and toxic than the organic forms in terrestrial environments (Zhao et al., 2010). In plants, As(V) is taken up by phosphate transporters (Tripathi et al., 2007), whereas As(III) and undissociated methylated As species are transported through the nodulin 26-like intrinsic (NIP) aquaporin channels (Ma et al., 2008; Li et al., 2009a,b). Rice is particularly efficient in As accumulation compared with other cereal crops (Williams et al., 2007), because of increased availability of soil As during anaerobic flooded conditions (Xu et al., 2008) and due to sharing the highly efficient Si uptake pathway (Zhao et al., 2010).

Silicon is essential for high and sustainable production of rice (Liang et al., 2007) and the Si transporter Lsi1 (OsNIP2;1) is a major uptake pathway for As(III) in rice (Ma et al., 2008). Bogdan and Schenk (2008) observed that indigenous silicic acid inhibits As uptake in rice while Norton et al. (2010) established a positive

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correlation between shoot As and Si concentration in agreement with the recent identification of two silicic acid transporters which also transport As(III) in rice (Ma et al., 2008).

Besides a potent risk for human health, As also causes significant yield losses due to As phytotoxicity (Panaullah et al., 2009). Lowering of As load by Si supplementation in rice plant is therefore likely to reduce As-induced oxidative stress, growth inhibition and finally would limit food chain contamination. Though, some authors demonstrated that Si addition reduces the As accumulation in shoot of rice plant (Guo et al., 2005, 2009; Li et al., 2009a,b), but no study has been carried out to investigate the role of Si on As induced oxidative stress and antioxidant system in rice. In order to obtain an insight into this perspectives two contrasting rice cultivars, Triguna (tolerant rice) and IET-4786 (sensitive rice) were selected on the basis of contrasting performance of growth, As accumulation, differential biochemical responses during As exposure (Dwivedi et al., 2010a; Rai et al., 2011; Tripathi et al., 2012a,b). It was hypothesized that reduction of As accumulation through Si addition must overcome the As induced oxidative stress through improvement of antioxidants. To test this hypothesis, the present study was conducted on two contrasting rice cultivars differing in As tolerance to elucidate the possible mechanisms of Si-associated As tolerance in terms of antioxidant enzyme activities and their isozymes.

2. Experimental

2.1. Treatment conditions

Rice seeds of two contrasting cultivars (Triguna and IET-4786) obtained from Rice Research Station, Chinsurah, West Bengal were grown in hydroponics. Seeds were disinfected in 0.1% HgCl_2 solution for 30 ss, followed by thorough washing with deionized water and soaking in milli-Q for 24 h. These seeds were then transferred to Petri-dishes (3–4 days) kept in culture room at 26 °C in dark for proper germination. Then uniform germinated seeds were selected and transplanted to tray having fixed PVC cups (4 cm diameter and 5 cm high, 10 plant per pot) and grown in modified 1/3 strength Hewitt nutrient medium (Liu et al., 2004) under hydroponic condition for 15 d and further exposed with three As(III) (NaAsO_2 ; 0, 10, 25 μM) level and three silicic acid levels (0, 0.5 and 1.0 mM Si) for 10 d to see the concentration dependent effect on growth and biomass. Further, to investigate the Si mediated biochemical changes the experimental design consisted of a control (no added Si and As) and three treatments (1.0 mM Si, 25 μM AsIII and 1.0 mM Si combination with 25 μM AsIII), arranged in a randomized block design with four replicates. The experiment was carried out in a controlled environment growth chamber with a 14-h light period (260–350 $\mu\text{E m}^{-2} \text{ s}^{-1}$) and temperatures of 28 °C day and 20 °C night with 70% relative humidity maintained by humidifier. All nutrient solutions were changed twice per week, and pH was adjusted to 5.5 using 0.1KOH or HCl. Shoot length was measured by metric scale. After that plants were harvested, washed with milli-Q, blotted and used for the study of various biochemical parameters.

2.2. Plant growth and biomass measurement

Shoot length was measured by metric scale of 25 d old rice seedlings. Plant biomass was measured on dry weight basis. Control and treated plants were harvested, washed with double distilled water, blotted gently to remove excess water. At harvest, plants were divided into roots and shoots, oven dried at 70 °C until constant weight and then weight of shoots were determined.

2.3. Arsenic quantification and quality control

For estimation of total As, dried plant samples (1 g) were powdered and digested in 3 mL HNO_3 at 120 °C for 6 h (Dwivedi et al., 2010b). The level of As was quantified by Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500 cx). The standard reference material of As (Agilent, Part No. 8500-6940) was used for the calibration and quality assurance for each analytical batch. Rice flour NIST 1568a was used as a reference material with known spiked samples and recovery of total As was 95.3% (± 2.8 ; $n=5$) and 92.5% (± 3.1 ; $n=5$) respectively. The detection limit of As was 1 $\mu\text{g L}^{-1}$.

2.4. Silicon estimation

The Si contents of shoots were determined by the colorimetric molybdenum blue method described by Vander Vorm (1987). Briefly, 300-mg samples of plant material were ashed in porcelain crucibles for 3 h at 550 °C, the ash was dissolved in 1.3% HF, and then the Si concentrations in the solutions were measured by the colorimetric molybdenum blue method at 811 nm with a spectrophotometer.

2.5. Estimation of superoxide radical ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), lipid peroxidation (MDA), and electrical conductivity (EC)

The measurement of $\text{O}_2^{\bullet-}$, H_2O_2 , MDA content and EC was done according to Tripathi et al. (2012a). The detailed methodologies were given in the supplementary material.

2.6. Assays of antioxidant enzymes

Control and As exposed plants were homogenized in buffers specific for each enzyme under chilled conditions. Homogenate was centrifuged at 12,000 $\times g$ for 15 min at 4 °C. Protein content of the supernatant was measured following the method of Bradford (1976), using BSA as standard. Supernatant thus obtained was used to measure the activities of various enzymes were expressed as units mg^{-1} protein. The activity of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2) was assayed according to Rai et al. (2011). Assay of ascorbate oxidase (AOX, EC 1.10.3.3) and glutathione-S-transferase (GST, EC 2.5.1.18) activity was done by according to Tripathi et al. (2012a).

2.7. Native page and activity stain

Native polyacrylamide gel electrophoresis (PAGE) was performed at 4 °C, 90 V, following Laemmli (1970). Protein measurement was performed according to Bradford (1976), using BSA as standard. For SOD, GPX, APX, DHAR and GR, the enzyme solutions were subjected to native PAGE with 10% polyacrylamide gel. Activity stain for each enzyme was carried out as follows. SOD activity was detected by the procedure described by Beauchamp and Fridovich (1971). The gel equilibrated with 50 mM K-phosphate buffer (pH 7.8) containing 2.8×10^{-5} M riboflavin and 0.028 M TEMED for 30 min. The gel was washed in distilled water for 1 min and submerged in the same solution (mentioned above) containing 2.45 mM NBT for 10–20 min with gentle agitation in the presence of light. GPX activity was revealed with a modification of the method described by Préstamo and Manzano (1993), the gel was covered with a solution containing 50 mL of 50 mM sodium acetate buffer (pH 6.0), 3.4 mL of 0.5% H_2O_2 and 3.4 mL of 0.25% (w/v) o-dianisidine. Finally, the gel

was agitated until a brown color appeared (30–60 min). APX activity was detected by the procedure described by Mittler and Zilinskas (1993). The gel equilibrated with 50 mM Na-phosphate buffer (pH 7.0) containing 2 mM ASC for 30 min was incubated in a solution composed of 50 mM Na-phosphate buffer (pH 7.0), 4 mM ASC and 2 mM H_2O_2 for 20 min. The gel was washed in the buffer for 1 min and submerged in a solution of 50 mM Na-phosphate buffer (pH 7.8) containing 28 mM *N,N,N,N*-tetramethyl ethylenediamine (TEMED) and 2.45 mM NBT for 10–20 min with gentle agitation in the presence of light. DHA-reducing protein activity was stained according to De Gara et al. (1997). After completion of the electrophoretic run, gels were incubated with 0.1 M phosphate buffer, pH 6.2, containing 4 mM GSH and 2 mM DHA. Gels were then washed with distilled water and stained by incubation for 10 min in a 0.125 M HCl solution containing 0.1% (w:v) ferrichloride and 0.1% (w:v) ferricyanide, their reaction with ASC yielding a coloured product. GR activity staining was adapted from Anderson et al. (1995) and was carried out in 0.4 mM NADPH, 3.4 mM oxidized glutathione (GSSG), 1.2 mM 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 0.3 mM 2,6-dichlorophenol-indophenol (DPIP) and 50 mM Tris–HCl buffer (pH 7.7).

2.8. Estimation of thiol compounds

The estimation of cysteine and glutathione (GSH) was done according to Tripathi et al. (2012a). The non-protein thiol (NPSH) content was measured according to the Rai et al. (2011). The detailed methodologies were given in the supplementary material.

2.9. Statistical analysis

Two-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments by using SPSS 16.0 software.

3. Result and discussion

3.1. Effect of Si supplementation on shoot length and biomass during As(III) stress

Growth parameters were measured in terms of shoot length and biomass (Table 1). Upon higher exposure (25 μ M) of As(III) growth parameters were decreased significantly in both the contrasting rice varieties ($P < 0.05$) but more significantly in sensitive rice (IET-4786). Various level of Si supplementation raised the shoot length and biomass of both the contrasting varieties, but more notably in tolerant rice (Triguna), either in the presence or absence of As(III), however induction were non-significant during As(III) exposure except biomass of tolerant rice. Addition of 1 mM Si during 25 μ M As(III) exposure showed best response and increased the shoot length and biomass by 10% and 20% respectively, in tolerant rice and 5% and 12% in sensitive rice, when compared with 25 μ M As(III) exposure alone. It is evident from various studies (Ma and Takahashi, 1990; Liang et al., 2003, 2007) that application of Si exerts beneficial effects on plant growth and production by alleviating both biotic and abiotic stresses including diseases, pests, lodging, drought, salt and nutrient imbalance.

3.2. Effect of Si supplementation on shoot Si level and As level during As(III) exposure

The genotypic differences were found for shoot As and Si level and their accumulation was found to correlate with given As and Si concentrations to both the varieties (Table 1). The maximum

As (μ g g⁻¹ dw) and Si (mg g⁻¹ dw) accumulation was observed in tolerant cultivar (183, As; 45, Si) than sensitive cultivar (106, As; 35, Si) at 25 μ M As(III) and 1 mM Si exposure. This result also validated that As(III) and Si share the similar transport pathway in rice (Ma et al., 2008) and also exhibited genotypic difference for As and Si accumulation in rice (Ma et al., 2007; Dwivedi et al., 2010b). In general, all doses of exogenous Si decreases the As accumulation in both the cultivar, but 1 mM Si addition reduces As content more significantly in tolerant (31% and 24%) and sensitive rice (24% and 18%) during 10 and 25 μ M As(III) exposure, respectively. However, no significant changes in Si uptake was noticed in both varieties ($P < 0.05$) at different As(III) exposure. These findings also validated the results of Guo et al. (2005). Thus our observation indicated that external Si-mediated reduction in As(III) uptake by rice is due to the direct competition between Si and As(III) during uptake (Ma et al., 2008; Guo et al., 2009).

3.3. Effect of Si supplementation on $O_2^{\bullet-}$, H_2O_2 , EC and MDA content during As(III) stress

As significant reduction in shoot As accumulation was observed during 1 mM Si supplementation, therefore the other various biochemical parameters were analysed at three treatments (1.0 mM Si, 25 μ M As(III) and 1.0 mM Si combination with 25 μ M As(III)) along with the control (no added Si and As). The various oxidative stress related parameters viz., $O_2^{\bullet-}$, H_2O_2 , EC and MDA content were measured during As(III) stress as well as Si supplementation condition. In general, during As(III) stress, these parameters were significantly elevated in both the cultivars, but induction was more prominent in sensitive rice (Fig. 1A–D). In rice As generates oxidative stress and thus causes lipid degradation of various biomolecules (Tripathi et al., 2012a). During As(III) stress, the chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content were significantly decreased in both the varieties, but more prominently in sensitive rice (supplementary Table S1). However, supplementation of 1 mM Si increased their content more markedly in tolerant rice either in presence or absence of As(III) ($P < 0.05$). The observed effects of Si during As stress might be due to the fact that plants treated with Si contain less As. Liang (1998) also reported that Si enhances the growth of various (mostly monocotyledonous) plants by providing rigidity to plant tissues and promoting photosynthesis. These slight differences in induction level of photosynthetic pigments of the contrasting varieties might be attributed to their genotypic difference for Si accumulation. Application of Si (1 mM) significantly ameliorates the oxidative stress, and affects were more significant in tolerant rice than sensitive rice ($P < 0.05$) either in the presence or absence of As(III). Addition of Si during As(III) exposure reduces the $O_2^{\bullet-}$, H_2O_2 , EC and MDA content by 16, 10, 17 and 18% in tolerant rice, while in sensitive rice reduction of only 11, 9, 13 and 13% was noticed in these respective parameters in comparison to As(III) exposure. It is well documented that Si overcomes the various types of biotic and abiotic stresses (Epstein, 1999; Liang et al., 2007) and also alleviates manganese and aluminium toxicity (Ma and Takahashi, 1990; Ma et al., 1997). In agreement with this, addition of Si reduces MDA content in maize (Moussa, 2006) and H_2O_2 radical in barley (Liang et al., 2003) during salt stress.

3.4. Effect of Si supplementation on antioxidant enzymes during As(III) stress

Various antioxidant enzymes SOD, CAT, GPX, APX, DHAR, GR and GST were analysed along with pro-oxidant enzyme AOX, in shoots of both the cultivars. Isozymes of SOD, GPX, APX, DHAR and GR

Table 1

Effect of Si supplementation on length, DW, As uptake and Si concentration of the shoots of rice under various level of As treatments. All the values are means of 4 replicate ($n=4$) $\pm$ SD.

Rice varieties	As level (μM)	Si level (mM)	Length (cm)	DW (gm plant^{-1})	As concentration ($\mu\text{g g}^{-1}$ dw)	Si concentration (mg g^{-1} dw)
Triguna	0	0	24.6 $\pm$ 2	0.018 $\pm$ 0.002	0	0
		0.5	26.4 $\pm$ 2	0.019 $\pm$ 0.002	0	27 $\pm$ 2
		1.0	28 $\pm$ 2	0.023 $\pm$ 0.002	0	45 $\pm$ 7
	10	0	25.7 $\pm$ 2	0.016 $\pm$ 0.001	116 $\pm$ 14	0
		0.5	27.1 $\pm$ 3	0.017 $\pm$ 0.001	96 $\pm$ 12	25 $\pm$ 2
		1.0	28.4 $\pm$ 2	0.02 $\pm$ 0.002	80 $\pm$ 10	40 $\pm$ 6
	25	0	20.9 $\pm$ 2.4	0.015 $\pm$ 0.001	183 $\pm$ 16	0
		0.5	22 $\pm$ 2	0.016 $\pm$ 0.00	155 $\pm$ 17	23 $\pm$ 2
		1.0	23 $\pm$ 2.5	0.018 $\pm$ 0.002	139 $\pm$ 14	38 $\pm$ 3
	Analysis of variance	Si level	NS	$P < 0.05$	$P < 0.01$	$P < 0.01$
		As level	$P < 0.05$	NS	$P < 0.01$	NS
		Six As level	NS	NS	$P < 0.05$	NS
IET-4786	0	0	27.7 $\pm$ 2	0.021 $\pm$ 0.002	0	0
		0.5	28.9 $\pm$ 1.3	0.022 $\pm$ 0.002	0	19 $\pm$ 1.7
		1.0	30.5 $\pm$ 1.7	0.026 $\pm$ 0.002	0	35 $\pm$ 2.3
	10	0	26 $\pm$ 2	0.019 $\pm$ 0.002	93 $\pm$ 11	0
		0.5	26.8 $\pm$ 2	0.02 $\pm$ 0.002	83 $\pm$ 10	19 $\pm$ 0.7
		1.0	27.4 $\pm$ 2	0.02 $\pm$ 0.002	71 $\pm$ 9	32 $\pm$ 2
	25	0	19.1 $\pm$ 1.7	0.017 $\pm$ 0.002	106 $\pm$ 9	0
		0.5	19.6 $\pm$ 1.8	0.018 $\pm$ 0.002	96 $\pm$ 11	17 $\pm$ 1.6
		1.0	20 $\pm$ 2	0.019 $\pm$ 0.001	87 $\pm$ 9	29 $\pm$ 2
	Analysis of variance	Si level	NS	NS	$P < 0.05$	$P < 0.01$
		As level	$P < 0.05$	NS	$P < 0.05$	NS
		Six As level	NS	NS	$P < 0.05$	NS

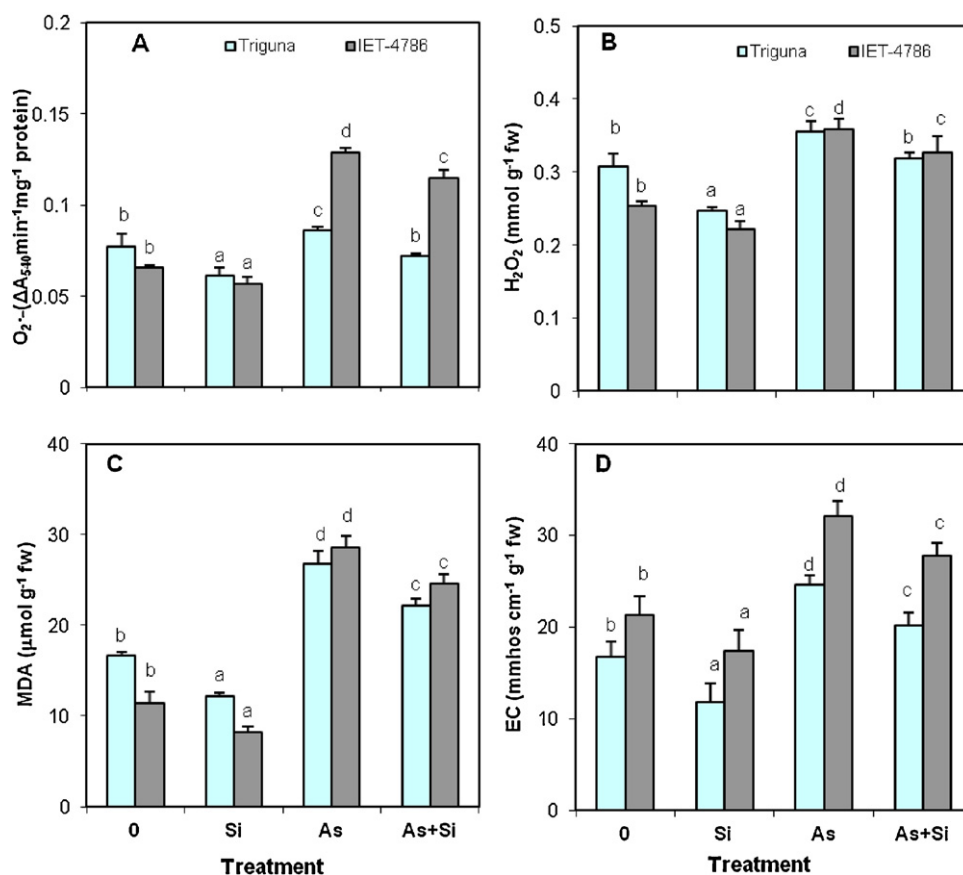


Fig. 1. Changes in the level of superoxide radicals ($\text{O}_2^{\cdot-}$) (A), hydrogen peroxide (H_2O_2) (B), malondialdehyde (MDA) (C) and ion leakage (EC) (D) in shoots of the rice seedlings after 10 d of treatments with (0.0, 1 mM Si, 25 μM As and 25 μM As + 1 mM Si). All the values are means of 4 replicate ($n=4$) $\pm$ SD. ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values between treatments (DMRT, $P \leq 0.05$).

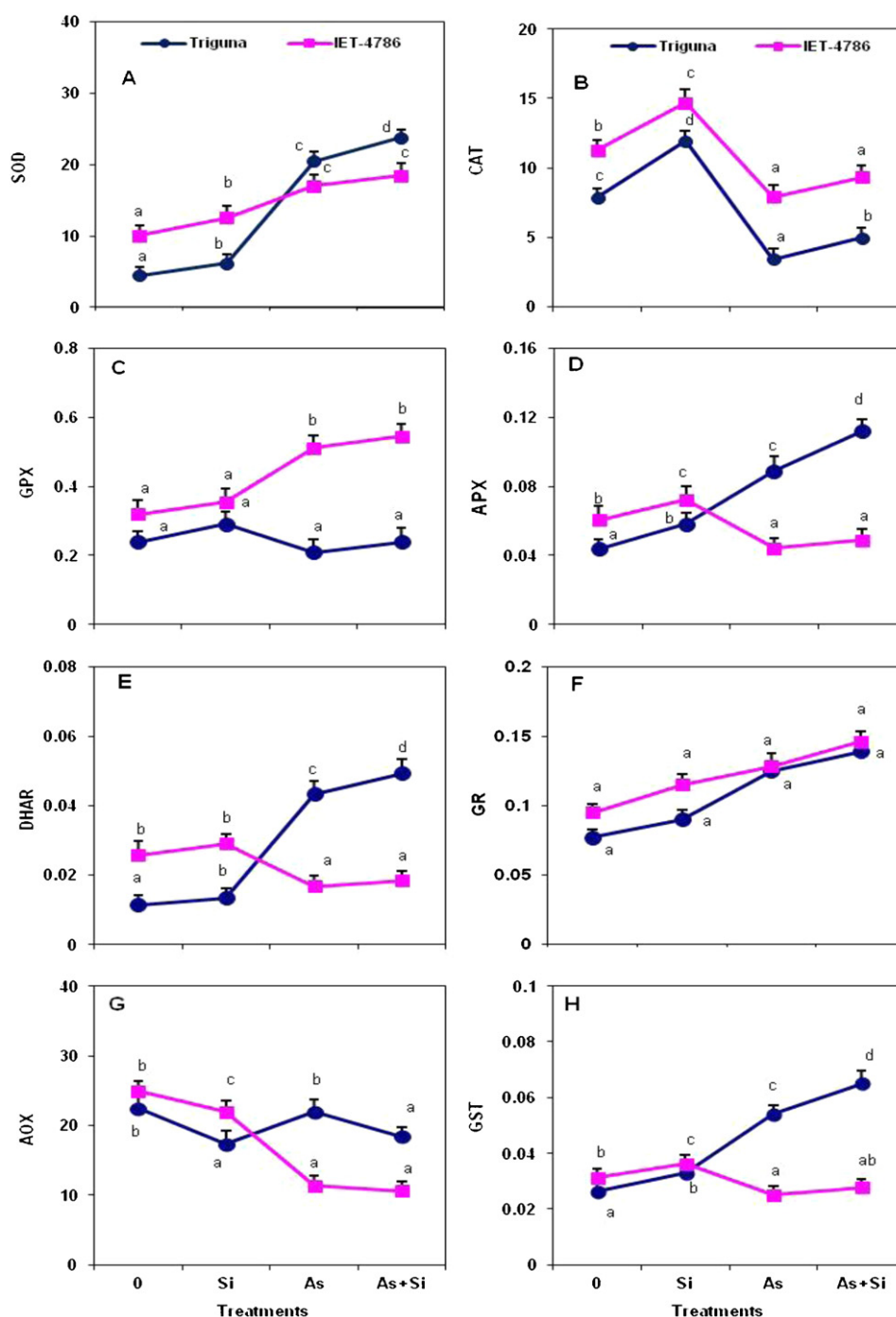


Fig. 2. Changes in the activities of superoxide dismutase (SOD) (A), catalase (CAT) (B), guaiacol peroxidase (GPX) (C), ascorbate peroxidase (APX) (D), dehydroascorbate reductase (DHAR) (E), glutathione reductase (GR) (F), ascorbate oxidase (AOX) (G), and glutathione-S-transferase (GST) (H) in shoots of the rice seedlings after 10 d of treatments with (0.0, 1 mM Si, 25 μ M As and 25 μ M As + 1 mM Si). All the values are means of 4 replicate ($n = 4$) $\pm$ SD. ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values between treatments (DMRT, $P \leq 0.05$).

were also examined in shoots of both the cultivar. In general, during As(III) exposure, the antioxidant enzymes viz., SOD (Fig. 2A), APX (Fig. 2D), DHAR (Fig. 2E), GR (Fig. 2F), and GST (Fig. 2H) were increased in tolerant rice except CAT (Fig. 2B) and GPX (Fig. 2C), while most of the antioxidants were decreased in sensitive rice except SOD, GPX and GR activities. Isozymes analysis also revealed that SOD activity was increased in both the varieties mainly due to two Mn-SOD and one Cu/Zn-SOD isozymes (Fig. 3A and A'). The SODs exist in three isoforms, viz., Mn-SOD, Fe-SOD and Cu/Zn-SOD,

which are localized in different cell organelles depending upon their prosthetic groups (Bowler et al., 1994). Shri et al. (2009) also observed induction of Cu/Zn-SOD and Mn-SOD in rice roots and shoots during As stress. Three isoforms of APX (Fig. 3B and B') and GPX (Fig. 3C and C') were observed in shoots of both the varieties. In tolerant rice, APX activity increased mainly due to APX-1 and APX-2 isozymes, while GPX activity decreased mostly due to GPX-2 and GPX-3 isozymes during As stress. Similarly, isozymes of APX and GPX also paralleled with their specific activity in the sensitive rice.

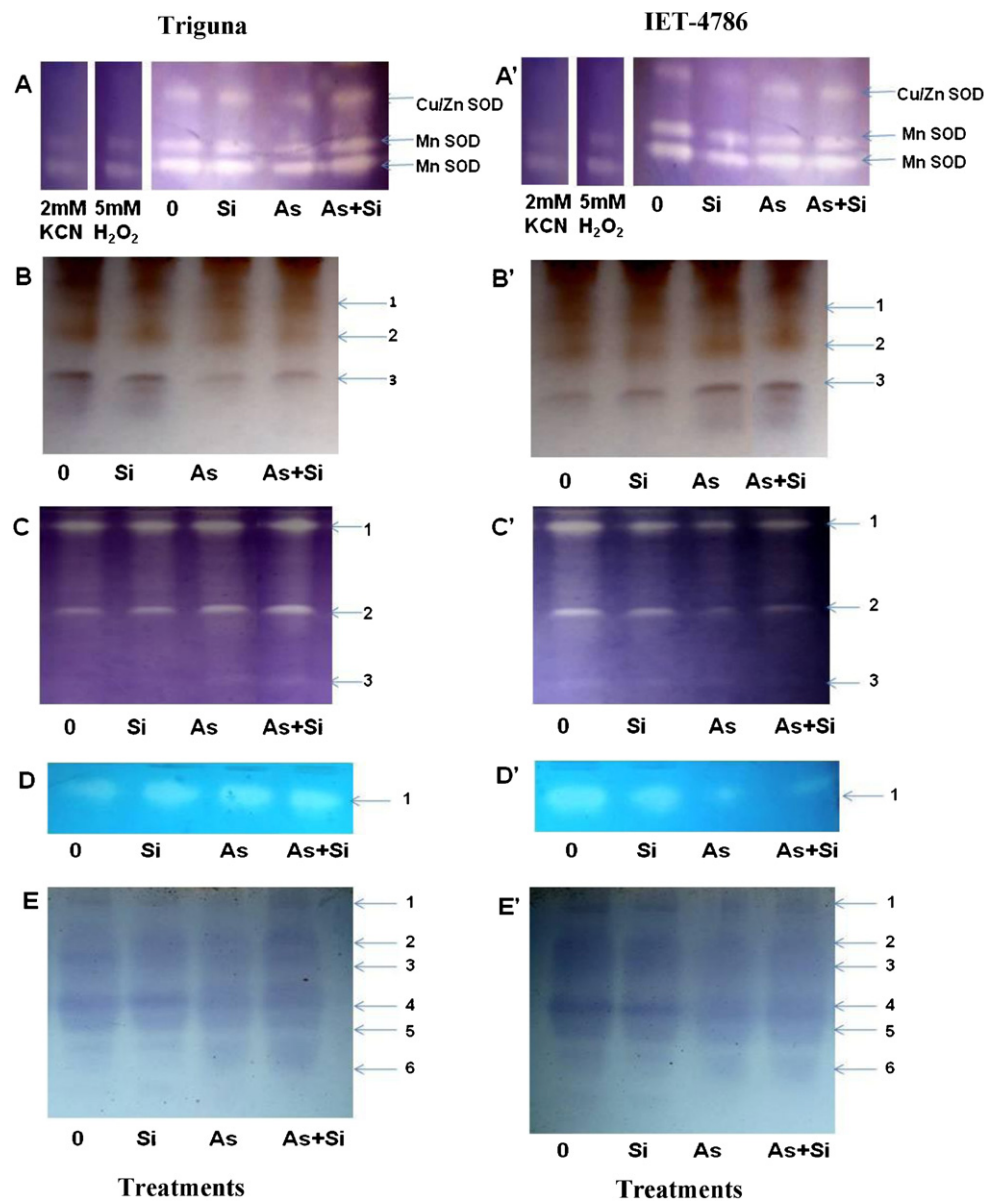


Fig. 3. Superoxide dismutase (A and A'; SOD), guaiacol peroxidase (B and B'; GPX), ascorbate peroxidase (C and C'; APX), dehydroascorbate reductase (D and D'; DHAR) and glutathione reductase (E and E'; GR) in gel assay of proteins from shoots of the rice seedlings after 10 d of treatments with (0.0, 1 mM Si, 25 μ M As and 25 μ M As + 1 mM Si). Samples were electrophoresed on a 10% native polyacrylamide gel. 150 mg total protein was loaded per each well. Assays were carried out as described in methods. The different isoforms are numbered from cathode to anode.

Interestingly, only one band observed for DHAR isoforms which was also correspond to its specific activity in tolerant (Fig. 3D) and sensitive rice (Fig. 3D'). Six isoforms for GR were found in both the varieties; however increase in GR activity during As stress is mainly due to GR-6 isoforms. The accumulation of H_2O_2 is prevented in cells by the ascorbate–glutathione cycle where APX reduces it to H_2O . At higher As(III) concentration, shoots experienced high ROS buildup and tolerant rice detoxified it by increased level of antioxidant enzymes and their isoforms. Similar to this result, various antioxidant enzymes were also showed varietal differences in rice along with enzymes of ascorbate–glutathione cycle viz., APX, DHAR and GR (Rai et al., 2011) during As stress. Plants actually possess several protective mechanisms to cope with As generated ROS, involving enzymes such as SOD, POD and CAT (Rai et al., 2011) as well as antioxidants such as ascorbate and glutathione (Tripathi et al., 2012a). Pro-oxidant enzyme AOX were found to decrease

in tolerant rice due to activation of various antioxidant enzymes, while induction was observed in sensitive rice (Fig. 2G). Similarly, during As(V) stress pro-oxidant enzymes viz., AOX and NADPH oxidase were decreased in tolerant rice and positively correlated to As exposure in sensitive rice (Tripathi et al., 2012a). Si supplementation enhanced the enzyme activities in both the genotypes, but more significantly in tolerant rice suggesting the higher amelioration of As induced oxidative stress in tolerant rice by increased antioxidant activities. Our isoforms study also substantiated that addition of Si during As stress induced the various isoforms of SOD (Cu/Zn-SOD and two Mn-SOD), GPX (GPX-2 and GPX-3), APX (APX-1 and APX-2), DHAR (DHAR-1), and GR (GR-4, GR-5 and GR-6) in both the cultivar but mainly in tolerant rice, when compared to As(III) treated plants. In agreement to this, Liang et al. (2003) also found that exogenous Si increases antioxidant enzymes activities in barley during salt stress condition. Various isoforms

Table 2

Changes in cysteine, NPSH and GSH content of the shoots of rice seedlings after 10 d of treatments with (0.0, 1 mM Si, 25 μ M As and 25 μ M As + 1 mM Si). All the values are means of 4 replicate ($n=4$) $\pm$ SD. ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values between treatments (DMRT, $P \leq 0.05$).

Rice varieties	Treatments	Cysteine content (nmol g ⁻¹ fw)	GSH content (μ mol g ⁻¹ fw)	NPSH content (mmol g ⁻¹ fw)
Triguna	0.0	100a $\pm$ 10	53b $\pm$ 5	0.063b $\pm$ 0.004
	Si	134b $\pm$ 8	63c $\pm$ 4	0.077c $\pm$ 0.006
	As	148b $\pm$ 13	31a $\pm$ 3	0.042a $\pm$ 0.004
	As + Si	178c $\pm$ 9	36a $\pm$ 3	0.05a $\pm$ 0.005
IET-4786	0.0	85a $\pm$ 7	59b $\pm$ 5	0.084b $\pm$ 0.007
	Si	98ab $\pm$ 8	65b $\pm$ 4	0.093b $\pm$ 0.006
	As	113bc $\pm$ 7	28a $\pm$ 3	0.045a $\pm$ 0.007
	As + Si	124c $\pm$ 10	30a $\pm$ 2	0.049a $\pm$ 0.005

of SOD and CAT enzymes were also induced in *Brassica chinensis*, when Si supplemented during Cd stress (Song et al., 2009). Supporting evidence also concluded that foliar application of silicate stimulated SOD activity in drought-stressed bentgrass (Schmidt et al., 1999) and exogenous Si also enhanced SOD, CAT, and GPX activity in maize for providing tolerance during salt stress (Moussa, 2006). Zhu et al. (2004) also noticed that addition of Si in salt stress condition increases the activities of SOD, GPX, APX, DHAR and GR in both salt tolerant and sensitive varieties of cucumber and thus ameliorates the oxidative stress.

3.5. Effect of Si supplementation on thiolic content during As(III) stress

Similar to other parameters, cysteine content was also increased in tolerant rice, while sensitive rice showed reduction in cysteine content upon As(III) exposure (Table 2). Tripathi et al. (2012a) reported that cysteine, a central metabolites for As detoxification, was also found to increased in tolerant rice along with the other thiolic ligands, conversely reduced in sensitive rice during As(V) exposure. Addition of 1 mM Si during As(III) exposure increased the cysteine content in both the cultivars, but more effective induction were seen in tolerant rice (20%) than sensitive rice (10%). The GSH and NPSH content were decreased in both the varieties upon As(III) exposure. The GSH content might be decreased due to As toxicity and its consumption in the phytochelatin synthesis (Tripathi et al., 2012a). Supplementation of Si during As(III) stress, increased the NPSH and GSH content in both the varieties, and showed induction of 19% and 16% in tolerant rice, whereas only 10% and 9% in sensitive rice, respectively. Moussa (2006) reported that 1 mM Si significantly induced the level of NPSH and GSH in maize and coincides with reduction in lipid peroxidation. Similarly, Song et al. (2009) observed that addition of Si intensified the NPSH and GSH content in contrasting varieties of *B. chinensis*, but more significantly in tolerant cultivar during Cd stress.

4. Conclusion

The present study demonstrated that addition of Si alleviates the As phytotoxicity in rice by subsequent reduction of shoot As accumulation and elevated levels of antioxidant enzymes and their isozymes. Moreover, addition of Si enhanced the concentrations of cysteine, GSH and NPSH, consequently reducing the As induced lipid peroxidation. It is apparent from the study that Si mediated As tolerance was more in Triguna than IET-4786, further indicating genotypic differences in Si accumulation and its subsequent affect on As reduction. It is well established that As(III), dimethylarsinic acid (DMA) and monomethylarsenic acid (MMA) taken up through the Si transport pathway in rice, therefore application of Si fertilization may be one of the important strategy to not only reduce the As_i from Asian rice but also the DMA content from US rice. Further field experiments are needed to understand the role of silicic

acid amendment on reduce uptake of different As species and its detoxification mechanism in the rice plant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2012.12.057>.

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