RESEARCH ARTICLE

A Comparative Study on Effect of Arsenic on Thiolic Ligands and Phytochelatins in Contrasting Arsenic Accumulating Rice Genotypes

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Abstract

Arsenic (As) is a carcinogenic element present in the environment, hence, detrimental to biota. Rice due to highly expressed silicon pathways accumulates As more than other cereals crops. Moreover, this cereal is the major staple diet of billions of people. The current study was done to analyze the responses of two contrasting rice (*Oryza sativa* L.) genotypes [high As accumulating rice genotypes (HAARG) and low As accumulating rice genotypes (LAARG)] in terms of thiol and phytochelatins (PCs) synthesis, during arsenate (AsV) and arsenite (AsIII) exposure. AsV and AsIII moderated the thiolic pathway which was evident by different metabolite levels in two genotypes. The synthesis of PCs and consequently the formation of PC-As complexes under As stress was more in HAARG than in LAARG. It may be considered that the plants triggered synthesis, as well as, consumption pathway of thiols to counter As toxicity. However, elevated concentration of AsV and AsIII caused disturbances of this balance resulting in phytotoxicity in HAARG even after the accumulation of a higher level of PCs. In contrast, LAARG showed less up-regulation of PCs, however, it was adequate to combat relatively low As concentrations. The results indicate that if the synthesis of thiols and PCs are stimulated to an optimum level, it helps rice plants tolerate As toxicity effectively.

Keywords: Arsenate, Arsenite, Phytochelatin, Rice, Thiol metabolism

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Introduction

Arsenic (As) is an environmental contaminant that is gaining attention due to its carcinogenicity (Naujokas *et al*., 2013). Agricultural and industrial activities, like the wide use of As loaded chemicals, *viz*., insecticides, pesticides, defoliants, wood preservatives, and soil sterility caused worldwide As contamination (Hue, 2015). Agricultural and industrial activities have also led to worldwide soil contamination (Azcue and Nriagu, 1994). In India, many states face As contamination problem, such as, West Bengal, Assam, Bihar, and Uttar Pradesh (Gupta *et al*., 2020; Shukla *et al*., 2020). IARC has categorized Asamongclass-1 carcinogen and it is considered as one of the most hazardous toxicants for human beings (IARC, 2019; Susan *et al*., 2019). High As concentration causes various neurological, hematological, cardiovascular troubles, hypertension, childbirth deformity, and even cancer in humans (Mitra *et al*., 2020). Reports documented a considerable amount of inorganic As is toxic to pancreatic beta cells and thereby disrupts glucose homeostasis (Hassan *et al*., 2017). Among various oxidation species of As, inorganic As (iAs) species, *viz*., arsenite (AsIII) and arsenate (AsV) prevail in soil (Kumar *et al*., 2016). The substantial effect of As toxicity is due to the enormous generation of reactive oxygen species (ROS), *viz*., superoxide radical, hydroxyl radical, and hydrogen peroxide (Kumar *et al*., 2014a; Gupta *et al*., 2018). These ROS disrupt necessary biomolecules, like lipids, proteins, carbohydrates, as well as, DNA (Singh *et al*., 2015; Singh *et al*., 2017). After entry into plants, As intervenes with the metabolism of plants and causes physiological and morphological abnormalities, which led to planting growth inhibition (Mokgalaka-Matlala *et al*., 2008). The proliferation, as well as, the extension of plant roots shows inhibition (Srivastava *et al*., 2019). Furthermore, As causes inhibition in the reproductive potential of plants (Farnese *et al*., 2014; Finnegan & Chen, 2012).

To combat heavy metals (HMs) toxicity plants showed various defense mechanisms, like generation of thiol, *viz*., cysteine (Cys), glutathione (GSH), and phytochelatin (PCs). The PCs are cysteine-rich HMs binding peptides that have the potentiality to sequestrate, as well as, detoxify HMs in plants. The phytochelatins are synthesized from glutathione (GSH) by

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the activity of the PC synthase enzyme (Jia *et al*., 2011; Guo *et al*., 2020). The increased rate of PC biosynthesis under As stress might induce sulfate uptake and reduction pathways (Rausch & Wachter, 2005) to fulfill the increased demand for cysteine and GSH. Rai *et al*. (2011) demonstrated a higher expression of sulfate transporter during AsV stress in comparison to AsIII.

Cysteine is synthesized in the final step of the sulfate reduction pathway by the enzyme cysteine synthase (CS). The amount of GSH in a given organ is the result of the combined action of biosynthesis, consumption, and degradation. GSH is synthesized in two ATP-dependent steps catalyzed by γ-glutamyl cysteine synthetase, a rate-limiting enzyme, and glutathione synthetase (GS) (Khullar & Reddy, 2018). It is consumed in a number of redox reactions to combat oxidative stress resulting in its oxidation to oxidized glutathione (GSSG), which is recycled into its reduced form by glutathione reductase (GR) (Mishra *et al*., 2008). In addition, GSH protects the plants against a range of toxicants by conjugating them or their metabolites through glutathione-*S*-transferases (GSTs) (Øverby *et al*., 2015). Degradation of GSH, an important step in its metabolism, is supposed to be initiated by the enzyme called γ-glutamyltranspeptidase (γ-GT), which is localized outside the cell membrane and in vacuoles (Cordente *et al*., 2015). This enzyme catalyzes the hydrolysis of uniquely linked N-terminal Glu from GSH, GSSG, GS-conjugates, and probably PCs (Połéc-Pawlak *et al*., 2005). The earlier studies on the protective effect of reduced GSH and cysteine (Cys) to As stressed rice seedlings showed that both GSH and Cys imparted enhanced tolerance to seedlings against As stress (Kumar *et al*., 2014a,b, 2016; Dixit *et al*., 2016). Germination percentage and seedling growth improved, while the level of malondialdehyde (MDA) declined significantly when GSH and Cys were supplemented to As treatments suggesting GSH and Cys mediated protection against oxidative stress (Singh *et al*., 2015). A detailed investigation of PC-based As detoxification with respect to its effect on whole thiol metabolism might improve our understanding of the involvement of PCs in As detoxification. In comparison to other cereals, rice accumulates more As (Williams *et al*., 2007). Exposure to both inorganic arsenic (iAs) species in roots and shoots of contrasting As accumulating rice genotypes (IET 19226 and BRG-12) exhibited 6-10 fold difference in As accumulation and grain AAs composition during field trials and pot experiments (Dwivedi *et al*., 2012; Tripathi *et al*., 2015). The present study was conducted to analyze the responses of contrasting rice genotypes in terms of thiol and PCs responses during AsV and AsIII exposure.

Materials and Methods

Selection, Plant Growth, and Treatments

Our previous field study concluded that West Bengal rice germplasms have great diversity for grain As accumulation (Tripathi *et al*., 2015). Two rice cultivars (IET-19226 and BRG-12) exhibited 7 to 11 fold difference in As accumulation. IET-19226 is low arsenic accumulating rice genotype (LAARG) and BRG-12 is high arsenic accumulating rice genotype (HAARG). The seeds of these two cultivars were obtained from RRS, Chinsurah, West Bengal. Seeds were disinfected in 0.1% $HgCl_2$ solution for 30 seconds, followed by thorough washing with deionized water and soaking in milli-Q for 24 hours. These seeds were transferred to Petri-dishes to germinate for 3 days at 26°C in dark. Then uniform germinated seeds were selected and transplanted to trays having 24 fixed PVC cups (4 cm diameter and 5 cm height, 8 plants per cup) and grown in modified Hewitt nutrient medium (Liu *et al*., 2004) for 10 days and were then exposed to AsIII ($NaAsO_2$; 0, 10, and 25 µM) and AsV (Na_2HAsO_4; 0, 10, and 50 µM) for 7 days. Plants were harvested, washed with milli-Q, separated into roots and shoots, blotted, and used for various parameters. Root and shoot lengths were measured by the metric scale.

Estimation of Arsenic Quantification and Quality Control

For total As an estimation, 0.25-gram oven-dried tissue was taken and digested in 3 mL of HNO_3 (Dwivedi *et al*., 2010a; Kumar *et al*., 2014a). Arsenic was quantified with the help of Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500 cx). The standard solution material of As (Agilent, Part # 8500-6940) was used for the calibration and quality assurance for each analytical batch. Rice flour NIST 1568a was used as reference material with known spiked samples and recovery of total As were 95.3% (± 2.8; n = 5) and 92.5% (± 3.1; n = 5) respectively. The detection limit of As was 1 $\mu g\ L^{-1}$.

Estimation of Thiols

The level of Cys, non-protein thiol (NPT), and reduced and oxidized glutathione (GSH and GSSG, respectively) was done according to Kumar *et al*. (2014a, 2016). Total glutathione (GSSG and GSH) content was determined fluorometrically in the supernatant after 15 minutes incubation with o-phthaldialdehyde (OPT). Fluorescence intensity was recorded at 420 nm after excitation at 350 nm by using the Hitachi fluorescence spectrophotometer (F-7000).

Assay of Phytochelatin and Phytochelatin Synthase

After homogenizing 0.2-gram of plant tissue in 1 mL of extraction buffer [6.3 mm DTPA containing 0.1% (v/v) TFA], the supernatant was collected by centrifugation at 13,000 × grams for 10 minutes and used for subsequent analyses. The precolumn derivatization of thiol compounds with monobromobimane (mBBr) was based on the methods described by Minocha *et al*. (2008). Standard samples of cysteine, GSH, and PCs (PC_2, PC_3, and PC_4; Sigma-Aldrich, USA) were run to identify the peaks. The concentration of PCs was estimated in terms of GSH equivalents and is expressed as nmoles of GSH equivalents g^{-1}fw. A step-by-step gradient was used with an increase of the proportion of solvent A until it reached 21% during 5 minutes at 1 mL min^{-1}, followed by an increase up to 100% in 10 minutes, with a flux of 2.5 mL min^{-1}. The column was then cleared and optimized to 100% B for 6 minutes at 1 mL min^{-1}.

Phytochelatin synthase (PCS; EC 2.3.2.15) activity was determined by quantification of the synthesized PCs following Loscos *et al*. (2006). Briefly, the reaction mixture contained 100 mm Tris-HCl (pH 8.0), 1 mm BME, 10, 50 µM AsV and 10, 25 µM AsV, 1, 2.5, or 5 mm GSH, and 200 µL enzyme extract, in a final volume of 1 mL. The reaction mixture was cleared by centrifugation and filtered, and 50 µL was injected on

the HPLC. A reaction mixture containing all the constituents except metalloid served as control. Only the synthesis of PC_2 could be detected whose amount was calculated in terms of GSH equivalents. Enzyme activity is expressed as nmoles PC_2 min^{-1} mg^{-1} protein.

Statistical Analysis

Analysis of variance (ANOVA) ($p \leq 0.01$) and Duncan's multiple range test (DMRT) ($p \leq 0.05$) were performed to determine the significant difference between treatments by using SPSS 17.0 software.

Results

Plant Growth and Arsenic Accumulation in Rice

Both the species of As significantly reduced the root length; maximum reduction being upon AsIII exposure of 25 µM in IET-19226 (40%). The lower concentration of AsV (10 µM) slightly induced the root length by 32 and 15% for BRG-12 and IET-19226, respectively. Shoot length of BRG-12 and IET-19226 increased at a lower concentration of AsIII and AsV, followed by a decline at the maximum doses in both IET-19226 (35–40%) and BRG-12 (20–30%). Similarly, the root and shoot biomass reduction were more in IET-19226 as compared to BRG-12 during AsIII exposure.

A significant genotypic variation was observed between IET-19226 and BRG-12 for total As accumulation, which also depended on the As species. The accumulation of As was two times greater in AsV exposed plants than AsIII at various concentrations in both the genotypes. The selected rice genotypes, IET-19226 (LAARG) and BRG-12 (HAARG), showed about 7-fold difference accumulation in root and 5-fold in the shoot for both the As species (Table 1).

Cysteine and NPTs Level

IET-19226 showed a significant decline in cysteine levels in both root (16 and 28%) and shoot (23 and 45%) at higher exposure of AsV and AsIII, respectively. In contrast, BRG-12 showed a reverse trend with a significant increase in cysteine content with increasing exposures of AsV. The increase in cysteine in response to 50 µM AsV was 91% in roots and 71% in shoots (Fig. 1A-B).

In response to AsV, IET-19226 showed a concentration-dependent decline in non-protein thiol level with the maximum reduction being 55% in root and8% in shoot at 50 µM AsV. The NPTs content remained at par to control in BRG-12 roots, while in shoots it declined progressively with the maximum reduction being 36% at 50 µM AsV. In AsIII exposed roots, more than two-fold increase was observed in NPTs level in BRG-12, while a decline of 58% was noticed in IET-19226 at 25 µM AsIIIas compared to control. In shoots, a concentration-dependent decrease in NPTs content was observed in both the cultivars under AsIII exposure (Figs. 1C and D).

GSH, GSSG, and GSH/GSSG Ratio

In AsV exposed plants, BRG-12 root showed induction of GSH content at lower doses, but a decline of 19% at 50 µM AsV, however, IET-19226 root showed a concentration-dependent decline in GSH content (40% at 50 µM AsV). A continuous increase in GSH content was observed in BRG-12 shoot (58% at

Table 1: Roots and shoots lengths (cm) and total As accumulation (mg kg^{-1} fw) under various level of AsV and AsIII treatments

Treatments	*Plant lengths (cm)*				*As accumulation (mg kg^{-1} fw)*			
	IET-19226 (LAARG)		*BRG-12 (HAARG)*		*IET-19226 (LAARG)*		*BRG-12 (HAARG)*	
	Root	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*
Control	$4.13^{b} \pm 0.406$	$15.78^{b} \pm 1.022$	$6.77^{b} \pm 0.871$	$21.78^{b} \pm 2.51$	-	-	-	-
10 µM AsV	$4.75^{b} \pm 0.548$	$19.56^{c} \pm 1.342$	$8.96^{c} \pm 0.548$	$29.34^{c} \pm 3.209$	$79.15^{b} \pm 11.22$	$40.37^{b} \pm 6.55$	$481.15^{b} \pm 39.66$	$201.01^{b} \pm 21.49$
50 µM AsV	$2.65^{a} \pm 0.423$	$10.97^{a} \pm 1.165$	$4.96^{a} \pm 0.548$	$17.4^{ab} \pm 3.209$	$175.06^{c} \pm 17.19$	$90^{c} \pm 5.25$	$1,282.8^{d} \pm 78.29$	$425.64^{d} \pm 37.13$
10 µM AsIII	$4.25^{b} \pm 0.837$	$19.14^{c} \pm 1.304$	$8.12^{c} \pm 1$	$27.2^{c} \pm 1.342$	$72.1^{a} \pm 8.24$	$38.73^{a} \pm 3.37$	$312.06^{a} \pm 29.68$	$129.38^{a} \pm 12.48$
25 µM AsIII	$2.44^{a} \pm 0.6$	$9.876^{a} \pm 1.84$	4.77 ± 0.867	$15.39^{a} \pm 1.45$	$111.2^{b} \pm 10.47$	$57.39^{b} \pm 7.69$	$679.26^{c} \pm 46.5$	$262.21^{c} \pm 19.64$

All the values are means of three replicate (n = 3) ± SD; ANOVA significant at $p < 0.01$; Different letters indicate significantly different values between treatments (DMRT, $p < 0.05$)

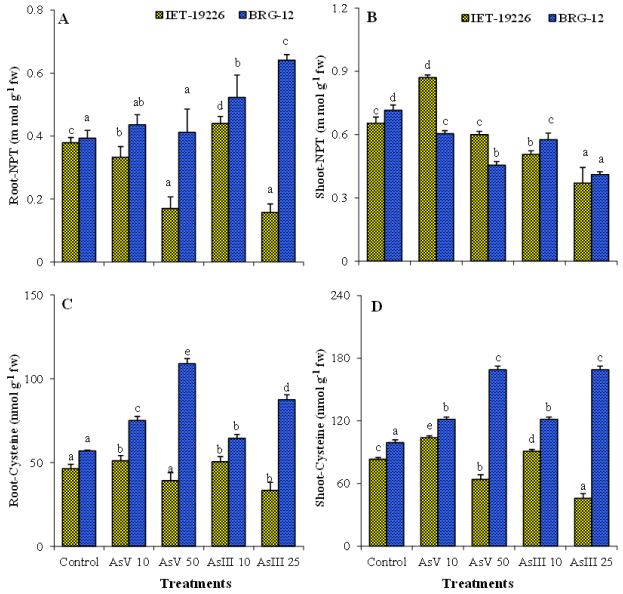

Fig. 1 (A–D): Effect of arsenate and arsenite on the non-protein thiol (A, B) and cysteine (C, D) in root and shoot of IET-19226 and BRG-12; All the values are means of three replicate (n = 3) ± SD; ANOVA significant at p ≤ 0.01; Different letters indicate significantly different values between treatments (DMRT, p ≤ 0.05)

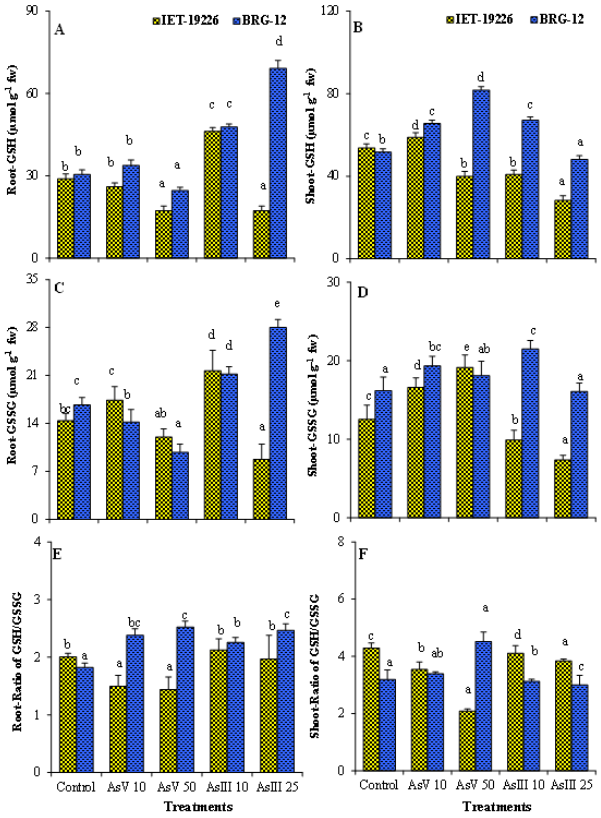

Fig. 2 (A–F): Effect of arsenate and arsenite on the reduced glutathione (GSH) (A, B) and oxidized glutathione (GSSG) (C, D) and GSH/GSSG ration (E, F) in root and shoot of IET-19226 and BRG-12; All the values are means of three replicate (n = 3) ± SD; ANOVA significant at p ≤ 0.01; Different letters indicate significantly different values between treatments (DMRT, p ≤ 0.05)

50 µM AsV), whereas the IET-19226 shoot also showed a decline in GSH level with a decrease being 26% at 50 µM AsV. During AsIII exposure, GSH level increased in a concentration-dependent manner in BRG-12 roots (127% at 25 µM AsIII), while IET-19226 roots showed induction only at a lower dose of AsIII, followed by a decline of 40% at 25 µM. A continuous decline of GSH content was observed in shoots of both the cultivar, i.e., BRG-12 (7%) and IET-19226 (38%) at 25 µM AsIII (Figs. 2A and B).

Arsenate exposure decreased the level of GSH in both genotypes with the maximum for BRG-12 (41%). In the shoot, an increase in the level of GSSG was observed in IET-19226 (53%) and BRG-12 (11%) at 50 µM AsV. The GSSG content significantly increased in roots (68%) of HAARG at a higher dose of AsIII, while no significant change was observed in its shoots upon AsIII exposure. Contrastingly, the roots of LAARG cultivar showed induction in GSSG level at a lower dose only, followed by a decline of 39% at 25 µM AsIII. However, in shoots, the GSSG level was negatively correlated (r = -0.467^{NS}) to As accumulation (Figs. 2C and D).

The ratio of GSH/GSSG increased upon AsV exposure in root and shoot of HAARG with maximum induction of 38 and 41%, respectively, at 50 µM AsV (Figs. 2E and F). However, GSH/GSSG ratio was negatively correlated with As accumulation in the root (r = -0.933*) and shoot (r = -0.967*) of LAARG. The ratio of GSH/GSSG was increased upon AsIII exposure only in roots of HAARG, while in roots of LAARG the ratio was not affected. However, in shoots of both the varieties, the GSH/GSSG ratio was declined.

Phytochelatin and Phytochelatin Synthase

Analysis of PCs was performed in plants after exposure 10 and 50 µM AsV, and 10 and 25 µM AsIII. The quantification of PCs in two contrasting rice varieties has been shown in Figs. 3A and B. Synthesis of PC_2, PC_3 and PC_4 showed a progressive increase in their levels with an increase in both As species, which was more prominent in BRG-12 than in IET-19226. Upon exposure to As species, total PCs were increased by more than 12- and 7-fold in the root of BRG-12 and 7- and 4-fold in roots of IET-19226 at higher concentrations of AsV and AsIII, respectively. Similarly, PCs content of BRG-12 shoot induced by 6-fold at 50 µM AsV, while IET-19226 showed an only two-fold increase in comparison to control. The PCs level were positively correlated with total As content in BRG-12 (r = 0.989* and r = 0.998**) and IET-19226 (r = 0.998** and r = 0.980*) in root AsV and AsIII, respectively.

The activity of PCS (Figs. 4A and B) was significantly affected by As accumulation in the IET-19226 and BRG-12 rice genotype. The PCS activity showed a significant positive correlation with total PCs content in IET-19226 root (r = 0.94*) and shoot (r = 0.961*) and increased by 77 and 120% in its root and shoot, respectively, at 50 µM AsV. Similarly, BRG-12 showed maximum induction of 141 and 174% in root and shoot, respectively, at 50 µM AsV and PCs content was positively correlated with PCS activity in its root (r = 0.93*) and shoot (r = 0.91*). Likewise, the PCS activity was increased in a concentration-dependent manner during AsIII exposure in IET-19226 and raised by 46 and 68% in its root

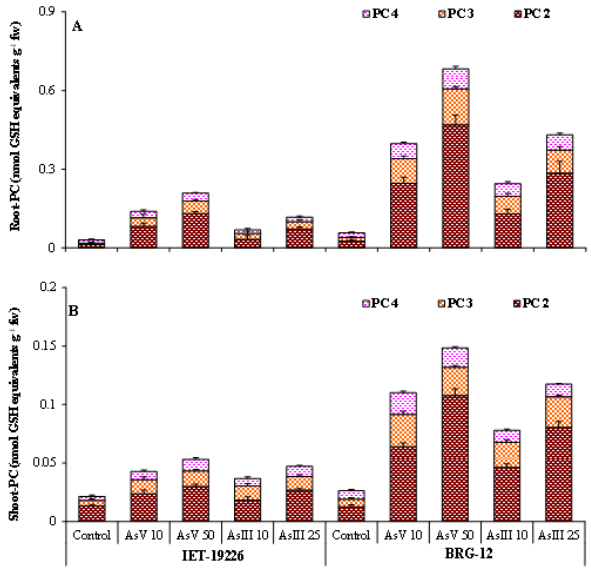

Fig. 3 (A–B): Effect of various concentrations of arsenate and arsenite on the level of phytochelatins; PC_2, PC_3, and PC_4 in crude extracts of IET-19226 and BRG-12 root (A) and shoot (B); All the values are means of three replicate (n = 3) ± SD

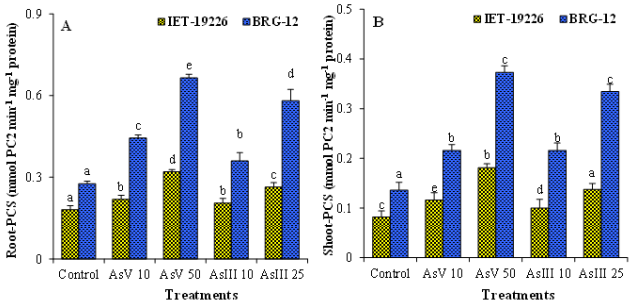

Fig. 4 (A–B): Effect of various concentrations of arsenate and arsenite on the level of phytochelatins synthase of IET-19226 and BRG-12 root (A) and shoot (B); All the values are means of three replicate (n = 3) ± SD

and shoot, respectively, while BRG-12 showed induction 146 and 110% in root and shoot at 25 µM AsIII.

Discussion

A great diversity for As accumulation prevails in rice genotypes with about 3 to 37 fold difference for As accumulation in rice cultivars grown across the countries (Norton *et al*., 2012). This study also demonstrates a significant genotypic variation (Dwivedi *et al*., 2010b; Tripathi *et al*., 2015) between IET-19226 and BRG-12 for total As accumulation, which was further dependent on the As species (Srivastava *et al*., 2009). The variation for IET-19226 and BRG-12 rice genotypes was also found different depending on the concentration of AsV and AsIII used. These differences may be related to differential As uptake mechanisms in rice plants for As species (Tripathi*et al*., 2007; Ma *et al*., 2008; Zhao *et al*., 2010). In this study, a higher accumulation of As was observed in plants exposed to AsV compared to AsIII, which may be attributed to higher AsV concentration than that of AsIII. Inorganic As species have been found to dominantly accumulate in root and shoot of rice varieties in the field and hydroponic culture (Dwivedi *et al*., 2012; Kumar *et al*., 2014b; Tripathi *et al*., 2015).

Several reports concluded that the As exposure significantly reduces the plant growth and biomass (Kumar *et al*., 2014a,b, 2016; Gupta *et al*., 2020). These results indicated that inhibition was stronger in the root than in the shoot when exposed to As, because the plant roots are the first point of contact for these toxic As species in the nutrient media. Higher accumulation of As in root may disturb the transport of minerals in roots leading to reduced biomass and plant growth (Dwivedi *et al*., 2010b). Srivastava *et al*. (2009) demonstrated the differential growth response of sensitive and tolerant variety of *Brassica* during As exposure. During the present study, the observed reduction of root length, shoot length and biomass was more in IET-19226 (LAARG) as compared to BRG-12 (HAARG).

Heavy metals exposure to plants leads to increased production of metal-binding cysteine-rich compounds, mainly being PCs (Koźmińska *et al*., 2018). Their formation from GSH is catalyzed by a γ-glutamylcysteinedipeptidyl transferase, called PC synthase, which is activated by HM ions and plays an important role in the cellular metal homeostasis (Grill *et al*., 2006). These thiolic compounds of the cells play a number of essential functions to maintain the physiological status necessary for various biochemical reactions. There are a multitude of functions that are directly or indirectly performed by the major sulfur metabolites, Cys, and GSH (Anjum *et al*., 2015). Considering the importance of thiols to combat the biotic and abiotic stresses, including the stress imposed by As (Gill *et al*., 2013; Kumar *et al*., 2014b; Gupta *et al*., 2020), this thiolic pathway was investigated in response to AsV and AsIII exposure in the two contrastingly As accumulating rice genotypes.

In crop plants mainly rice, it is important that the cultivar should be efficient in ameliorating As induced oxidative stress through detoxification and chelation of As for better nutritional yield with minimal damage to proteins. Thus, tolerance against As stress in the plant might be a conglomeration of thiolic potency and stress-responsive amino acids to provide the due resistance to plant. NPTs and GSH are also important non-enzymatic antioxidants. Previously, Mishra *et al*. (2008) has demonstrated the role of thiols in As tolerance and detoxification in aquatic plant, *viz*., *Ceratophyllum demersum*. In the present study, BRG-12 showed a continuous increase in NPTs content in roots under both the species of As. However, increasing As concentration significantly decreased the NPT level in IET-19226 indicating that As caused the toxicity in plant cells and thus, reduced the NPTs (Chakrabarty *et al*., 2009). Similarly, PCs content was also increased with As accumulation at all exposure. Chelation with PCs and subsequent compartmentalization in the vacuole is considered to be a primary strategy to detoxify metal(loid)s, including As (Meharg & Hartley-Whitaker, 2002; Song *et al*., 2010). However, the increased synthesis of PCs under stress conditions may result in increased demand for upstream metabolites of the pathway (Srivastava *et al*., 2016). In this relation, the rate-limiting step is considered to be the supply of cysteine. The importance of cysteine and PCs in As detoxification is such

that in almost zero supply of sulfur also, rice plants synthesis cysteine and PCs to tackle As load (Srivastava *et al*., 2016). In the present investigation, a significant increase in the level of Cys was observed in response to both AsV and AsIII in BRG-12, however, in IET-19226 increase occurred at a lower concentration of As but the decrease was noticed at higher concentration. In addition, stimulation of the activity of sulfate transporters and other enzymes of the pathway might also be responsible for the observed increase in cysteine levels (Sung *et al*., 2007; Srivastava *et al*., 2016). Since CS is the key enzyme in the cysteine biosynthetic pathway, it is conceivable that PC synthesis might be regulated by CS activity (Harada *et al*., 2001).

Glutathione (GSH) plays important roles not only in relieving the oxidative stress but also in metalloid detoxification (Mittler, 2002; Clemens, 2006; Grill *et al*., 2006). It is utilized in the detoxification of ROS through its participation in the ascorbate-glutathione cycle, and GST and GSH peroxidase dependent reactions (Mittler, 2002; Mittova et al., 2003; Moons, 2003). GSH, a tripeptide, is synthesized by two ATP-dependent steps: (i) The rate-limiting step-γECS catalyzes the synthesis of γEC using cysteine and γ-glutamic acid as substrates; (ii) glutathione synthetase (GS) catalyzes the synthesis of GSH using γEC and glycine as substrates. High cellular GSH/GSSG ratio makes an important contribution to the redox state of the cell along with ascorbate/dehydroascorbate and NADPH/NADP (Srivastava *et al*., 2013). In the present study, exposure of both genotypes to AsV and AsIII showed a significant increase in the levels of GSH, GSSG, and GSH/GSSG ratio with the higher increases being in HAARG. Exposure to AsIII produced a higher increase in these parameters as compared to AsV exposed plants. GSH is one of the most crucial reductants in the cell and also helps in AsV to AsIII reduction, which is necessary for complex formation with PCs (Raab *et al*., 2004; Bleeker *et al*., 2006). The ratio of GSH/GSSG was analyzed to see how they were modulated by oxidative stress (Srivastava & D'Souza, 2010). An increase in the ratio of GSH/GSSG was observed in roots of HAARG, while LAARG showed reduced regeneration of GSH from GSSG during AsV and AsIII stress.

An increase in the synthesis of PCs under As stress is a crucial step for the detoxification of As by complexing it in the form of AsIII (Srivastava *et al*., 2016). In the present investigation, PCs content was also increased in both varieties at all As(III) treatments. Various species of PCs (PC_2, PC_3, and PC_4) were first time identified in rice genotypes, however, synthesis of PC_2 was much enhanced at higher doses of AsIII and AsV in roots of HAARG (BRG-12). Earlier, various species of PCs (PC_2 and PC_3) were reported in different aquatic plants (Srivastava *et al*., 2007; Mishra *et al*., 2008) during As exposure. Arsenite exposure significantly affected the responses of thiol metabolism ($p < 0.01$) in both the contrasting genotypes. Analysis of exposed plants showed that PC_2, PC_3, and PC_4 were present more in the roots than shoots of the plants in LAARG and HAARG. The total PC content significantly correlated with As accumulation in both the genotypes BRG-12 ($r = 0.989^{*}$ and $r = 0.998^{**}$) and IET-19226 ($r = 0.998^{**}$ and $r = 0.98^{*}$) in root AsV and AsIII, respectively. Duan *et al*. (2011) demonstrated low shoot PC biosynthesis was in accordance with high As concentration in rice grain. Similarly, during the present study low level of PC in the root of LAARG (IET-19226) results in more shoot As accumulation. In the present study, As build-up was found to be positively correlated with the amount of PCs ($p < 0.05$) in HAARG. Thus, enhanced tolerance in BRG-12, most probably attributed to detoxification of some part of AsV through reduction to AsIII and subsequent complexation via PCs in the root (Raab *et al*., 2004; Liu *et al*., 2010).

Biosynthetic enzymes of PCs, *viz*., PCS was significantly affected by As accumulations. The PCS activity was found correlated with PC synthesis in LAARG and HAARG roots and shoots at both the As species exposures. Expression analysis of the genotypes showed that higher exposure to AsIII sulfate transporter was up-regulated most significantly in HAARG than in LAARG roots corresponding to higher accumulation of As and PCs. While, higher upregulation of sulfate transporter may be explained by assuming that more rapid As accumulation may lead to higher sulfur demand for localized As complexation (Srivastava & D'Souza, 2010).

Induction of PCs was supported by a significant increase in PCS activity at different AsV and AsIII exposure as reported in an aquatic plant, *viz*., *Ceratophyllum demersum* (Mishra *et al*., 2008). Additionally, overexpression of *PCS1* enhanced PCs synthesis and thereby stimulated Cd resistance in *Vicia sativa* (Zhang *et al*., 2018). Moreover, *OsPCS5/-15* expressed under As and Cd stress causes tolerance for HMs, which evident the supporting role of PCs biosynthesis to combat heavy metals toxicity (Park *et al*., 2019). Hence, it could be demonstrated that PCs have remediating potential to overcome HMs contamination (Wei *et al*., 2020).

Conclusion

Arsenate and arsenite appear to have modulated the whole pathway of thiolic metabolism in both genotypes evaluated in terms of various metabolites. The study reinforces the importance of thiols and PCs which enhanced the tolerance capacity of two contrasting rice variety at a diverse level. Moreover, these insights provide mechanistic details of contrasting rice lines which may prove useful to identify the HAARG or LAARG characteristics in rice plants exposed to As stress. Furthermore, the results might prove useful in the development of As resistant variety with desirable agronomic characters through genetic engineering.

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