H₂O₂ pretreated rice seedlings specifically reduces arsenate not arsenite: difference in nutrient uptake and antioxidant defense response in a contrasting pair of rice cultivars

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Abstract The study investigated the reduction in metalloid uptake at equimolar concentrations (~53.3 µM) of As(III) and As(V) in contrasting pair of rice seedlings by pretreating with H₂O₂ (1.0 µM) and SA (1.0 mM). Results obtained from the contrasting pair (arsenic tolerant vs. sensitive) of rice seedlings (cv. Pant Dhan 11 and MTU 7029, respectively) shows that pretreatment of H₂O₂ and H₂O₂ + SA reduces As(V) uptake significantly in both the cultivars, while no reduction in the As(III) uptake. The higher growth inhibition, higher H₂O₂ and TBARS content in sensitive cultivar against As(III) and As(V) treatments along with higher As accumulation (~1.2 mg g⁻¹ dw) than in cv. P11, unravels the fundamental difference in the response between the sensitive and tolerant cultivar. In the H₂O₂ pretreated plants, the translocation of As increased in tolerant cultivar against AsIII, whereas, it decreased in sensitive cultivar both against AsIII and AsV. In both the cultivars translocation of Mn increased in the H₂O₂ pretreated plants against As(III), whereas, the translocation of Cu increased against As(V). In tolerant cultivar the translocation of Fe increased against As(V) with H₂O₂ pretreatment whereas, it decreased in the sensitive cultivar. In both the cultivars, Zn translocation increased against As(III) and decreased against As(V). The higher level of H₂O₂ and SOD (EC 1.15.1.1) activ-

ity in sensitive cultivar whereas, higher, APX (EC 1.11.1.11), GR (EC 1.6.4.2) and GST (EC 1.6.4.2) activity in tolerant cultivar, also demonstrated the differential anti-oxidative defence responses between the contrasting rice cultivars.

Keywords Hydrogen peroxide · Salicylic acid · Arsenite · Arsenate · *Oryza sativa*

Introduction

Arsenic is a non-threshold carcinogen, estimated to be transferred into the rice paddy fields to the tune of 1,000 tonnes annually in the rice growing areas of Indian subcontinent (soil As level ranging between 10.4 and 15.5 µg g⁻¹) during dry season (Dwivedi et al. 2012) as a result of irrigation with As laden ground-water. Hydrogen peroxide (H₂O₂) is present endogenously as an important intermediate of the metabolic pathways in plants. It has an important role in plant's response towards biotic and abiotic stresses. Primarily, H₂O₂ functions as a messenger in ethylene and salicylic acid (SA) signal transduction pathway during plant stress response (Foyer et al. 1997; Chamnopol et al. 1998). Several studies have highlighted the role of H₂O₂ in enhancing plant's tolerance towards abiotic stresses (Murphy et al. 2002; Uchida et al. 2002; Azevedo Neto et al. 2005; Wahid et al. 2007; Liu et al. 2010; Chou et al. 2012). At the molecular level H₂O₂ regulates expression of several genes encoding antioxidants, defense proteins, kinase signaling proteins, phosphatases and transcription factors that are individually as well as contentedly involved in plant defense and hypersensitive reactions (Kovtun et al. 2000; Hung et al. 2005). Salicylic acid (a hormone like endogenous regulator) acts with the involvement of H₂O₂ in one of several ways including gene expression during plant defense against biotic and abiotic stresses

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(Szalai et al. 2000). It induces the synthesis of antioxidants ascorbate (AsA) and glutathione (GSH), antioxidant enzymes glutathione-S-transferase (GST), glutathione peroxidase (GPX), ascorbate peroxidase (APX). Endogenous SA protects rice plants from oxidative damage caused by aging, biotic and abiotic stress (Yang et al. 2004). The mode of SA action is largely through generation of reactive oxygen species (ROS) and by inhibiting the activity of catalase (CAT), which is a H_2O_2 scavenging enzyme (Janda et al. 2012). Alternatively, H_2O_2 -triggered programmed cell death (PCD) has been recognized as an essential process to maintain tissue or organ homeostasis in concert with cell proliferation, growth and differentiation (Mittler et al. 2004).

The role of H_2O_2 in stress response has been directly assessed through its exogenous application. This is reported to increase chilling tolerance (Murphy et al. 2002), salt tolerance (Wahid et al. 2007), tolerance to osmotic stress (Liu et al. 2010), and low light (Zhang et al. 2011). Alleviation of heavy metals induced oxidative stress have also been demonstrated during Cd treatment in rice, barley, maize, pea seedlings, *Arabidopsis thaliana* and *Glycine max* (Pal et al. 2002; Metwally et al. 2003; Choudhury and Panda 2004; Drazic and Mihailovic 2005; Noriega et al. 2012; Guo et al. 2007; Zawoznik et al. 2007; Popova et al. 2009; Xiao-Juan et al. 2010). Reduction of Pb and Hg induced membrane damage in *Oryza sativa* have been studied by pretreatment with SA (Mishra and Choudhuri 1999), pre-treatment of a rice cultivar with H_2O_2 has been reported to increase its tolerance towards Cd toxicity by increasing GSH, phytochelatin and non-protein thiols (Chou et al. 2012). However, the role of H_2O_2 or both H_2O_2 and SA, towards alleviation of heavy metal(loid) induced toxicity in plants has not been extensively studied. Therefore, investigating the role of H_2O_2 and SA in reducing heavy metal(loid) related toxicity in plants is of great importance, particularly with arsenic (As) stress in rice plants.

Among various strategies, pre-soaking and priming of seeds with stress alleviating compounds has been proposed as an easy, low cost, low risk and effective approach to overcome the abiotic stresses (Wahid and Shabbir 2005). It is well established that most of the biotic and abiotic stress response results into generation of H_2O_2 , hence, priming rice seedlings with H_2O_2 would induce a well developed antioxidative defence mechanism prior to As exposure, and treatment with H_2O_2 + SA could also have additive effect, as the mode of SA in plants is through generation of H_2O_2 . As exposure to rice interferes with uptake of mineral elements such as Cu, Zn, Fe, Ni and Se (Duan et al., 2013; Dwivedi et al. 2012). However, there are no reports about the influence of the plant hormones such as jasmonic acid, salicylic acid or molecules like H_2O_2 on the uptake or translocation of these minerals in As challenged rice plants. In this backdrop, the current study attempts to explore the role of H_2O_2 and SA in abatement of As toxicity in contrasting cultivars of rice plants that differ in tolerance towards arsenic. Additionally, the study

also investigates the translocation of As and essential metal nutrients, antioxidant pool and anti-oxidative enzyme activities in the H_2O_2 or H_2O_2 + SA pretreated plants.

Materials and methods

Growth conditions and experimental design

Popular rice cultivars, MTU 7029 (*Nati mansuri*) and cv. Pant Dhan 11 (P11) were obtained from Narendera Dev Agricultural University, Faizabad, Uttar Pradesh, and from Govind Ballav Pant Agricultural University, Panthnagar, Uttarakhand, respectively. The seeds were germinated and cultured as explained in Kumar et al. (Kumar et al. 2013). 50 uniform (10 cm) seedlings were placed in 150 ml beaker, containing 100 ml of 100 % Hewitt nutrient medium (HNM), prepared with Milli-Q water (pH 6.8–7.0) (Hewitt 1966). The composition ($\mu\text{g ml}^{-1}$) of Hewitt nutrient solution included N (168), P(41), K (156), Mg (36), Ca (160), S (48), Fe (2.8), Mn (0.55), B (0.54), Cu (0.064), Zn (0.065), Mo (0.048). After 7 d of growth in the treatments were provided as As(III), As(V) (both at the rate of $4 \mu\text{g ml}^{-1}$ which is equivalent to $\sim 53.3 \mu\text{M}$) in HNM using salts of $\text{NaHAsO}_4 \cdot 7\text{H}_2\text{O}$ (Merck) and NaAsO_2 (Sigma, USA), respectively for another 14 days and harvested for morphological and biochemical analysis. Morphological parameters were also recorded after 7 days in a similar set of experiment separately.

For convenience, the treatments were abbreviated as (P11) for Pant Dhan 11, MTU for MTU 7029, “C” for control, “AsIII(4)” for arsenite $4 \mu\text{g ml}^{-1}$ ($\sim 53.3 \mu\text{M}$), “AsIII(4) H_2O_2 ” for arsenite $4 \mu\text{g ml}^{-1}$ pretreated with H_2O_2 , “AsIII(4) H_2O_2 + SA” for arsenite $4 \mu\text{g ml}^{-1}$ pretreated with SA and H_2O_2 . Similarly, “AsV(4)” for arsenate $4 \mu\text{g ml}^{-1}$ ($\sim 53.3 \mu\text{M}$), “AsV(4) H_2O_2 ” for arsenate $4 \mu\text{g ml}^{-1}$ pretreated with H_2O_2 , “AsV(4) H_2O_2 + SA” for arsenate $4 \mu\text{g ml}^{-1}$ pretreated with both SA and H_2O_2 . The pre-treatment of the seedlings with H_2O_2 was made with 1 mM of H_2O_2 prepared in HNM for 48 h and for H_2O_2 + SA, pretreatments were made by 1 mM of SA + 1 mM of H_2O_2 prepared in HNM for 48 h, prior to exposure to various As treatments as mentioned above.

Biochemical analysis

Chlorophyll and protein content was estimated by Arnon (1949) and Lowry et al. (1951) methods, respectively. TBARS content ($\text{mmol g}^{-1} \text{fw}$) was estimated according to Heath and Packer (1968) using $\epsilon = 0.155 \text{ M g}^{-1} \text{fw}$ for MDA-TBA adduct. H_2O_2 content ($\text{nmol g}^{-1} \text{fw}$) by Velikova et al. (2000) and ascorbic acid ($\mu\text{M g}^{-1} \text{fw}$) by Shukla et al. (1979). Reduced and total glutathione (GSH and total GSH) were estimated by Anderson (1985) methods by estimating the

stoichiometric formation of 5-thio-2-nitrobenziodic acid (TNB) at $\lambda=412$ nm and comparing with a standard curve prepared with GSH (Sigma).

For estimation of antioxidant enzyme activities, fresh samples of leaves (~300 mg each) were used following Kumar et al. (2013). Superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured spectrophotometrically at 560 nm following Beauchamp and Fridovich (1971) and presented as U mg^{-1} protein, where 1 U of SOD activity is the amount of protein required to inhibit 50 % of initial reduction of nitro-blue tetrazolium (NBT) under light. Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was measured following Nakano and Asada (1981) using $\epsilon=2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ and the enzyme activity was expressed as μ moles of ascorbate oxidized $\text{min}^{-1} \text{ mg}^{-1}$ protein. Catalase (CAT) (EC 1.11.1.6) activity was measured by method of Scandalios et al. (1983) and was expressed as $\text{mM min}^{-1} \text{ mg}^{-1}$ protein. Guaiacol peroxidase (POD) (EC 1.11.1.7) activity was assayed at 470 nm following Kato and Shimizu (1987) using $\epsilon=26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as μ moles of guaiacol oxidized $\text{min}^{-1} \text{ mg}^{-1}$ protein. Glutathione reductase (GR) (EC 1.6.4.2) activity was assayed following Smith et al. (1988) and represented as U mg^{-1} protein, where 1U is conversion of 1 mM of oxidized glutathione (GSSG) min^{-1} to reduced glutathione (GSH). Ascorbate oxidase (AO) (EC 1.10.3.3) activity was assayed following Oberbacher and Vines (1963) and represented as U mg^{-1} protein, where 1U is oxidation of 1 μ mol of the ascorbic acid min^{-1} . Monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) activity was assayed following Vanacker et al. (1998) by monitoring the formation of monodehydroascorbate at $\lambda=340$ nm, due to the action of ascorbate oxidase (0.4 unit; 1 unit; 1 mmol of ascorbate oxidized per min) and represented as mM mg^{-1} protein. Dehydroascorbate reductase (DHAR; EC 1.8.5.1) activity by Doulis et al. (1997) at $\lambda=265$ nm using $\epsilon=7.0 \text{ mM}^{-1} \text{ cm}^{-1}$ and represented as $\mu\text{M mg}^{-1}$ protein. Glutathione S-transferase (GST; EC 2.5.1.13) activity by Habig et al. (1974) using the $\epsilon=9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ of the product formed and was expressed as μM of CDNB conjugated mg^{-1} protein.

Elemental and radical analysis

The metal(loid) contents (As) were determined following Kumar et al. (2013). Plant tissues (leaf~500 mg and root~300 mg) were digested using HNO_3 : HClO_4 (3:1) in borosilicate glass beakers (50 ml) kept on hot plate temperature not exceeding 120°C . Cu, Fe, Zn and Mn ($\mu\text{g ml}^{-1}$) were analysed using AAS (GBC Avanta Σ), whereas for As ($\mu\text{g l}^{-1}$) AAS was fitted with a hydrate generator (MDS 2000) using NaH_2BO_4 + NaOH (3 M) and HCl (3 M). The values are presented in $\mu\text{g g}^{-1}$ dw (dry weight) and the translocation factor (TF) is the ratio of elements in leaves upon its roots.

Statistical analysis and analytical quality control

The whole experiment was set in randomized block design with four replicates for each treatment. The presented data is an average of two experiments conducted separately at different time intervals. The data were subjected to one way ANOVA through Duncan's Multiple Range Test (DMRT) for the analysis of significant difference between the treatments. Analytical data quality of the elements, was ensured through repeated analysis ($n=6$) of Standard Reference Material (CRM 028–050) Resource Technology Corporation, USA (Lot No. IH 028). The values obtained (10 times) varied between –3.97 to 22.86 % error. The blanks were used to eliminate background noise.

Morphological parameters

The fresh-weight (fw) was measured by blot-drying roots, on an electrical balance (Mettler-Toledo) in milligrams (mg), shoot length and roots in millimetres (mm). All the growth parameters were measured after both 7th and 14th day after treatments, whereas, the biochemical parameters were measures only after 14d.

Results

Growth parameters

Pretreatment of the cultivars P11(tolerant) and MTU(sensitive) with H_2O_2 and H_2O_2 + SA did not show any significant change in root and shoot lengths (Table 1; S Fig. 1). However, in case of fresh weight a marginal increase was observed at both 7d and 14d of treatment in cultivar P11. Relative to the As(III) treatment, in this cultivar, pretreatment with H_2O_2 and H_2O_2 + SA showed ca. 14 % and ca. 35 % increase respectively at 7d, while corresponding values at 14d were ca. 17 and ca. 18 %, respectively.

Arsenic accumulation and variation on essential elements

In the tolerant cultivar (P11), in comparison to the As accumulation ($\mu\text{g g}^{-1}$ dw) in the leaves of AsV(4), the accumulation in the H_2O_2 pretreated plants receiving AsV(4), was significantly lower (ca. 42 %) (Fig. 1a). Similarly, significant reduction (ca. 30 %) in As accumulation was also observed in sensitive cultivar (MTU) with H_2O_2 pretreatment and also with H_2O_2 + SA pretreatment (ca. 16 %), in comparison to its AsV(MTU). On the contrary, the uptake and accumulation of As in As(III) treated plants was not reduced by pretreatment of H_2O_2 and SA in any of the cultivars. Arsenic uptake in the sensitive cultivar pretreated with H_2O_2 + SA receiving AsIII [AsIII(4) H_2O_2 + SA(MTU)], increased (ca. 63 %), as

Table 1 Effect of the pretreatment with H₂O₂ and H₂O₂ + SA in AsIII and AsV treated on the growth parameters of *Oryza sativa* seedlings, cv. P11 and cv. MTU. The values are mean of four independent replicates + SD

Treatments	Root length (cm)		Shoot length (cm)		Fresh weight (gm)		Total Chlorophyll (mg g ⁻¹ Fw)		Carotenoid (mg g ⁻¹ Fw)		Protein (mg g ⁻¹ Fw)	
	7d	14d	7d	14d	7d	14d	7d	14d	7d	14d	7d	14d
C(P11)	3.8±0.5 ^{ab}	3.4±0.8 ^{abcde}	24.5±1.7 ^a	32.5±0.6 ^e	10.9±2.1 ^{abcd}	18.1±1.2 ^c	3.8±0.2 ^{bcd}	3.4±0.2 ^a	1.2±0.11 ^{abc}	1.1±0.0 ^b	3.3±0.1 ^{bc}	5.5±0.0 ^e
AsIII(4)(P11)	3.0±0 ^a	4.1±0.3 ^{def}	22.0±2.9 ^a	30.1±2.1 ^{de}	10.3±2.2 ^{abcd}	17.1±1.4 ^c	3.4±0.1 ^{ab}	4.2±0.2 ^{ab}	1.1±0.12 ^a	1.4±0.1 ^c	2.8±0.1 ^a	4.4±0.7 ^{abc}
AsIII(4)H ₂ O ₂ (P11)	3.8±0.9 ^{ab}	3.9±0.5 ^{cdef}	22.5±3.3 ^a	30.7±0.5 ^{de}	12.1±2.5 ^{bcd}	18.7±1.5 ^c	3.3±0.3 ^a	4.1±0.1 ^a	1.2±0.16 ^{ab}	1.3±0.1 ^c	3.4±0.1 ^c	3.9±0.1 ^{ab}
AsIII(4)H ₂ O ₂ + SA (P11)	4.0±0.8 ^{ab}	4.3±0.5 ^{ef}	23.5±0.6 ^a	32.0±1.6 ^e	13.9±1.4 ^d	20.1±1.4 ^c	3.6±0.2 ^{abc}	3.4±0.2 ^a	1.2±0.08 ^{ab}	1.2±0.1 ^b	3.2±0.2 ^{bc}	3.8±0.1 ^a
AsV(4)(P11)	3.58±0.6 ^{ab}	3.75±0.3 ^{bcddef}	24.0±1.1 ^a	32.0±0.82 ^e	13.8±1.5 ^d	19.1±0.9 ^c	3.6±0.0 ^{abc}	3.4±0.2 ^a	1.2±0.03 ^{ab}	1.1±0.1 ^b	3.4±0.3 ^c	4.3±0.0 ^{abc}
AsV(4)H ₂ O ₂ (P11)	3.8±0.5 ^{ab}	4.8±1.0 ^f	23.5±1.0 ^a	30.0±0.82 ^{de}	13.0±1.4 ^{cd}	17.7±0.8 ^c	3.7±0.3 ^{abc}	3.9±0.3 ^a	1.2±0.06 ^{abc}	1.2±0.1 ^b	3.0±0.0 ^{ab}	5.4±0.0 ^e
AsV(4)H ₂ O ₂ + SA(P11)	3.8±0.5 ^{ab}	3.7±0.7 ^{bcddef}	24.5±1.2 ^a	31.5±1.2 ^e	13.1±0.9 ^{cd}	20.2±1.8 ^c	3.5±0.1 ^{abc}	3.4±0.1 ^a	1.1±0.02 ^a	1.1±0.0 ^b	3.3±0.0 ^{bc}	4.5±0.1 ^{bc}
C(MTU)	3.8±0.9 ^{ab}	4.8±0.5 ^f	22.5±1.0 ^a	27.2±1.2 ^{cd}	8.6±0.4 ^{ab}	12.1±2.1 ^b	4.1±0.1 ^d	3.8±0.1 ^a	1.4±0.06 ^c	1.2±0.1 ^b	3.4±0.2 ^c	5.3±0.0 ^{de}
AsIII(4)(MTU)	5.0±0.0 ^b	4.9±0.3 ^f	20.2±2.2 ^a	21.8±3.2 ^{abc}	8.0±1.7 ^a	9.7±2.8 ^{ab}	3.9±0.2 ^{cd}	3.8±0.1 ^a	1.3±0.08 ^{abc}	1.2±0.1 ^b	3.8±0.2 ^d	4.3±0.0 ^{abc}
AsIII(4)H ₂ O ₂ (MTU)	4.7±0.5 ^b	2.5±0.4 ^{ab}	23.2±1.2 ^a	24.0±1.4 ^{abc}	9.4±1.0 ^{abc}	7.9±1.2 ^a	3.7±0.1 ^{bcd}	3.8±0.0 ^a	1.2±0.04 ^{ab}	1.1±0.1 ^b	3.2±0.1 ^{bc}	4.7±0.0 ^{cd}
AsIII(4)H ₂ O ₂ + SA(MTU)	4.5±0.6 ^{ab}	2.8±0.3 ^{abc}	21.7±3.1 ^a	21.6±1.2 ^{ab}	8.5±1.9 ^{ab}	9.7±1.5 ^{ab}	3.7±0.1 ^{abc}	3.5±0.7 ^a	1.1±0.05 ^a	1.0±0.0 ^a	3.2±0.1 ^{bc}	4.3±0.0 ^{abc}
AsV(4)(MTU)	4.3±0.5 ^{ab}	2.9±0.3 ^{abcd}	24.0±1.8 ^a	24.5±1.7 ^{bc}	8.6±1.1 ^{ab}	9.9±1.0 ^{ab}	3.8±0.1 ^{cd}	3.6±0.2 ^a	1.2±0.02 ^{ab}	1.1±0.1 ^b	3.4±0.3 ^c	4.4±0.2 ^{abc}
AsV(4)SA(MTU)	4.8±0.5 ^b	2.1±0.5 ^a	22.0±1.4 ^a	20.6±1.2 ^a	8.5±1.8 ^{ab}	7.4±1.5 ^a	3.9±0.1 ^{cd}	3.7±0.3 ^a	1.3±0.01 ^{bc}	1.1±0.0 ^b	4.1±0.2 ^d	4.6±0.4 ^c
AsV(4)H ₂ O ₂ + SA (MTU)	4.3±0.9 ^{ab}	2.1±0.3 ^a	22.0±2.9 ^a	23.7±1.7 ^{abc}	7.4±1.2 ^a	7.5±0.5 ^a	3.6±0.1 ^{abc}	3.5±0.0 ^a	1.2±0.09 ^{ab}	1.2±0.0 ^b	3.4±0.2 ^d	4.5±0.4 ^{bc}

Values marked with same letter are not significantly different (DMRT, $p < 0.05$)

Fig. 1 Levels of As, ($\mu\text{g g}^{-1}$ dw) in the leaves and roots of H_2O_2 and SA pretreated seedlings of rice cv. P11 and cv. MTU (A); PO_4^{3-} level (mg g^{-1} dw) of cv. P11 and cv. MTU (B), Cu level

(C), Fe level (D), Mn level (E), Zn level (F). All bars are mean of four replicates + SD. Bars marked with same letters are not significantly different (Duncan's test, $p < 0.05$).

compared to AsIII(4)MTU. Comparison of the levels of As accumulation in the leaves of AsIII(4) and AsV(4) between the two cultivars, show that accumulation in the sensitive cultivar was ca. 16 % and ca. 64 % higher than their respective As(III) and As(V) treatments in tolerant cultivar. On the contrary, As level in the roots of tolerant cultivar, of AsIII(4) and AsV(4) treatments were ca. 54 % and ca. 56 % higher than those in sensitive cultivar, respectively. Hence, the TF of As in

the tolerant cultivar against As(III) (0.31) and As(V) (0.49), were lower than those of sensitive cultivar, which was 0.79 and 0.88, respectively (Table 2). However, the reduction of TF of As with H_2O_2 pre-treatments in AsV(4)(P11) was ca. 12 % and for AsIII(4)(MTU) and AsV(4)(MTU) was ca. 38 % and ca. 33 %, respectively.

Among the essential elements, the level of Cu ($\mu\text{g g}^{-1}$ dw) in the roots of H_2O_2 pretreated plants of tolerant cultivar

Table 2 Effect of the pretreatment with H₂O₂ and H₂O₂+SA in AsIII and AsV treated on the TF of various nutrient, in *Oryza sativa* seedlings, cv. P11 and cv. MTU

Treatments	As Leaves/Root	Cu Leaves/Root	PO ₄ ³⁻ Leaves/Root	Fe Leaves/Root	Mn Leaves/Root	Zn Leaves/Root
C(P11)	–	0.29±0.01 ^b	1.31±0.02 ^d	0.34±0.01 ^a	2.09±0.02 ^g	0.19±0.04 ^a
AsIII (4) (P11)	0.35±0.01 ^a	0.35±0.01 ^{de}	1.74±0.11 ^{ef}	0.56±0.02 ^{bc}	3.17±0.02 ⁱ	0.37±0.03 ^b
AsIII (4) H ₂ O ₂ (P11)	0.54±0.01 ^d	0.41±0.02 ^g	1.76±0.07 ^f	0.52±0.03 ^b	3.59±0.07 ^j	0.66±0.02 ^{ef}
As III (4) H ₂ O ₂ + SA (P11)	0.47±0.02 ^c	0.37±0.01 ^e	1.57±0.02 ^e	0.57±0.02 ^{bcd}	4.28±0.04 ^k	0.72±0.06 ^{fg}
AsV (4) (P11)	0.37±0.01 ^a	0.37±0.02 ^e	1.17±0.01 ^c	0.51±0.02 ^b	3.25±0.03 ⁱ	0.73±0.05 ^{fg}
AsV (4) H ₂ O ₂ (P11)	0.36±0.03 ^a	0.42±0.02 ^g	0.95±0.02 ^a	1.08±0.01 ^h	1.67±0.07 ^e	0.59±0.02 ^{de}
AsV (4) H ₂ O ₂ + SA (P11)	0.40±0.01 ^b	0.40±0.03 ^g	1.03±0.01 ^b	0.61±0.02 ^{de}	1.30±0.04 ^d	0.56±0.02 ^d
C (MTU)	–	0.19±0.01 ^a	1.40±0.05 ^d	1.04±0.04 ^h	1.17±0.06 ^c	0.48±0.03 ^c
AsIII (4) (MTU)	0.69±0.01 ^f	0.35±0.02 ^{de}	1.40±0.03 ^d	0.62±0.05 ^e	0.54±0.05 ^a	0.77±0.07 ^g
AsIII (4) H ₂ O ₂ (MTU)	0.51±0.02 ^d	0.34±0.01 ^d	1.39±0.02 ^d	0.79±0.05 ^g	1.18±0.07 ^c	1.00±0.05 ^h
As III (4) H ₂ O ₂ + SA (MTU)	0.40±0.03 ^b	0.31±0.01 ^d	1.62±0.01 ^{ef}	0.70±0.06 ^f	0.93±0.02 ^b	0.63±0.03 ^{ef}
AsV (4) (MTU)	0.79±0.01 ^g	0.34±0.02 ^c	1.08±0.03 ^b	0.69±0.04 ^f	1.78±0.03 ^f	1.13±0.04 ⁱ
AsV (4) H ₂ O ₂ (MTU)	0.53±0.05 ^d	0.39±0.02 ^f	1.22±0.02 ^c	0.67±0.05 ^{cde}	2.12±0.06 ^g	0.72±0.04 ^{fg}
AsV (4) H ₂ O ₂ + SA (MTU)	0.64±0.02 ^e	0.31±0.01 ^c	1.59±0.01 ^e	0.71±0.06 ^f	2.47±0.08 ^h	1.28±0.04 ^j

receiving AsIII(4), decreased by ca. 26 %, against that in AsIII(4) (Fig. 1c). However, when compared with the control, the TF of Cu increased in H₂O₂ pretreated plants receiving AsIII(4) and AsV(4), were 0.41 and 0.42, respectively (Table 2). The level of Fe increased significantly in the leaves of the sensitive cultivar (ca. 57 %) treated with AsV(4), as compared to AsIII(4) (Fig. 1d). The TF of Fe in all the plants of tolerant cultivar receiving AsIII(4) and AsV(4), were higher than its control, whereas, in sensitive cultivar the TF declined. The level of Mn ($\mu\text{g g}^{-1}$ dw) in H₂O₂ pretreated plants of the tolerant cultivar receiving AsIII(4), was significantly higher (ca. 25 %) than its control [P11(C)], whereas, in the sensitive cultivar, it was significantly lower (ca. 42 %) in AsIII(4) and As(III) + H₂O₂ + SA treated plants (Fig. 1e). Compared between the Zn level ($\mu\text{g g}^{-1}$ dw) in the leaves of control plants of the two cultivars, the level in sensitive cultivar was significantly higher (ca. 122 %) than that in the tolerant cultivar (Fig. 1f). With respect to the level of Zn in control of tolerant cultivar, except for AsIII(4) the levels in the leaves of all the treated plants was significantly higher and that in the roots were significantly lower, however, same pattern was not evident in sensitive cultivar. Comparing the Zn levels between AsIII(4) and AsV(4) treated seedlings of the two cultivars, in sensitive cultivar the level increased significantly (ca. 45 %) in plants receiving AsV(4) than in AsIII(4). In tolerant cultivar, the TF of Zn (0.37) in AsIII(4) receiving plants was higher than C(P11) which increased further consistently with H₂O₂ (0.65) and H₂O₂+SA treatment (0.72), whereas, in AsV(4) treated plants, the TF although higher than C(P11), but the values in H₂O₂ (0.59) and H₂O₂+SA(0.55) treated plants were less than AsV(4) (0.73). The TF of Zn in the sensitive cultivar (0.48) was relatively higher than that in tolerant cultivar (0.19) as compared between the control plants, and

also the TF in AsIII(4) (0.77) and AsV(4) (1.13) were higher than the respective treatments in the tolerant cultivar. Similarly, the TF of Zn in AsIII(4)+H₂O₂ (1.0) and AsV(4)+H₂O₂ (0.71) in sensitive cultivar, were higher than the respective values in tolerant cultivars. The levels of PO₄ in the leaves and roots of the two cultivars did not vary significantly, as compared to their respective controls or between the various As treatments (Fig. 1b).

Effect on H₂O₂ and TBARS

The level TBARS (mmol g^{-1} fw) in the sensitive cultivar were relatively higher than in the tolerant cultivar (Fig. 2a). No significant difference was observed between the treatments, except in H₂O₂ and H₂O₂+SA pretreated plants of sensitive cultivar receiving AsIII(4), where, it was higher by ca. 80 % and ca. 72 % respectively, as compared to AsIII(4) (Fig. 2a). Unlike in sensitive cultivar, the level of lipid peroxidation in tolerant cultivar receiving AsIII(4), decreased with H₂O₂ and H₂O₂+SA pretreatment, as compared to plants receiving only AsIII(4). Alternatively, while comparing with its control [C(P11)], the TBARS levels of the tolerant cultivar increased by ca. 43 % and ca. 36 % in AsIII(4) of AsV(4), respectively.

The level of H₂O₂ (nmol g^{-1} fw) in the control plants of the two cultivars did not vary significantly, however, its level in As(III) and As(V) treated seedlings of the sensitive cultivar, were relatively higher than the corresponding values in tolerant cultivar (Fig. 2b). In the tolerant cultivar, the H₂O₂ content in both the As treatments, i.e. AsIII(4)(P11) and AsV(4)(P11) were significantly lower than its control, i.e. ca. 42 % and ca. 54 %, respectively. Whereas, in the sensitive cultivar, significant increase was observed only in plants treated with

Fig. 2 Effect of pretreatment of H₂O₂ and H₂O₂+SA on the activities of different antioxidant enzymes activity in leaves of AsIII and AsV treated rice cv. P11 and MTU after 14d. A, leaf TBARS level (mmol g⁻¹ fw); B, leaf H₂O₂ level (nmol g⁻¹ fw); C: APX: (μmol min⁻¹ mg⁻¹ protein); D,

POD (μmol min⁻¹ mg⁻¹ protein); E: CAT (M min⁻¹ mg⁻¹ protein); F: SOD (U mg⁻¹ protein). All the values are means of four replicates + SD. Bars indicated by same letters are not significantly different (DMRT, $p < 0.05$)

AsIII(4) (ca. 36 %), whereas, with AsV(4) treatment, it increased by ca. 21 %, as compared to C(P11). H₂O₂ levels in the H₂O₂ pre-treated plants receiving AsV(4), were higher over their respective AsV(4) values in both the cultivars i.e. ca. 42 % in cv. P11 and ca. 10 % in cv. MTU.

Antioxidant activity

Specific activity of SOD (U mg⁻¹ protein) of the tolerant cultivar was lower than that in the sensitive cultivar, as evident from the difference in the values between control plants

(Fig. 2f). When compared between plants treated with AsIII(4) and AsV(4) without any pretreatments, the enzyme activity in As(V)(4) treated plants of tolerant cultivar was significantly lower (ca. 62 %) than in AsIII(4). Whereas, in the sensitive cultivar, it was ca. 9 % lower in AsV(4) than AsIII(4). The specific activity of SOD in AsIII(4) treated plants of tolerant cultivar was ca. 42 % higher, while, it was ca. 24 % lower in sensitive cultivar, as compared to their respective controls. The activity of SOD in H₂O₂ pretreated plants receiving AsV(4), of both tolerant and sensitive cultivars was ca. 80 % and ca. 20 %, higher than their respective plants receiving only AsV(4) without any pretreatment.

The specific activity of APX ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in the H₂O₂ pretreated plants of tolerant cultivar receiving AsV(4) was ca. 10 % and ca. 137 % higher than its AsV(4) and C(P11), respectively (Fig. 2c). On the contrary, sensitive cultivar, the values in the corresponding treatments were ca. 6 % and ca. 42 % lower than its AsV(4)(MTU) and C(MTU), respectively. Similarly, when compared with their respective AsIII(4) treatments, the APX activities in AsV(4)P11 and AsV(4)MTU were ca. 39 % and ca. 15 % lower, respectively. In the sensitive cultivar, the activity was significantly higher (ca. 68 %) in plants treated with AsV(4), as compared to AsIII(4), whereas, it was lower (ca. 35 %) in plants pretreated with H₂O₂+SA receiving AsV(4), as compared to AsV(4) without pretreatment (Fig. 2e). The specific activity of POD ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in tolerant cultivar pretreated with H₂O₂ and H₂O₂+SA receiving AsIII(4), were ca. 76 % and ca. 106 % respectively, higher than in AsIII(4) without pretreatment. On the contrary in sensitive cultivar, the activity decreased by ca. 23 % and ca. 56 %, in the corresponding treatments, as compared to its AsIII(4) values (Fig. 2d).

The specific activity of GR (U mg^{-1} protein) was relatively higher in tolerant cultivar than that in sensitive cultivar, both in the control and treated plants (Fig. 3a). In both, H₂O₂ and H₂O₂+SA pretreated plants of tolerant cultivar receiving AsIII(4), the GR activity were significantly higher by ca. 27 % and ca. 50 %, respectively, as compared to AsIII(4), while plants receiving AsIII(4) without any pretreatment, the increase was ca. 38 % over its control [C(P11)]. Similarly, with AsV(4) treatments, the activity increased significantly (ca. 72 %) in H₂O₂ pretreatment, as compared to AsV(4). On the contrary, in the sensitive cultivar, the activity decreased significantly (ca. 53 %) in plants pretreated with H₂O₂+SA and receiving AsIII(4). Quite similar to the pattern of GR activity, the specific activity of GST ($\mu\text{M mg}^{-1}$ protein) was higher in the tolerant cultivar than the sensitive cultivar, as compared between their treatments and their control plants (Fig. 3b).

The specific activity of DHAR ($\mu\text{M mg}^{-1}$ protein) was observed to significantly decline in H₂O₂ and H₂O₂+SA pretreated plants of the sensitive cultivar receiving AsIII(4), viz. ca. 54 % and ca. 67 %, respectively (Fig. 3c). On the

Fig. 3 Effect of the pretreatment of H₂O₂ and H₂O₂ + SA on the activities of different biochemical parameter antioxidant enzymes activity in leaves of AsIII and AsV treated rice cv. P11 and MTU after 14d. A: GR activity (U mg^{-1} protein); B, GST activity ($\mu\text{M mg}^{-1}$ protein); C, DHAR activity ($\mu\text{M mg}^{-1}$ protein); D, MDHAR activity (mM mg^{-1} protein); E: AO (U mg^{-1} protein); F, AsA level ($\mu\text{M g}^{-1}$ fw); G, GSH level ($\mu\text{g g}^{-1}$ fw); H, total GSH ($\mu\text{g g}^{-1}$ fw). All values are mean of four replicates + SD. Bars marked with same letters are not significantly different (Duncan's test, $p < 0.05$)

contrary, the specific activity of MDHAR (mM mg^{-1} protein), decreased significantly in H₂O₂ pretreated plants receiving AsV(4), in either of the cultivars viz. ca. 44 % in both tolerant and ca. 44 % in sensitive, as compared to their respective AsV(4) treated plants which was without any pretreatment (Fig. 3d). The specific activity of AO (U mg^{-1} protein) was significantly higher in AsV(4)H₂O₂(P11), in comparison to AsV(4)(P11), while no such change was observed in cv. MTU. The activity in AsIII(4)H₂O₂+SA was significantly higher in either of the cultivars with respect to their respective control plants (Fig. 3e).

Ascorbate, GSH and GSSG

In the tolerant cultivar, the level of AsA ($\mu\text{M g}^{-1}$ fw) in AsIII(4)H₂O₂ and AsIII(4)H₂O₂+SA were significantly higher i.e. ca. 85 % and ca. 88 %, respectively than C(P11). However, the level in AsV(4)(P11) were significantly lower than AsIII(4)(P11) (Fig. 3f). On the contrary, in sensitive cultivar, the level was significantly higher (ca. 26 %) in AsV(4) plants than C(MTU), contrarily, the level in AsV(4)H₂O₂(MTU) and AsV(4)SA+H₂O₂(MTU) was significantly lower than AsV(4)(MTU). The level of total GSH ($\mu\text{g g}^{-1}$ fw) in either of the cultivars were significantly higher than their respective controls (Fig. 3g, h). However, the level did not differ significantly between the AsIII(4) and AsV(4) treated seedlings.

Discussion

Comparison of the growth parameters between the tolerant (P11) and sensitive (MTU) cultivars, reveals that the tolerant cultivar was phenotypically more profound in growth than the sensitive cultivar, as the root length, shoot length and fresh weight were higher in the tolerant than sensitive cultivar. The growth inhibition in sensitive cultivar was higher after 14d, against different treatments of both As(III) and As(V), compared to the same parameters in the respective treatments of tolerant cultivar. Similarly, higher translocation of As(III) and As(V) in the leaves of tolerant cultivar than in the sensitive cultivar, along with higher levels of TBARS and H₂O₂, shows cv. MTU is sensitive than cv. P11. Besides, the accumulation

of As being relatively higher in cv. P11 along with its lower level of H_2O_2 and lipid peroxidation in comparison to cv. MTU, exhibits its tolerance towards As. Doncheva et al. (2009) considered Mn tolerant and Mn sensitive cultivars of maize, based upon its tolerance towards high concentrations Mn in the tissue and not by virtue of avoidance but by exclusion. On the contrary in rice, Dave et al. (2013) have reported that, As sensitive rice cultivar accumulates higher As but has low Thiol-As molar ratio as compared to as tolerant cultivar having low As accumulation but higher thiol-As molar ratio. Hence, the higher As accumulation cv. P11 and less toxic manifestation qualifies it as tolerant than the other cultivar towards As stress.

Decline in fresh weight and shoot length of plants receiving AsIII(4) and AsV(4), both after 7d and 14d, in either of the cultivars, is attributed to the As induced toxicity. However, exception being increase in the root length in AsIII(4) receiving plants. Also, the root length in the H_2O_2 pre-treated plants of tolerant cultivar receiving AsV(4), significantly increased after 14d, in comparison to the plants treated only with AsV(4) without any pretreatment, which exhibits the amelioration of the toxicity due to H_2O_2 pretreatment. Whereas, in absence of any such phenomenon in the plants pretreated with both H_2O_2 +SA and receiving AsV(4), demonstrates that there is no added advantage with SA pretreatment. Hence, the pretreatment of H_2O_2 could induce tolerance and enhance growth of the plants. On the contrary, the significant decline in root lengths in all the treatments in sensitive cultivar after 14d, demonstrates the higher sensitivity of the cultivar towards As toxicity. Alternatively, increase in fresh weight of tolerant cultivar, exposed to both AsIII(4) and AsV(4) with H_2O_2 and SA+ H_2O_2 pretreatment, also confirms its enhanced tolerance. Increase of shoot lengths in plants of tolerant cultivar pretreated with H_2O_2 and H_2O_2 +SA receiving AsIII(4), on the other hand, absence of similar effect in sensitive cultivar supports the conclusion. Several studies indicate that either exogenous application or pretreatment of H_2O_2 or SA can alleviate abiotic stress in plants. Murphy et al. (2002) observed that exogenous application of H_2O_2 increases chilling tolerance by enhancing the GSH level in mung bean seedlings. Azevedo Neto et al. (2005) reported that addition of H_2O_2 to the nutrient solution induces salt tolerance by enhancement of antioxidants activities and reduced peroxidation of membrane lipids in leaves and roots of maize. Guo et al. (2007) have also observed that pretreatment with SA significantly alleviated the growth inhibition and transpiration suppression in rice plants caused by Cd stress, although the underlying mechanism was not fully explained. Increase in the length of shoot and root of rice seedlings was observed by Jing et al. (2007) by pre-treating seeds with SA (0.1 mmol L^{-1} SA for 48 h before Pb stress) against Pb, however under long-term exposure the effect was not maintained. Although, there are reports of increase in photosynthetic rate in mustard plant (Fariduddin

et al. 2003), wheat (Arfan et al. 2007), and in tomato plants (Poor et al. 2011), however, non significant increase in the total chlorophyll content in H_2O_2 pre-treated seedlings of the present study, thus remains inconclusive.

With H_2O_2 and H_2O_2 +SA pretreatment, As translocation to the leaves of tolerant cultivar increased in plants receiving As(III), this shows that the P11 being tolerant could overcome the toxicity due to the increased As(III) uptake translocation in the leaves. An important observation result of in the present study was that in both the cultivars, which showed significant decrease in As translocation in plants receiving As(V) by pretreatment with H_2O_2 for 48 h. This is in agreement with the earlier studies where plants with exogenous application of H_2O_2 or SA have ameliorated Cd toxicity (Guo et al. 2007, 2009), where they have attributed this to SA-induced H_2O_2 signaling in mediating Cd tolerance. Several studies have supported a major role of SA in modulating plant responses to abiotic stresses including heavy metal toxicity (Guo et al. 2007; Janda et al. 2012; Shi and Zhu 2008). The higher As level in the leaves of sensitive cultivar and the reverse in tolerant cultivar can be explained in the light of leaf GSH level. The leaf GSH level in tolerant cultivar was less than sensitive cultivar, hence the higher GSH level in sensitive cultivar could have chelated the As in roots and rendered it inactive, on the contrary the reverse happened in tolerant cultivar. The transport of As(III) takes place across aquaporin and As(V) across PO_4 transporters. It is quite likely that the PO_4^- transporters are down regulated with H_2O_2 and H_2O_2 +SA pretreatments, across which transportation of both As(V) and PO_4 takes place. Consecutively, the TF of PO_4 in As(V) followed by H_2O_2 pretreated plants were lower than that of AsIII receiving plants. Hence, it could also be inferred that H_2O_2 pretreatment in rice plants may reduce As(V) translocation albeit however, compromising the uptake of PO_4^- .

Among the essential elements, lower level of Cu in the roots of the tolerant cultivar receiving both AsIII(4) and AsV(4), as compared to C(P11), and further reduction with H_2O_2 and H_2O_2 +SA pretreatment along with their reduced translocation to leaves, all signifies that Cu translocation in rice is reduced both by As(III) and As(V) and pretreating the plants with H_2O_2 compliments in reducing the translocation further. Similar, trend in the sensitive cultivar, (cv. MTU) also supports the fact. However, in sensitive cultivar the increase in Cu level against AsIII(4) treatment is in congruence with the corresponding increase in SOD levels, where Cu acts as a co-factor (CuSOD isoform). Apart from Cu, Fe and Zn are also cofactors of a SOD, hence, the concomitant increase in translocation of these elements in the tolerant cultivar against AsV(4) treatments supports the rise in the specific activity of SOD. In spite of the relatively higher TF of Fe in the leaves of sensitive cultivar, however, its translocation in the tolerant cultivar did not decline upon treating with As or pre-treating with H_2O_2 . This could be due to inter-varietal difference of Fe

accumulation with response to As(III) or As(V) and not due to the pretreatments. Similar inter-varietal difference in Fe content has been earlier reported in different rice cultivars (Dwivedi et al. 2010). The translocation of both Mn and Zn increased with As(V) treatments in both the cultivars, however, in the tolerant cultivar pretreating plants with H_2O_2 and H_2O_2 +SA, the translocation of both the elements decreased in plants with AsV(4) exposure, on the contrary it increased with As(III) exposure. In agreement to this, Williams et al. (2009) surveyed the concentrations of As and trace mineral elements in rice grains collected from Bangladesh and found that Zn content in rice grain significantly declined with increasing As concentration. Hence, it can be concluded that there exists a certain degree of specificity imparted by As(III) and As(V) towards influencing the translocation of elements in rice plant. Hayat and Ahamed (Hayat and Ahmad 2007) have reported that decrease in PO_4 uptake in plants upon exogenous application of SA. Although, in the present study, PO_4 content in roots of both the cultivars treated with As(III) declined. Similarly, PO_4 content was also declined by SA, which signifies that SA and As(III) could inhibit PO_4 transport. However, in sensitive cultivar, the higher accumulation of As could be due to higher expression of PO_4 transporters and thus contributing towards higher As(V) accumulation. Thus, it is apparent that H_2O_2 has a role in down regulation of PO_4 transporters which manifesting in reduced uptake of both As(V) and PO_4^- .

Decreased level of H_2O_2 in the leaves of the tolerant cultivar when treated with both AsIII(4) and AsV(4), could be correlated with increased activity of APX. Whereas, the higher level of H_2O_2 in sensitive cultivar treated with AsV(4), correlates well with its lower APX activities. Guo et al. (2007) have attributed the SA signaling pathway in plants being associated with increased H_2O_2 levels. In both the cultivars, the higher level of H_2O_2 in plants pretreated with H_2O_2 +SA receiving AsIII(4), over their respective plants pretreated only with H_2O_2 receiving AsIII(4), and no such increase in their corresponding As(V) treatments [AsV(4) H_2O_2 +SA and AsV(4) H_2O_2 , respectively], show that with AsV there is little role of exogenous H_2O_2 pretreatment on the plant's endogenous H_2O_2 level. Alternatively, meager increase of H_2O_2 levels in both As+ H_2O_2 treated seedlings along with their non significant increase in APX and SOD activities, over their AsIII(4) or AsV(4) values, supports that exogenous H_2O_2 pretreatment does not elevate the endogenous H_2O_2 levels significantly. Similar observation was also reported by Popova et al. (2009) where they observed no major changes in H_2O_2 level in Cd- treated plants or pretreated with SA before exposure to Cd.

The observed variation in SOD activity between the two contrasting cultivars shows that the tolerant cultivar, was responsive towards As(III) stress than the sensitive cultivar, however, the progressive increase in SOD activity from

AsIII(4) to AsIII(4) H_2O_2 and further to AsIII(4) H_2O_2 +SA in the sensitive cultivar can be attributed to its higher sensitivity towards As(III). Similarly, higher APX activity in tolerant cultivar than in sensitive cultivar conjunction with lower H_2O_2 levels, also demonstrates the existence of differential response between the two cultivars. Higher level of POD and GR activities in As(III) treated plants of tolerant cultivar than in sensitive cultivar, also supports existence of differential detoxification mechanism between the two cultivars. Alternatively, significantly higher POD and GR activities in AsIII(4)+ H_2O_2 *vis-a-vis* AsV(4)+ H_2O_2 seedlings demonstrates a differential response towards the two species of As, as well. Increase in DHAR activity in AsIII(4) H_2O_2 and AsIII(4) H_2O_2 +SA treated seedlings of tolerant cultivar in contrast to decrease in its activity in sensitive cultivar, could be due to similar reason. Similarly, the significant decline in MDHAR activity in AsV(4) H_2O_2 as compared to AsV(4) in both the cultivars and not with AsIII(4) H_2O_2 is yet another evidence supporting the differential response towards two species of As.

Among the antioxidant levels, the significantly higher levels of AsA in AsIII(4) and AsIII(4) H_2O_2 treated seedlings of the tolerant cultivar than AsV(4) and AsV(4)+ H_2O_2 shows that tolerant cultivar is more responsive towards As(III) than As(V), whereas the sensitive cultivar is more responsive towards As(V). The higher level of total GSH in the treated seedlings of sensitive cultivar than in tolerant cultivar shoes that GSH plays a greater role in the sensitive cultivar.

Analysis of the results thus demonstrates that the pre-treatment of the rice seedlings with H_2O_2 increases fresh-weight against As(III) treatment whereas, it significantly reduces the As(V) translocation to the leaves. The study also differentiates sensitive verses tolerant cultivar on the basis of the biochemical and growth parameters, where growth inhibition, lipid peroxidation and H_2O_2 content in the sensitive cultivar (cv. MTU) was higher than the tolerant (cv. P11) observed against As(III) and As(V) stress. The As accumulation against AsIII and AsV treatments, were lower in sensitive cultivar, however, the As translocation to its shoots were higher than that in tolerant cultivar. In both the cultivars, pretreatment of the seedlings with H_2O_2 decreased the As(V) uptake and translocation significantly. Cu uptake and translocation decreased considerably in tolerant cultivar with both As(III) and As(V) which increased with H_2O_2 and H_2O_2 +SA pre-treatments. The higher uptake of Cu in the sensitive cultivar could be attributed to higher activity of CuSOD. In absence of any additive effect with H_2O_2 +SA pretreatment, it is concluded that both H_2O_2 or H_2O_2 +SA yields similar response in terms of reduction of As(V) uptake. CAT had little role in either of the cultivars, however, APX played a significant role towards H_2O_2 detoxification in tolerant cultivar. On the contrary, higher H_2O_2 levels in different treatments of sensitive cultivar were attributed to higher

activity of SOD. Also the higher APX, GR and GST activity in tolerant cultivar contributed towards higher tolerance towards As. Redundancy of antioxidant enzymes existed between tolerant cultivar and sensitive cultivar, where, APX, GR and GST dominated in the former and SOD in the later.

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