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Evaluation of amino acid profile in contrasting arsenic accumulating rice genotypes under arsenic stress

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Abstract

Amino acids (AAs) play significant roles in metal binding, antioxidant defense, and signaling in plants during heavy metal stress. In the present study, the essential amino acids (EAAs), non-essential amino acids (NEAAs), as well as the enzymes of proline and cysteine biosynthetic pathways were studied in contrasting arsenic accumulating rice genotypes grown in hydroponic solutions with addition of arsenate (As^V) or arsenite (As^{III}). Under a mild As stress, the total AAs content significantly increased in both the rice genotypes with a greater increase in a low As accumulating rice genotype (LAARG; IET-19226) than in a high As accumulating rice genotype (HAARG; BRG-12). At the equimolar concentration (10 µM), As^{III} had a greater effect on EAAs than As^V. Conversely, As^V was more effective in inducing a proline accumulation than As^{III}. Among NEAAs, As significantly induced the accumulation of histidine, aspartic acid, and serine. In contrast, a higher As concentration (50 µM) reduced the content of most AAs, the effect being more prominent during As^{III} exposure. The inhibition of glutamate kinase activity was noticed in HAARG, conversely, serine acetyltransferase and cysteine synthase activities were increased which was positively correlated with the cysteine synthesis.

Additional key words: arsenate, arsenite, cysteine synthase, glutamate kinase, *Oryza sativa*, proline, serine acetyltransferase.

Introduction

Arsenic (As) is an environmental contaminant gaining attention due to its carcinogenicity irrespective of any threshold (Naujokas *et al.* 2013). Inorganic As species [arsenite (As^{III}) and arsenate (As^V)] are dominant forms in soil and cause morphological and physiological disorders in plants (Tripathi *et al.* 2007, Ahsan *et al.* 2008). Rice is an efficient As accumulator in comparison to other cereals (Williams *et al.* 2007) due to growth under flooded conditions that leads to As uptake through the nodulin 26-like intrinsic (NIP) aquaporin channel as As^{III} (Ma *et al.* 2008). Arsenate uptake is mediated through phosphate transporters (Zhao *et al.* 2010). Several studies have demonstrated that the accumulation of As in different parts of rice including grain is several fold higher than the maximum tolerable daily intake (MTDI) limit (Norton *et al.* 2009, Dwivedi *et al.* 2010a, Norton *et al.* 2013).

The proportion of various amino acids (AAs) in rice is relatively well balanced (Duan and Sun 2005). The content of AAs like histidine (His), proline (Pro), cysteine (Cys), and glycine (Gly) increases after heavy metal exposure in various plants species (Davies

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Abbreviations: AA - amino acid; Ala - alanine; Arg - arginine; Asp - aspartic acid; BCAAs - branched chain amino acids; CS - cysteine synthase; Cys - cysteine; EEAs - essential amino acids; GK - glutamate kinase; Glu - glutamic acid; Gly - glycine; HAARG - high As accumulating rice genotype; His - histidine; Ile - isoleucine; LAARG - low As accumulating rice genotype; Leu - leucine; Lys - lysine; Met- methionine; NEEAs - non essential amino acids; Phe - phenylalanine; Pro - proline; RDI - recommended daily intake; SAT - serine acetyl transferase; Ser - serine; Thr - threonine; Tyr - tyrosine; Val - valine.

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et al. 1987, Rai 2002, Kerkeb and Krämer 2003, Dwivedi *et al.* 2010b). Amino acids play a crucial role in osmotic adjustment or as phytochelatins (PCs) (Tripathi *et al.* 2012b, Zagorchev *et al.* 2013). Proline is known as osmoregulator and protects plants under heat, drought, and heavy metal stress (Paleg *et al.* 1981, Nayyar and Walia 2003, Mishra and Dubey 2006, Simon-Sarkadi *et al.* 2006). Dave *et al* (2013) demonstrated that glutamic acid (Glu), Gly, and Cys are involved in As detoxification through the formation of glutathione (GSH) and PCs. On the other hand, Pavlík *et al* (2010) showed that As changes the Glu content and consequently inhibits the glutamate kinase (GK) activity in *Spinacia oleracea*. Dwivedi *et al* (2010b) demonstrated that a higher exposure to As^V significantly reduces the valine (Val), threonine (Thr), and alanine (Ala) content in rice grains. Dwivedi *et al* (2012) reported that high As accumulating rice genotypes contain the insufficient amount of AAs and may not be able to fulfil the daily AAs requirement in a rice based diet.

Inorganic As species act differently, *e.g.*, As^V influences the antioxidant system more than A^{III}, whereas As^{III} activates more thiol metabolic pathways than As^V (Srivastava *et al.* 2007). A comparative transcriptome analysis revealed that the expression of a few genes involved in AA metabolism, such as Tyr aminotransferase (*Os06g11290*) and genes for AAs transport (*Os04g39489* and *Os04g56470*) is downregulated under As^V but not in As^{III} stress (Chakrabarty *et al.* 2009). Several studies have confirmed that rice genotypes have a great diversity for the grain As accumulation (Norton *et al.* 2009, Dwivedi *et al.* 2010a, 2012). In this context, our group conducted rice field trials in As prone areas and data indicate that IET 19226 exhibited 7 - 11 fold lower grain As accumulation than BRG-12 (Dwivedi *et al.* 2012). In light of this, it was hypothesised that the AAs response in contrasting As accumulating genotypes might differ with As species. Further, the modulation of some important enzymes involved in the biosyntheses of Pro and Cys by different As species in contrasting As accumulating rice genotypes was investigated.

Materials and methods

Two rice (*Oryza sativa* L.) genotypes (IET-19226 and BRG-12) contrasting in As accumulation were obtained from the Rice Research Station, Chinsurah, West Bengal. Seeds were disinfected in a 0.1 % (m/v) $HgCl_2$ solution for 30 s, followed by thorough washing with deionized water and soaking in *milli-Q* (Thermo Fisher Scientific, Waltham, USA) water for 24 h. Then the seeds were transferred to Petri-dishes for germination at 26 °C in the dark. Three-d-old seedlings were grown in a modified hydroponic medium (Liu *et al.* 2004) for 10 d and then exposed to As^{III} ($NaAsO_2$; 0, 10, and 25 µM) or As^V (Na_2HAsO_4; 0, 10, and 50 µM) for 7 d. The experiments were conducted twice with three replicates for each treatment. After that, the plants were harvested, washed with *milli-Q* water, blotted dry, and used for the study of various parameters. Root and shoot lengths and fresh masses were noted.

Plant samples (0.5 g of dry mass) were taken and digested in 3 cm^3 of HNO_3 at 120 °C for 6 h (Dwivedi *et al.* 2010b). Total As was quantified using an inductively coupled plasma mass spectrometer (ICP-MS, *Agilent 7500 cx*, Tokyo, Japan). Determination of As species in roots and shoots was performed following Zheng *et al.* (2011). The standard reference materials of metals (*Merck*, Darmstadt, Germany) were used for calibration and quality assurance for each analytical batch. The total As estimation, quality assurance and controls of the measurement were checked by analyzing rice flour *NIST1568a* as reference material with known spiked samples, and recovery of total As was 91.3 ±2.1 % ($n = 6$). For As speciation, standard compounds of As^V, As^{III}, dimethylarsenic acid (DMA) and monomethylarsonic acid (MMA) were used to obtain retention times. Matrix-matched DMA standards were used to calibrate the instrument. As species in samples were identified by comparison with the retention times of the standard compounds and quantified using external calibration curves with peak areas (Dave *et al.* 2013). The detection limit for As was 1 µg dm^{-3}.

The Pico Tag method (Bidlingmeyer 1984) was followed for the estimation of AAs. Chromatograms were integrated using the *Empower 2* HPLC software *v. 6.0.* The AA content was expressed in mg kg^{-1} (f.m.). The AAs analysed were isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), valine (Val), asparagine (Asp), glutamine (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), alanine (Ala), proline (Pro), tyrosine (Tyr), and cysteine (Cys). Tryptophan (Trp), asparagine (Asn), and glutamine (Gln) could not be analysed by this procedure, as these are heat labile.

The GK (EC 2.7.2.11) activity was assayed by Hayzer and Leisinger (1980) with a slight modification (Jaleel *et al.* 2007). Plant samples (1 g of fresh mass) were extracted in a 50 mM Tris-HCl buffer and centrifuged at 40 000 *g* and 4 °C for 30 min. Then 8 mM ATP was added and 1 cm^3 of the final mixture was incubated at 37 °C for 30 min. After that, 2 cm^3 of a stop buffer ($FeCl_3$) was added. The GK activity was measured at 535 nm with a spectrophotometer (*Spectrascan UV 2700*, *Thermo Fisher Scientific*). One unit of enzyme activity was defined as amount (µg) of γ-glutamylhydro-xamate formed per milligram of protein per minute. The serine acetyl-transferase (SAT, EC 2.3.1.30) and cysteine synthase (CS; EC 2.5.1.47) activities were assayed according to Tripathi *et al* (2012a). The protein content of the supernatant was measured following the method of Bradford (1976).

The analysis of variance (*ANOVA*) and Duncan's multiple range test (DMRT) were performed to determine significant differences between treatments. The correlation analysis was performed for all the data which has been given within the text at relevant places (*** - $P < 0.001$, ** - $P < 0.01$, * - $P < 0.1$, ^{NS} - non-significant).

Results and discussion

The selected rice genotypes, IET-19226 (a low As accumulating rice genotype, LAARG) and BRG-12 (a high As accumulating rice genotype, HAARG), showed about 7-fold difference for As accumulation in roots and 5-fold in shoots during both the As species exposures (Table 1).

However, in both the genotypes, the As accumulation was about two times greater at the As^V than As^{III} stress. Increasing the As concentration in the solution increased the As accumulation in both the rice genotypes which was highest in roots of BRG-12 at the 50 µM As^V exposure. Total As accumulations in IET-19226 and BRG-12 were positively correlated with increasing As^V ($r = 0.954$** and $r = 0.992$***) and As^{III} ($r = 0.994$*** and $r = 0.999$***) concentrations in the medium, respectively. Norton *et al* (2013) found a great diversity for As accumulation in rice. Our previous study also demonstrated a significant genotypic variation (Dwivedi *et al.* 2010b) between IET-19226 and BRG-12 for total As accumulation which was further dependent on the As species (Srivastava *et al.* 2009). The As accumulations in IET-19226 and BRG-12 were different at different

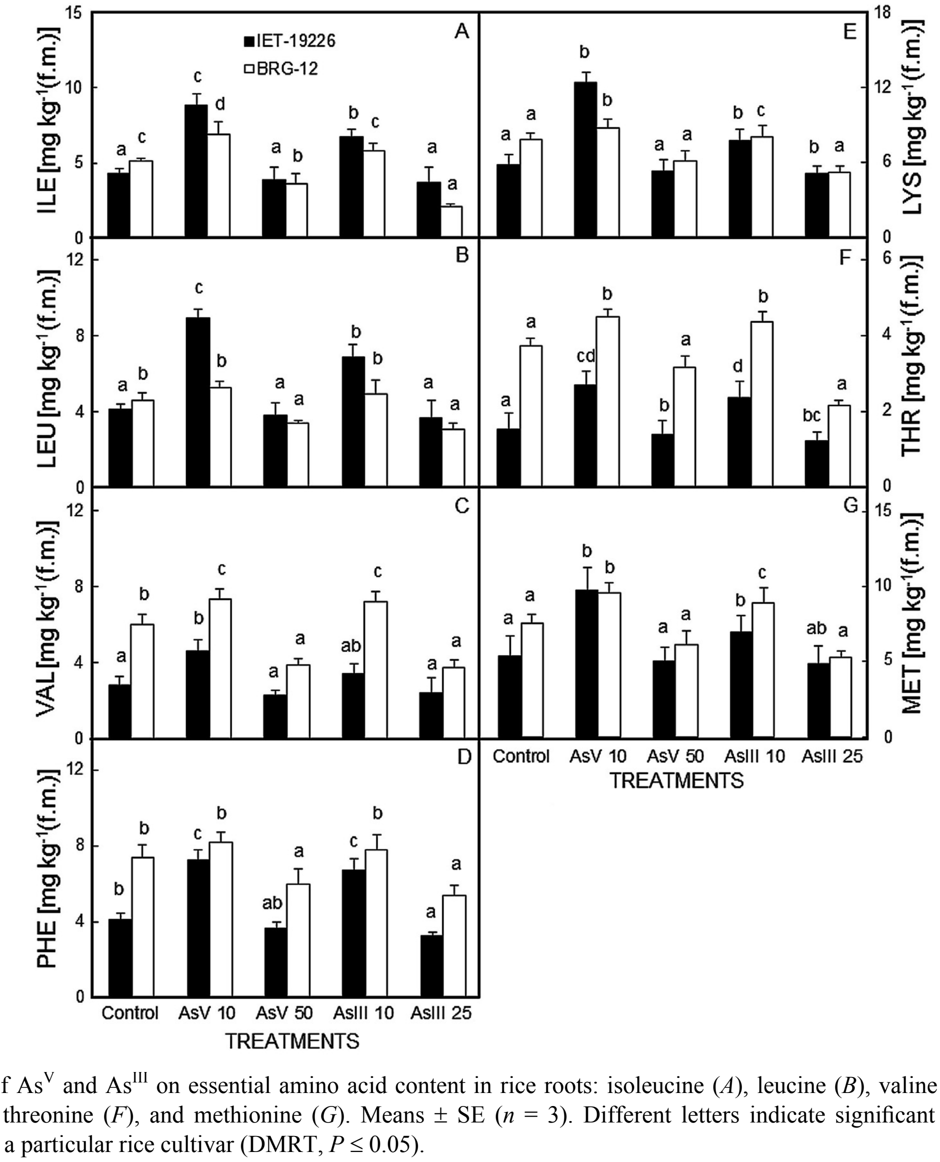

Fig. 1. Effects of As^V and As^{III} on essential amino acid content in rice roots: isoleucine (*A*), leucine (*B*), valine (*C*), phenylalanine (*D*), lysine (*E*), threonine (*F*), and methionine (*G*). Means ± SE ($n = 3$). Different letters indicate significant differences among As treatments in a particular rice cultivar (DMRT, $P \leq 0.05$).

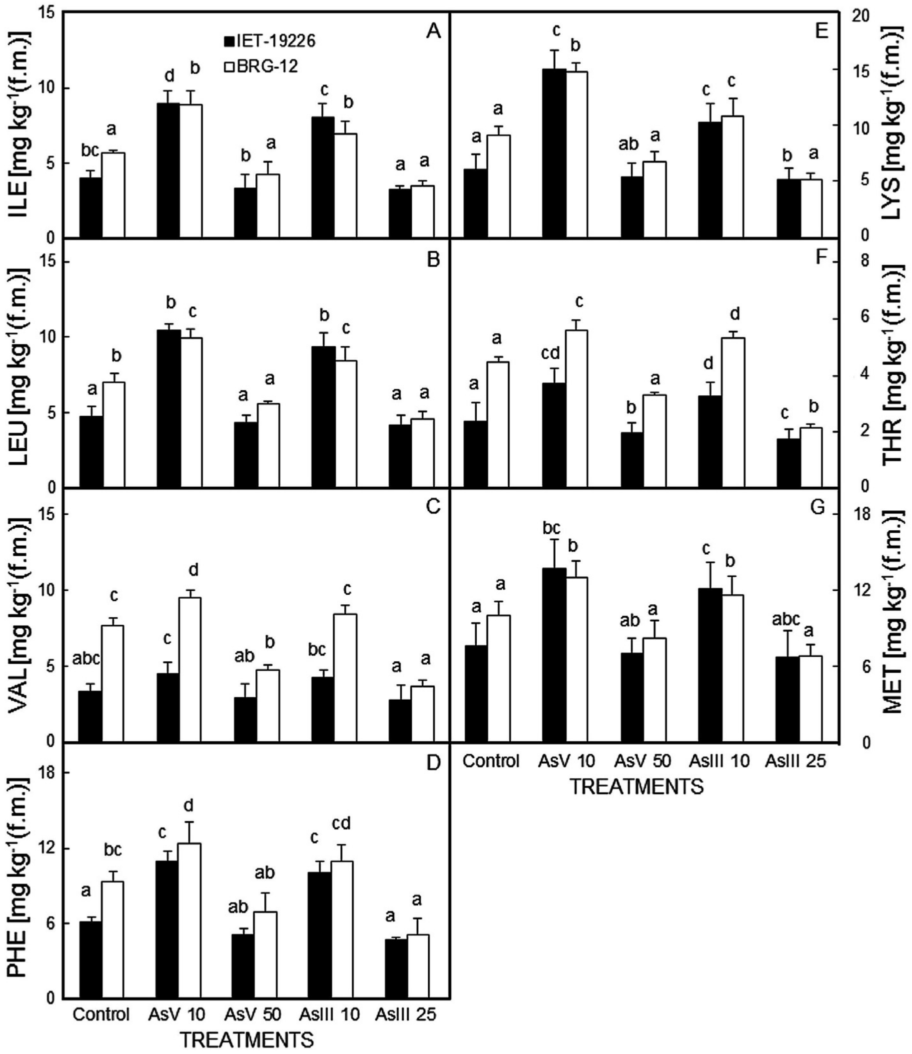

Fig. 2. Effects of As^{V} and As^{III} on essential amino acid content in rice shoots: isoleucine (*A*), leucine (*B*), valine (*C*), phenylalanine (*D*), lysine (*E*), threonine (*F*), and methionine (*G*). Means ± SE (n = 3). Different letters indicate significan differences among As treatments in a particular rice cultivar (DMRT, $P \leq 0.05$).

Table 1. Effects of As^{V} and As^{III} applications on a total As accumulation [mg kg^{-1}(f.m.)] in roots and shoots. Means ± SE (n = 3). Significant differences in individual columns at $P \leq 0.01$ are marked by different letters (DMRT).

Treatments	IET-19226 (LAARG) root	IET-19226 (LAARG) shoot	BRG-12 (HAARG) root	BRG-12 (HAARG) shoot
Control	-	-	-	-
10 µM As^{V}	90.52 ± 11.82^{b}	55.87 ± 6.55^{b}	498.48 ± 39.70^{b}	201.08 ± 22.60^{b}
50 µM As^{V}	161.06 ± 17.47^{c}	89.29 ± 5.57^{c}	1182.33 ± 78.41^{d}	445.38 ± 38.04^{d}
10 µM As^{III}	62.00 ± 8.09^{a}	35.56 ± 3.73^{a}	307.12 ± 29.11^{a}	143.10 ± 13.80^{a}
25 µM As^{III}	101.80 ± 10.72^{b}	55.19 ± 7.89^{b}	663.98 ± 46.24^{c}	252.84 ± 18.34^{c}

concentrations of As^{V} and As^{III}. These differences might be related to different As^{V} and As^{III} uptake mechanisms in different rice genotypes. In the present study, we also observed a higher reduction of root and shoot lengths and of biomass in IET-19226 as compared to BRG-12. The excess of As in roots may disturb the transport of other minerals leading to reduced biomass and plant growth (Dwivedi *et al.* 2010a). These results support the finding that an As exposure reduces plant growth and biomass in dependence on rice genotypes (Tripathi *et al.* 2012a).

Amino acids play a pivotal role in detoxification of heavy metals (Sharma and Dietz 2006). In roots, the content of all EAAs elevated at the lower dose (10 µM) of As^V in both the rice genotypes, but more in IET-19226 as compared to BRG-12. Similarly, Dwivedi *et al.* (2010b) demonstrated that a lower dose of As^V increases the EAAs content in different rice genotypes. In IET-19226, a maximum increase was observed for Leu (116 %) followed by Lys (112 %), Ile (107 %), Met (81 %), Thr (75 %), Phe (75 %), and Val (63 %), whereas in BRG-12, a maximum increase was observed for Ile (35 %) followed by Met (27 %), Val (22 %), Thr (21 %), Leu (14 %), Phe (11 %), and Lys (11 %) at 10 µM As^V as compared to the control (Fig 1*A*-*G*). Contrarily, the higher concentration of As^V (50 µM) decreased the EAAs content in roots of both the genotypes, however, this decrease was lower in IET-19226 than in BRG-12. In IET-19226, a maximum reduction was observed for Val (18 %) followed by Phe (12 %), Ile (10 %), Thr (10 %), and Lys (9 %), however, in BRG-12 a maximum reduction was observed for Val (35 %), followed by Ile (29 %), Leu (26 %), and Lys (22 %). These results validate that the excess of soil As decreases the EAAs content more in HAARGs than in LAARGs (Dwivedi

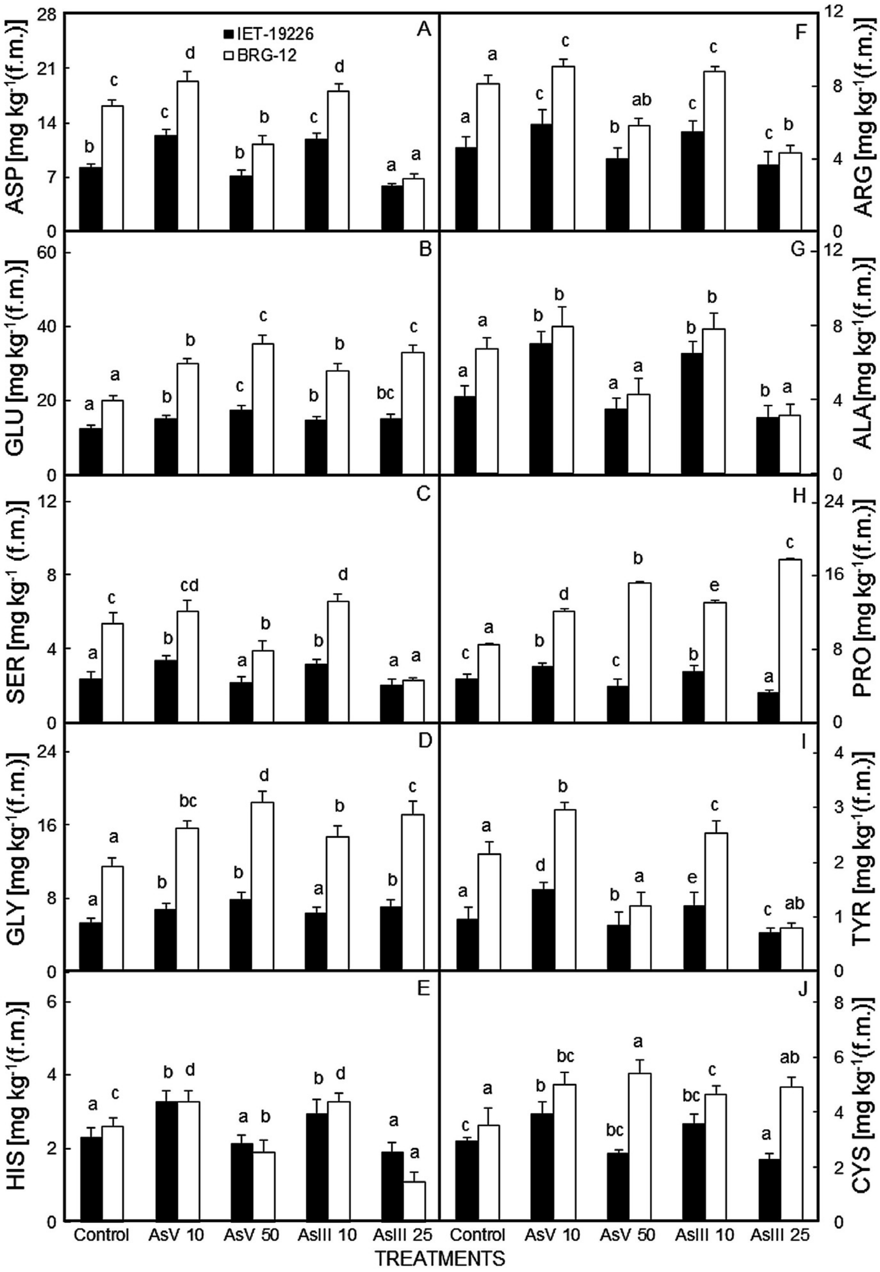

Fig. 3. Effects of As^V and As^{III} on non-essential amino acid content in rice roots: asparagine (*A*), glutamine (*B*), serine (*C*), glycine (*D*), histidine (*E*), arginine (*F*), alanine (*G*), proline (*H*), tyrosine (*I*), and cysteine (*J*). Means ± SE ($n = 3$). Different letters indicate significant differences among As treatments in a particular rice cultivar (DMRT, $P \leq 0.05$).

et al. 2010b, 2012). The effects of As on EAAs in shoots of both the rice genotypes were similar as in roots (Fig 2*A-G*). At 10 µM As^{V}, the maximum elevation of the EAAs content corresponded to a lower As accumulation in IET-19226 than in BRG-12. This increase was 145, 123, 121, and 80 % for Lys, Ile, Leu, and Met in IET-19226, whereas only 62, 57, 41, and 29 % for Lys, Ile, Leu, and Met, respectively, in BRG-12. Conversely, inhibitory effects on EAAs coincided with a higher As accumulation in BRG-12 where the content of Val (38 %), Thr (27 %), Phe (26 %), and Ile (25 %) were significantly reduced at 50 µM As^{V}, and these results are in agreement with previous ones (Dave *et al.* 2013). As^{III} at a lower concentration (10 µM) also increased the EAAs content more in IET-19226 in comparison to BRG-12, whereas at a higher concentration (25 µM), the reduction of the EAAs content was more apparent in BRG-12 than in IET-19226. At a lower dose, significant increases of Leu (66 %), Phe (62 %), Ile (57 %), Thr (54 %), Lys (33 %), Met (30 %), and Val (21 %) were observed in IET-19226 roots, however, only of Leu (8 %), Phe (6 %), Ile (14 %), Thr (18 %), Lys (2 %), Met

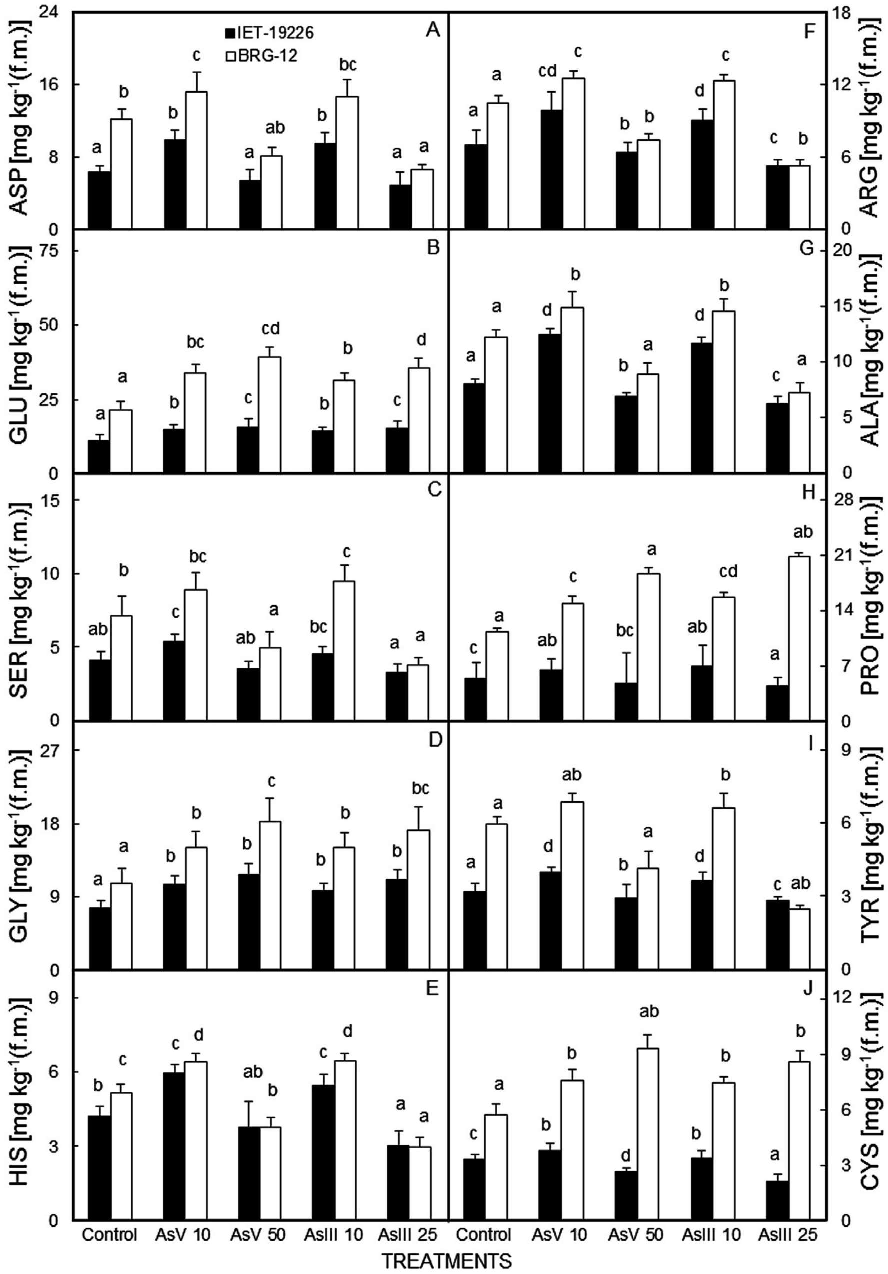

Fig. 4. Effects of As^{V} and As^{III} on non-essential amino acid content in rice shoots: asparagine (*A*), glutamate (*B*), serine (*C*), glycine (*D*), histidine (*E*), arginine (*F*), alanine (*G*), proline (*H*), tyrosine (*I*), and cysteine (*J*). Means ± SE ($n = 3$). Different letters indicate significant differences among As treatments in a particular rice cultivar (DMRT, $P \leq 0.05$).

(19 %), and Val (20 %) in BRG-12 roots which confirm genotypic differences in response to As^{III} exposure found by Tripathi *et al.* (2012b). At 25 µM As^{III} exposure, a reduction in the Ile and Thr content was 58 and 42 %, respectively, in BRG-12 roots, whereas only 12 and 20 %, respectively, in IET-19226 roots. In shoots, the content of Ile at 10 µM As^{III} was 101 and 24 % of the control in IET-19226 and BRG-12, respectively. Among all EAAs, Thr was reduced more in BRG-12 in comparison to IET-19226 at the 25 µM As^{III} treatment.

The content of Ile, Leu, and Val significantly increased by 10 µM As^{III} or As^{V} indicating their response against As stress. The similar observation has been earlier reported by Dwivedi et al (2010b). However, higher doses of As adversely affected the EAAs, suggesting toxicity to plant metabolism, which was also evident by hampered root and shoot growth. The toxicity may be attributed to As-induced production of ROS (Davies *et al.* 1987) which affected AAs synthesis. Phe is a precursor of numerous metabolites, which play crucial roles in plant growth and development (Maeda *et al.* 2011). In the present study, increase in Phe content in roots of IET-19226 (75 and 62 %) and BRG-12 (11 and 6 %) at the equimolar (10 µM) dose of As^{V} and As^{III}, respectively, indicating their responsiveness during As stress. Lys is a nutria-tionally important EAA (Galili 2002), and its enhanced content at the lower doses of As^{V} and As^{III} might be important in stress response (Dwivedi *et al.* 2010b). In contrast, higher dose of As significantly reduced the Lys content which may be due to its catabolism to Glu, as observed in some plants under stress conditions (Galili 2002). A branch of the Asp family pathway also leads to the synthesis of two additional EAAs, Met and Thr (Galili *et al.* 2005). In the present study, it was observed that increased content of Met and Thr at lower doses of As and subsequent increase in Lys supported that it was by product of this pathway. Dwivedi *et al.* (2010b) has also concluded that accumulation of Met is both, As concentration dependent and genotypic specific trait in rice, hence the higher accumulation of As leading to reduced Met content in roots and shoots of HAARG (BRG-12) is in agreement to the earlier findings (Dwivedi *et al.* 2012)

Recent findings indicate that some NEAAs, such as Pro, Cys, Glu, and Gly are induced upon heavy metal exposure and involved in detoxification of As (Tripathi *et al.* 2012b, Dave *et al.* 2013). In this study, the content of most NEAAs (Asp, Ser, His, Arg, Ala, and Tyr) increased in both the genotypes at lower doses and decreased at higher doses (Fig 3*A-J*). In roots and shoots, the content of NEAAs at 10 µM As^{V} was higher in IET-19226 than in BRG-12 indicating a genetic

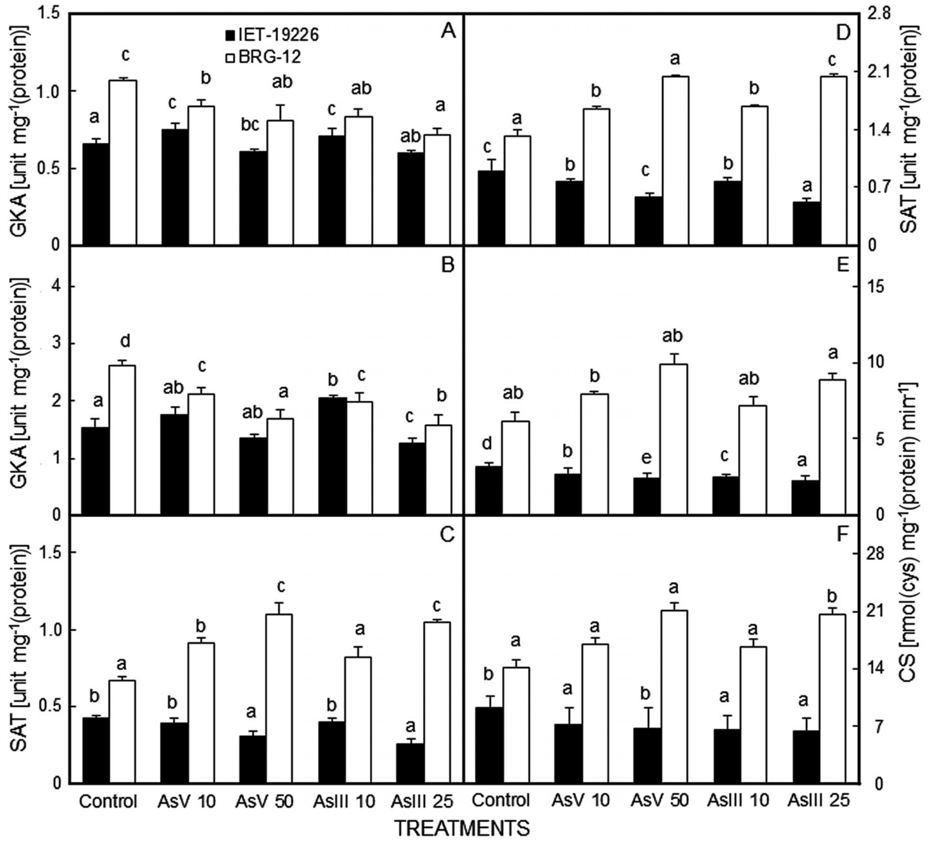

Fig. 5. Effects of As^{V} and As^{III} on assays of proline and cysteine biosynthesis enzymes in rice. GK activity in roots (*A*) and shoots (*B*), SAT activity in roots (*C*) and shoots (*D*), CS activity in roots (*E*) and shoots (*F*). Means ± SE ($n = 3$) ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values among As treatments in a particular rice cultivar (DMRT, $P \leq 0.05$).

difference between the genotypes for NEAAs during the As^{V} exposure. Exceptionally, the content of some stress responsive NEEAs, such as Pro (79 and 65 %), Glu (78 and 82 %), Gly (60 and 72 %), and Cys (54 and 64 %), were higher in roots and shoots of BRG-12 than of IET-19226, respectively. However, the reduction of the Pro (16 and 11 %) and Cys (16 and 21%) content was apparent in IET-19226 roots and shoots, respectively, at 50 µM As^{V}.

In roots and shoots exposed to lower concentrations of As^{III}, the content of a few NEAAs (Asp, Ser, His, Arg, Ala, and Tyr) increased more in IET-19226 than in BRG-12. The higher concentration of As^{III} (25 µM) decreased the content of most NEAAs like Tyr (63 %), Ser and Asp (58 %), His (57 %), and Ala (53 %) in BRG-12 roots, and Asp (29 %), Ala (28 %), and Tyr (26 %) in roots of IET-19226. Though 25 µM As^{III} decreased the contet of Pro (33 %) and Cys (24 %) in IET-19226, it enhanced the content of Pro (110 %), Glu (66 %), Gly (49 %), and Cys (40 %) in BRG-12 roots. Similarly in IET-19226 shoots, 10 µM As^{III} increased the NEAAs content, and 25 µM As^{III} decreased the content of most NEEAs except Glu and Gly. However, the increased content of Pro (84 %), Glu (65 %), Gly (62 %), and Cys (51 %) at 25 µM As^{III} were observed in BRG-12 shoots (Fig. 4*A*-*J*).

Proline is a well-known stress responsive AA predominately synthesised from Glu (Sharma and Ditez 2006). The induction of Pro has been reported in plants including rice during heavy metal stress (Mishra and Dubey 2006, Sharma and Ditez 2006, Dwivedi *et al.* 2010b). In the present study, the Pro accumulation was higher in the As^{V} exposed plants than in the As^{III} exposed ones at equimolar concentrations in both the rice genotypes. The increase in Pro content in the present study was also supported by the increase in the plant growth at lower doses of As. The content of Glu, Cys, and Gly were also enhanced upon the exposure to different As concentrations, especially during the As^{V} exposure indicating their roles for As detoxification through enhanced syntheses of GSH and PCs. The roles of GSH and PCs for As detoxification has been well documented (Srivastava *et al.* 2007, Tripathi *et al.* 2012b). Recently, Cys has also been reported to take part in As complexation (Mishra *et al.* 2013). NEAAs, particularly Asp and His, are known to accumulate in plant tissues under metal stress (Herrera-Rodriguez *et al.* 2007) and play protective and regulatory roles (Sharma and Dietz 2006). The present results demonstrate that the His accumulation was positively correlated with the low As content in the plants. Though Asp synthesised by transamination of oxaloacetate is NEEA, it is also an important precursor of some EAAs (Pavlík *et al.* 2010).

The GK activity increased in IET-19226 roots and shoots at 10 µM As^{V}, however, it decreased at both As^{V} concentrations in BRG-12 by 24 % in roots and 35 % in shoots at 50 µM As^{V} (Fig. 5*A*-*B*). In case of As^{III}, a maximum inhibition was observed in BRG-12 roots (32 %) and shoots (39 %) at the higher As dose (25 µM). The SAT activity increased at 50 µM As^{V} by 48 and 54 % in roots and shoots of BRG-12, respectively, whereas IET-19226 showed a concentration dependent decline in the SAT activity (up to 29 and 36 % in roots and shoots, respectively, at 50 µM As^{V}; Fig. 5*C*-*D*). In the As^{III} exposed plants, a similar response was observed; the SAT activity decreased in IET-19226 roots (39 %) and shoots (43 %), and increased in BRG-12 at 25 µM As^{III}. The CS activity increased in BRG-12 roots (60 %) and shoots (48 %), whereas decreased in IET-19226 roots (14 %) and shoots (28 %) at 50 µM As^{V} (Fig. 5*E*-*F*). As concerns the 25 µM As^{III} exposure, the CS activity increased in roots and shoots (46 and 44 %) of BRG-12, however, it decreased in roots (32 %) and shoots (30 %) of IET-19226.

The first step of Pro biosynthesis from L-glutamate is catalysed by GK (Pavlík *et al.* 2010) and the content of Pro and GK activity are positively correlated in *Catharanthus roseus* (Jaleel *et al.* 2007). Similarly, the GK activity was positively correlated with the Pro content in rice cv. IET-19226 roots (r = 0.982**) and shoots (r = 0.989**), whereas a continuous decrease in the GK activity was noticed in As-treated BRG-12 in contrast to the increased proline content in roots (r = -0.981**) and shoots (r = -0.984**). The activity of GK is regulated in plants *via* a feedback mechanism by a higher concentration of Pro (Vašáková and Štefl 1982). It may be likely that Glu is involved in the PC synthesis for As complexation in BRG-12 (Pavlík *et al.* 2010, Tripathi *et al.* 2012b) which might be further responsible for lowering the GK activity. Free Pro is also released from proteins under As stress (Mishra and Dubey 2006, Dave *et al.* 2013) rather than *de-novo* synthesised. Proline biosynthesis in plants involves not only glutamate pathway but also the other alternative pathway involving arginine/ornithine (Adams *et al.* 1980, Miflin and Lea 1990). In the plant, glutamic-γ-semialdehyde (GSA) is derived directly from ornithine using the enzyme ornithine δ-aminotransferase (δ-OAT) (Kishor *et al.* 2005). In the present study increased Pro content in HAARG (BRG-12) was indicative of prevalent ornithine dependent pathway for Pro biosynthesis, as the glutamate dependent Pro biosynthesis pathway involving GK was inhibited during As stress in rice.

The Cys biosynthesis is catalyzed by SAT and *O*-acetylserine (thiol) lyase (OAS-TL) (Wirtz *et al.* 2001). In the present study, the SAT and CS activities were positively correlated with the content of Cys in roots (r = 0.917*, 0.904*, respectively) and shoots (r = 0.977*, 0.971*, respectively) of BRG-12. However in IET-19226, the Cys content increased at lower As doses despite a non-significant decline in the SAT and CS activities with the As accumulation in roots (r = -0.748^{NS}, r = -0.808^{NS}) and shoots (r = -0.796^{NS}, r = -0.769^{NS}). Similarly to the present investigation, genotypic variations in SAT and CS activities has been reported in *Brassica juncea* and rice upon As exposure (Srivastava *et al.* 2009, Tripathi *et al.* 2012b).

In summary, the present findings indicte the modulation of various AAs and their metabolic pathways during the As exposure in rice. An increase in AAs content was observed in both the genotypes at 10 µM As^{V} and As^{III}, however, a decline at higher As doses. It is concluded that some NEAAs, such as Pro, Glu, Gly, and Cys played a role as As detoxifying metabolites. The enzymes of the Pro and Cys metabolic pathways responded differentially to the As exposure. The total As content significantly increased in both the rice genotypes during the As exposure and the As accumulation was more pronounced in LAARG (IET-19226) than HAARG (BRG-12). Thus, human consumption of LAARG grown in As contaminated paddy fields may decrease As exposure without compromising the amounts of EAAs.

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