DOI: http://dx.doi.org/10.47829/JRS.2020.1202

ISSN: 2690-5183

Journal of Rice Science

Research Article

Differential expression of As^{iii} uptake, Antioxidant System, and As^{iii}-Conjugating Compounds of Two Iranian Rice Cultivars Adapted With Two Different Climates

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Volume 1 Issue 2 - 2020

Received Date: 07 Dec 2019

Accepted Date: 30 Dec 2019

Published Date: 06 Jan 2020

2. Keywords

Aquaporin; Antioxidant system; Arsenite; Rice; Thiol compounds

1. Abstract

Arsenite (As^{III}) is a highly toxic inorganic and the dominant species of arsenic in anaerobic and paddy soils. Although arsenic is adventitiously absorbed via several types of transporters, distinct strategies are employed by various plant species in order to either reduce the accumulation or increase its detoxification. In the present study, the response of the rice plant to As^{III} was compared in two indigenous cultivars ;one adapted to amild and humid climate (Oryza sativa Indica cv. Hashemi) and the other one adapted to a dry and hot climate (O. sativa Indica cv. Amber). Differential responses of the roots of both cultivars in terms of the rate of As^{III} uptake and its translocation to shoots were evaluated as well. Twenty days old plants were exposed to 75 µM As^{III} for 6 hours. During the treatment, the levels of aquaporin transcripts OsNIP 2;1 (LSI1), OsPIP1;3, and OsPIP2;6 significantly decreased in both cultivars. A more prominent reduction of aquaporin transcripts was observed in the Amber cultivar and was accompanied by a lower rate ofAs^{III} uptake as compared to the other cultivar. The enhanced activity of antioxidant enzymes i.e., superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase observed in both cultivars indicate their attempts to protect against As^{III}-induced oxidative damage. These antioxidant responses were more pronounced in the Amber cultivar and were accompanied by remarkably enhanced levels of reduced glutathione (a non-protein thiol) and phytochelatin, particularly in shoots. Based on the data presented here, it is likely that long-term adaptation of Amber cultivar to hot and dry climate has driven genomic changes resulting in As^{III} sequestration and other protective strategies against As^{III} toxicity.

3. Introduction

Arsenic (As) is one of the most harmful toxic metalloids that is naturally present in many soils and ground waters and also deposited in the environment due to human industrial activities. In flooded paddy soils and rice fields, As is reduced from arsenate (As^{V}) to arsenite (As^{III}) which is the most toxic inorganic species of As. Therefore, crops such as rice (Oryza sativa) are the main As^{III}

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Citation: Ghanati F, Differentialexpression of As^{iii} uptake, Antioxidant System, and As^{iii}-Conjugating Compounds of Two Iranian Rice Cultivars Adapted With Two Different Climates Journal of Rice Science. 2020; 1(2): 1-9.

entrance to the human food chain [24]. A general observation is that As uptake systems are adventitious [13]. Most plants would prefer not to accumulate As, but it enters cells via uptake systems for other nutrients and minerals such as aquaporins (AQPs). In rice plants, the efficient Si uptake pathway allows inadvertent passage of As^{III} due to their chemical similarity; they both exist as neutral species in paddy soils, i.e., arsenous acid-$As(OH)_3$ and silicic acid-$Si(OH)_4$ [20]. Recent studies have shown that As^{III} is taken up through silicon transporters e.g., Lsi1 (OsNIP2;1) which is the major entry route of As^{III} into rice roots [24, 25]. Other NIPs including OsNIP1;1, OsNIP2;2, OsNIP3;1, and OsNIP3;2 also show permeability to As^{III} [3]. Moreover, plasma membrane intrinsic proteins (PIPs, another subfamily of the AQPs), including OsPIP2;4, OsPIP2;6 and OsPIP2;7, are also involved in As^{III} transport [25].

Arsenic is not only nonessential element for plants, but also adversely affects their growth and development. Damage of cell membranes, reduction of transpiration intensity, and increased production of Reactive Oxygen Species (ROS) are major consequences of As toxicity to plants. Oxidative stress interrupts the balance between oxidizing reactions and antioxidants and leads to oxidation and modification of cellular amino acids/proteins, and ultimately cell death [32]. Oxidative stress is combatted by plants through a well-developed enzymatic and nonenzymatic antioxidant system. Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR) are the most important enzymatic antioxidants with different affinities to their substrates. Ascorbate, α-tocopherol, and reduced glutathione (GSH) are well known representatives of non-enzymatic antioxidants. Meanwhile, GSH and another sulfur-containing ligand, phytochelatin (PC) are able to detoxify As via conjugationand subsequent transportation of As into vacuoles by ABC transporters [31, 32].

To the best of our knowledge, the differential uptake and detoxification strategies of rice cultivars from different habitats after a short exposure to a high concentration of As^{III} have not been studied yet. In the present study the response to As^{III} exposure was evaluated during a 6h treatment of two Iranian rice cultivars; Hashemi which is adapted to amild and humid climate, and Amber which is adapted to a dry and hot climate. Harmonized strategies from reducing As^{III} uptake by increasing its conjugation accompanied by improvement of antioxidant system in restoring redox stability of rice plants are discussed.

4. Material and Methods

4.1. Plant materials, As^{III} Treatment and Determination of its Content

Seeds of two rice indigenous rice cultivars; Oryza sativa Indica cv. Hashemi and O. sativa Indica cv. Amber were used in this study. Hashemi is a popular local rice variety characterized by high yield potential and long kernels, cultivated in the north of Iran with a moderate-humid climate, whereas Amber is cultivated in southern-west of Iran with a very warm, dry, long summers and moderate winters [34]. The seeds were surface sterilized by immersing in 1% sodium hypochlorite (NaClO) solution for 5 min followed by rinsing with deionized water three times. The seeds were then allowed to germinate between two layers of moistened culture paper for seven days. Uniform seedlings were selected and transferred to aerated hydroponic cultures containing Kamachi nutrient solution [19], pH 5.5. The plants were grown in growth chamber with a 16 h light/8 h dark photoperiod, 24±2°C, relative humidity 60% and 107 μmol $m^{-2}s^{-1}$PPFD (Photosynthetic Photon Flux Density). Nutrient solutions were renewed every 3 days. Arsenite was supplied in form of sodium arsenite (Na_2HAsO_2). A preliminary study was conducted applying different concentrations from 0 to 250 μM of As^{III}, and the plant growth was monitored in a time course manner. Based on the results of this study, 75 μM was selected as a concentration at which the plant growth was inhibited. Higher concentrations were severely detrimental leading to death of the plants after 12h treatment. Therefore, twenty days old plants were treated with 75μM As^{III} for 6h. The plants were harvested at different intervals, thoroughly washed with deionized water, gently blotted, frozen with liquid N_2, and stored at -80°C untilthey were used for biochemical analysis.

Total arsenic concentrations in plant samples were determined by ICP-MS following closed-vessel microwave digestion. In brief, the samples were weighed into digestion tubes and digested in high purityconcentrated nitric acid (HNO_3, 65%) and hydrogen peroxide (H_2O_2, 30%) at a temperature up to 200˚C. The samples were then diluted and analyzed using an ICAP Q (Thermo Fischer Scientific, Waltham, USA) with a FAST sample introduction system (Elemental Scientific, Omaha, USA). Calibration was performed using single element standard solutions and a 1 ppb in solution was used as internal standard. Analysis was performed in both standard and collision (i.e. Kinetic Energy Discrimination, KED) modes, using helium as a collision gas for the latter. Comparison of standard and collision modes demonstrates that potential

Table 1: The content of thiol containing compound of rice plants before and after exposure to 75 μMAs^{III}.

		GSH	NPT (μmol/g FW)	GSH/GSSG	PC
Root	Control	4.09±0.1^{d}	22.45±1.2^{d}	13.53±0.3^{c}	18.36±1.2^{c}
	1 h	4.89±0.2^{b}	27.68±0.8^{c}	14.82±0.2^{b}	22.79±1.6^{b}
	3 h	6.91±0.2^{a}	36.23±0.8^{b}	18.67±0.3^{a}	29.32±1.2^{a}
	6 h	4.52±0.2^{c}	40.23±1.4^{a}	10.71±0.4^{d}	32.41±1.4^{d}
Shoot	Control	6.22±0.1^{c}	62.24±1.3^{d}	16.19±0.2^{c}	56.02±1.2^{c}
	1 h	6.91±0.1^{b}	66.25±1.6^{c}	17.08±0.3^{b}	59.34±1.8^{b}
	3 h	9.14±0.3^{a}	74.59±2.0^{b}	19.95±0.3^{a}	65.45±2.0^{a}
	6 h	6.33±0.2^{c}	92.18±2.8^{a}	11.20±0.2^{d}	81.48±3.4^{d}

Data are presented as mean ±SD, n=3. Bars with different letters in each graph are significantly different at p ≤ 0.05 according to LSD test, GSH; glutathione, GSSG; non-reduced glutathione; NPT; non-thiol proteins, PC; phytochelatin

Table 2: The content of certain amino acids in rice plants before and after exposure to 75 μMAs^{III}.

		Glu	Gly	Cys	Pro
		μg/g FW			
Root	Control	45.40±0.7^{b}	25.73±1.3^{b}	28.04±0.8^{b}	17.02±0.4^{d}
	1 h	42.19±1.2^{c}	24.23±2.2^{b}	24.33±1.3^{c}	19.23±0.6^{c}
	3 h	66.14±2.0^{a}	40.32±1.8^{a}	41.23±0.7^{a}	25.23±0.4^{b}
	6 h	35.03±0.9^{d}	20.32±0.9^{c}	16.01±0.9^{d}	31.23±0.6^{a}
Shoot	Control	57.60±0.7^{d}	55.44±1.7^{c}	25.46±1.2^{c}	23.27±0.8^{d}
	1 h	59.66±1.1^{c}	62.10±1.1^{b}	30.33±0.6^{b}	25.23±0.5^{c}
	3 h	70.21±0.8^{a}	66.84±0.8^{a}	36.71±0.9^{a}	30.25±03^{b}
	6 h	65.81±2.1^{b}	63.42±1.6^{b}	31.57±1.3^{b}	32.62±0.6^{a}

Data are presented as mean ±SD, n=3. Bars with different letters in each graph are significantly different at p ≤ 0.05 according to LSD test.

interference from ArCl on ^{75}As is not an issue for these samples. Concentrationsobtained using the KED mode were used in this study.

4.2. ROS and Antioxidant Assay

The samples were extracted with 50 mM Na-Pi buffer. The measurement of hydroxyl radical (OH•) was conducted using 2-deoxyribose. This compound is highly sensitive to degradation by OH• radicals, and the resulting compounds react with hiobarbituric acid (TBA) [35]

Determination of hydrogen peroxide (H_2O_2) was conducted based on the reaction of the sample extract with Trichloracetic Acid

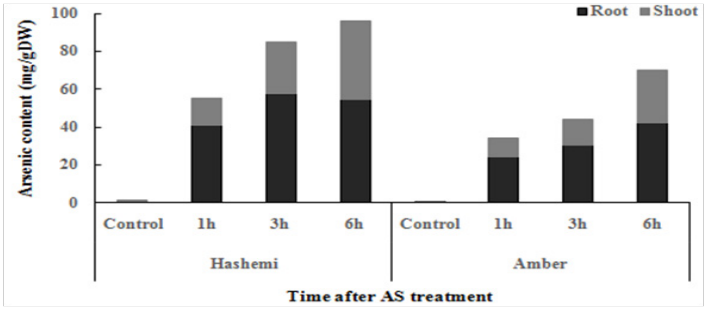


Figure 1: Arsenite content of rice cultivars, Hashemi and Amber afterexposure to 75 μM As^{III}for 0, 1, 3, and 6h. Data are presented as mean of three independent experiments with 20 plantseach. The standard deviations are not shown, however their ratios to corresponding mean values were less than 4%.

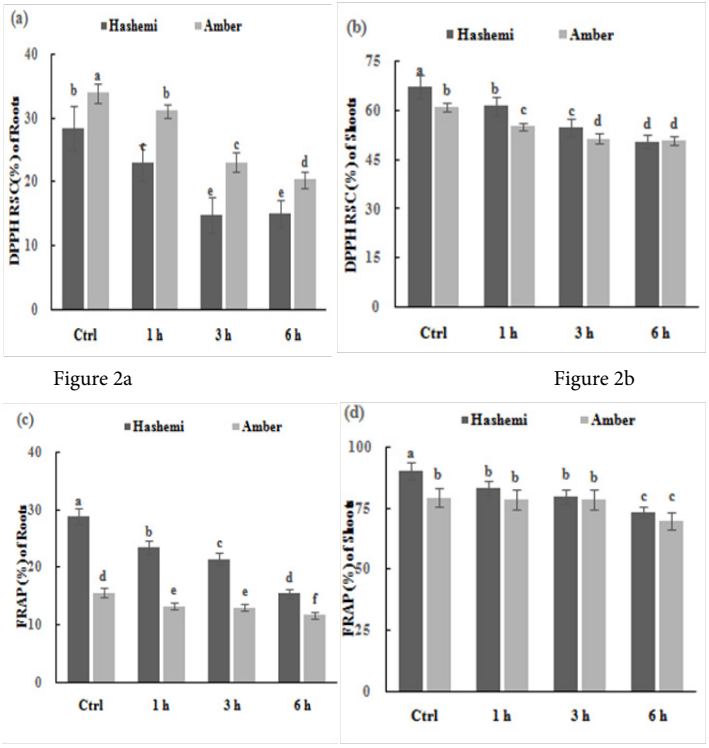


Figure 2a Figure 2b

Figure 2c Figure 2d

Figure 2: Radical scavenging and ferric ion reducing potential of two rice cultivars, before and after exposure to 75 μM As^{III}. Data are presented as mean ±SD, n=3. Bars with different letters in each cultivarshowsignificant different at p ≤ 0.05 according to LSD test.

(TCA) in the presence of KI. Absorbance of the mixture solution was read at 390 nm by a double beam spectrophotometer (Cintra 6, GBC, Dandenong, Vic., Australia) [29].

Ferric ion Reducing Antioxidant Power (FRAP) of sample extracts was determined based on oxidation-reduction of potassium ferricyanide and ferric chloride in the presence of TCA. Ascorbic acid was used as a positive control [27].

Free radical scavenging capacity of samples extracts was determined using the stable 2.2'diphenylpicrylhydrazyl radical (DPPH). Ascorbic acid (10 mgmL^{-1}) was used as a positive control [2].

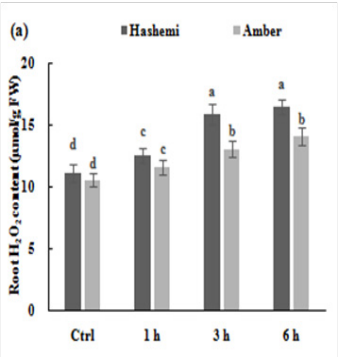
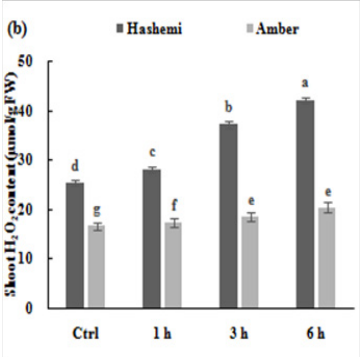


Figure 3a



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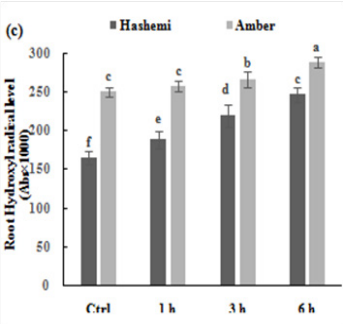

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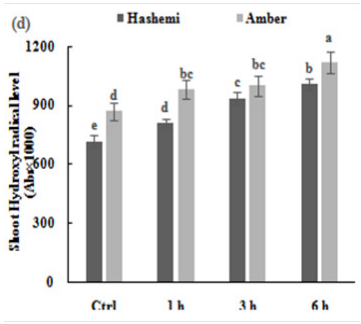

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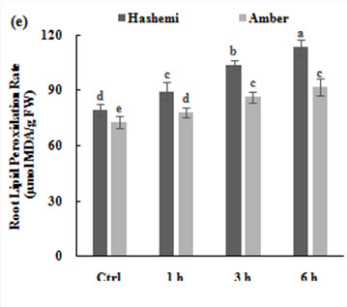

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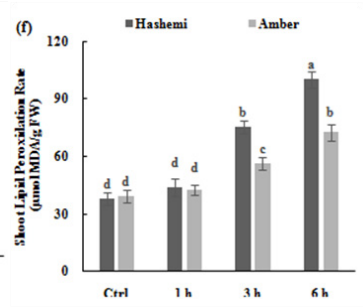

Figure 3f

Figure 3: Effect of As^{III} on H_2O_2 and hydroxyl radical contents and membrane lipid peroxidation rate of Hashemi and Amber rice cultivars before and after treatment with 75 µM As^{III}. Data are presented as mean ±SD, n=3. Bars with different letters in each cultivarshow significant different at $p \leq 0.05$ according to LSD test.

For determination of antioxidant enzymatic activities, the samples were extracted with HEPES-KOH buffer (50 mM, pH 7.8) containing 0.1 mM EDTA. Protein content of the extract was measured by [5]. The activity of superoxide dismutase was determined based on the inhibition of reduction of nitroblue tetrazolium chloride (NBT) and production of formazan. One unit of SOD activity was defined as the amount of enzyme that resulted in 50% inhibition of the rate of NBT reduction at 560 nm. The activity of catalase was monitored by measuring H_2O_2 consumption and decreasing of absorbance at 240 nm. Peroxidase activity of the sample extract was determined by measuring oxidation rate of guaiacol in the presence of H_2O_2 at 470nm. The activity of APX was monitored by oxidation of ascorbate as a specific substrate and the

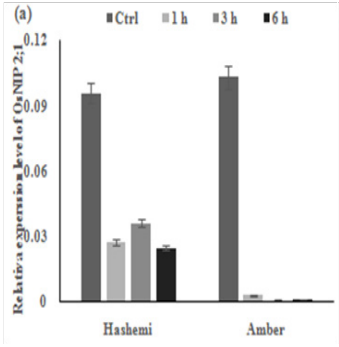

Figure 4a

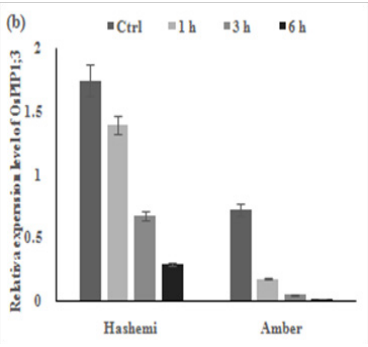

Figure 4b

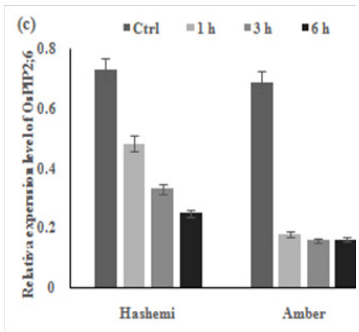

Figure 4c

Figure 4: Expression levels of OsNIP2;1 (a), OsPIP1;3 (b), and OsPIP 2.6 genes in roots of Hashemi and Amber rice cultivars. Data are presented as mean ±SD, n=3. Bars with different letters in each cultivarshow significant different at $p \leq 0.05$ according to LSD test.

decrease in absorbance at 290 nm. The rate constant was calculated using the extinction coefficient of 2.8 mM^{-1} cm^{-1}. Glutathione reductase activity was determined following oxidation of NADPH at 340 nm in 1 mL of reaction mixture containing 100 mM Na-phosphate buffer (pH 7.8), 2 mM EDTA, 0.2 mM NADPH and 0.5 mM oxidized glutathione (GSSG) [28].

In order to measure GSSG and GSH contents of the samples, ophthaldialdehyde (OPA) was added to the extract and the absorbance was read by a fluorescence spectrophotometer (Perkin Elmer LS55, USA) with excitation at 350 nm and emission at 420 nm (Hissin and Hilf 1976). Non-Protein thiols (NPTs) content was measured according to the method of [11]. The content of phytochelatin was calculated as PCs=NPT – (GSH + GSSG) [10]. Peroxidation of membrane lipids was measured by measuring complex of TBA with malondialdehyde (MDA) at 532nm. Corrections were used for the interference of complexation of TBA with non-specific substrates at 440 and 600nm. An extinction coefficient of 157 mM^{-1} cm^{-1} was used [9].

4.3. Determination of Amino Acids

The samples were extracted with EtOH 80% followed by

centrifugation, evaporation of the supernatant, and adding full name (OPA). Amino acids were eluted on a Prevail C18 column (250 mm × 4.6 mm I.D., 5 µm, Alltech) using HPLC system (Agilent Technologies 1260, CA, USA), equipped with a FLD HP 1100 detector. Mobile phase was composed of 25 mM Na-pi containing 35% MeOH and 15% acetonitrile. Fluorescence detection and quantification were carried out by excitation at 230 nm and emission at 460 nm [4]. For measurement of proline content, the samples were extracted with 3% sulfosalicylic acid. Acetic acid and ninhydrin were added to the supernatant and boiled for 1 h and the absorbance was read at 520 nm [1].

4.4. Quantitative Expression Analysis of As Transporters

In order to determine the role of AQPs in As^{III} uptake, quantitative RT–PCR (qRT–PCR) was performed and the changes in transcripts of OsNIP 2;1 (LSI1) and OsPIP1;3, and OsPIP2;6 (as representative members of PIP1 and PIP2) were analyzed in rice roots. Total RNA was isolated using Hybrid-R™ kit (Cinna Gen, Iran) following the manufacturer's instructions. Reverse transcription was performed to synthesize cDNA by using the RT Kit. Specific primers were designed on the basis of sequence information obtained from NCBI for Oryza sativa as follows: OsPIP1;3 forward primer: CTGGTGATCGATGAAGCTAG; reverse primer: ACACAAGTACCATTTCTCACAC; OsPIP2;6 forward primer: GCCAGGTGCATGATTTGTT; reverse primer: GCCGAAGCAGTTTGTATCTC; OsNIP 2;1 forward primer: GCCAGCAACAACTCGAGAACAA; reverse primer: CATGGTAGGCATGGTGCCGT. Quantitative real time qRT-PCR was carried out in ABI step one real-time PCR detection system using SYBR-Green. Relative expression level was calculated using $2-\Delta\Delta CT$ method [18]. Rice Beta actin was used as an internal control to normalize genes expression.

4.5. Statistical Analysis

All experiments were carried out three times, each of them using at least three plant, and all data are expressed as the mean values ± the Standard Deviation (SD). Statistical analysis was performed using LSD (Least Significant Difference), and the differences between the treatments were expressed as significant at a level of $p \leq 0.05$.

5. Results

5.1. Arsenite Uptake

Rice plants were exposed to 75 mM AsIII during 6h and harvested at different time points. Negligible amount of As^{III} was found in the rice seeds and were transferred to the seedlings before any treatment (Figure 1). During the treatment, As^{III} was absorbed reaching maximal levels of 96 and 70 µg/g DWin Hashemi and Amber, respectively (Figure 1). A major portion of the absorbed As^{III} was found in the roots and less in the shoots through out the treatment, indicating that both cultivars may use strategies to prevent as translocation to the upper part of the plant. Nevertheless, after 6h of treatment, an almost even amount of as was found through out the plant, suggesting that the rate of As^{III} translocation from roots to shoots may be higher in Hashemi.

5.2. Antioxidant Parameters and As^{III} Detoxifying Agents

The DPPH radical scavenging capacity (DPPH RSC) of shoots was overall higher than of roots in both examined cultivars (ca. 2.5-3 folds) (Figuare 2a-b). Exposure to As^{III} significantly reduced the DPPH RSC of roots to 53% and 75% of their corresponding controls in Hashemi and Ambers, respectively, (Figure 2a). Arsenic treatment also reduced the DPPH RSC of shoots, but again the reduction was more pronounced in Hashemi than in Amber (Figure 2b). A remarkable reduction was observed in FRAP of rice roots after exposure to As^{III} (54% and 23% of the controls in Hashemi and Amber, respectively, at 6h) (Figure 2c). The FRAP was also reduced in shoots of As^{III}-treated plants albeit with a much moderate tendency. Although the FRAP of shoots of both cultivars after As^{II} treatment were almost identical, again shoot FRAP reduction was more prominent in Hashemi than Amber (20% and 11% of the controls in Hashemi and Amber, respectively, at 6h) (Figure 2d).

The initial activity of SOD was lower in Hashemi roots than in Amber (Table 1). Treatment with As^{III} significantly increased SOD activity of roots in both cultivars, and the rate of increase was found more pronounced in Amber. The initial activity of CAT was identical in both cultivars, but the arsenite treatment increased it up to 2.8 and 2 folds of their control levels in roots and shoots of Hashemi, respectively. In Amber, CAT activity increased up to 3.3 and 2.3 fold of their corresponding controls in roots and shoots, respectively.

The activity of GR in both cultivars was similar before treatment with As^{III}. Exposure of plants to As^{III} significantly increased GR activity of roots and shoots in both cultivars, although the rate of increase was more pronounced in Amber. The initial activity of POD was higher in roots of Hashemi than of Amber. Treatment with As^{III} significantly enhanced POD activity in both cultivars, but

the rate of increase was more prominent in Amber. An increase of about 2-3 folds of the controls was observed in the activity of APX of both cultivars after exposure to As^{III}, but the rate was found higher in Amber.

The content of GSH and the GSH/GSSG ratio significantly increased in both roots and shoots of Hashemi upon exposure of the plants to As^{III} up to 3h of the treatment (Table 2). The same tendency was observed in the content of GSH and the GSH/GSSG ratio of Amber but with a more prominent rate which persisted toward the end of treatment.

Exposure to As^{III} significantly and continuously increased the contents of PC and NPT during the treatment in both rice cultivars. It was noticeable however, that the rates of enhancement of PC and NPT were more pronounced in shoots than roots and in particular of Amber than Hashemi.

In comparison with the control condition, exposure to As^{III} increased H_2O_2 content of roots of Hashemi and Amber to 149% and 135% of their control, respectively (Figure 3a). The rate of increase of H_2O_2 content of shoots was more outstanding in Hashemi than in Amber (Figure 3b). Despite that the initial content of $OH^{\bullet}$ in roots and shoots of Hashemi was lower than in Amber, the rate of its increase upon As^{III} treatment was more prominent (Figure 3. c,d). The rate of membrane lipid peroxidation of root sand shoots of Amber, particularly after 6h exposure to As^{III} was remarkably lower than in Hashemi (Figure 3. e,f).

Among all detected essential amino acids of rice plants, the alterations of Glu, Cys, Gly, and Pro were more prominentin As^{III}-treated plants relative to the amino acid levels in control plants. The Pro content of both cultivars increased during the period of exposure to As^{III} (Table 3). In Hashemi cultivar the highest contents of Glu, Cys, Gly were observed 3h after As^{III} treatment whereas in Amber cultivar the increase of aforementioned amino acids was more pronounced and lasted for 6h (Table 3).

5.3 Expression Level of As Transporters:

Quantitative RT–PCR analysis of AQPs as putative As transporters showed that under control conditions, the expression levels of OsNIP2;1 and OsPIP2;6 were almost identical in the roots of both cultivars, whereas the expression level of OsPIP1;3 was about 2-foldhigher in Hashemi compared to Amber (Figure 4). 1 hour of arsenite treatment resulted in reduced expression level of OsNIP2;1to 26% and 1.4% of the corresponding control levels in Hashemi and Amber, respectively (Figure 4a). At the 6h of the treatment the expression of OsPIP1;3 was only 4% and 2.5% of their corresponding control levels in Hashemi and Amber, respectively (Figure 4b). Duringthe As^{III} treatment, transcripts of OsPIP2;6 gradually decreased in Hashemi roots to 34% of the control, whereas in Amber they decreased to 24% of the control at 1h and did not significantly change for the rest of the treatment period (Figure 4c).

6. Discussion

Arsenite was easily and rapidly absorbed by both examined rice cultivars and accumulated more in roots than in shoots. Involvement of Lsi1 (OsNIP2;1), OsPIP1;3, and OsPIP2;4 in transport of As^{III} into the rice plant have previously been shown [20, 25]. Diminished expression of these genes, although to some extentmay be resulted from down regulated metabolism, indicates that the plants attempt to restrict As^{III} uptake. Regulation of as uptake systems to restrict influx of this toxic metalloids has been reported previously in yeast and mammalian cells [16, 13, 37].

The highest rate of down regulation was observed forOsNIP2;1 as compared to OsPIP1;3 and OsPIP2;4, and was more prominent in the Amber cultivar. The observed down regulation of OsNIP2;1in amber was accompanied bya 30% lower As^{III} accumulation in this cultivar than in Hashemi. Expression of other NIPs e.g., OsNIP1;1, OsNIP2;2, OsNIP3;1, and OsNIP3;2 to As^{III} was not examined in the present work but should not be overlooked [3, 20]. It has been suggested that PIP1 functions more in water conductance than in solute transport, and this function is modulated by heteromerization with PIP2 [38, 36]. This hypothesisis supported by the observation that the rate of reduction in OsPIP2;4 expression was much lower than for other examined AQPs. Interestingly, no significant change was observed in OsPIP2;4 expression in amber between 1 to 6h of As^{III} treatment, resulting in a higher water content (66% of control) as compared to Hashemi (49% of control, data not shown).

Plant root is the first organ which encounters As^{III}, accumulates it and expresses its toxicity [8]. A significant correlation has been reported between the reduced growth attributes raised from As toxicity and the as accumulation rate. The total As^{III} accumulated in Amber cultivar was about 70% of Hashemi.

One inevitable toxic effect of as for plants is over production of ROS that adversely affects plant metabolism. A clear relationship between as stress, redox homeostasis and antioxidant capacity has been reported [30]. Exposure to As^{III} significantly reduced DPPH

and FRAP of both cultivars, indicating As^{III}- induced oxidative stress and the rate of their reduction was more outstanding in roots than shoots. Superoxide anions ($O_2^{\bullet-}$) are the first destructive ROS which are produced.Catalytic dismutation of $O_2^{\bullet-}$ to H_2O_2 and water is carried out by SOD and serves as a source for production of other species i.e., $OH^{\bullet}$ and H_2O_2. The latter is relatively long-lived (1ms) and therefore more dangerous than other ROS. Increase of SOD activity in plants must be followed by increased activity of enzymes that are responsible for H_2O_2 elimination, such as CAT, APX, and POD [30]. Although all H_2O_2 scavenger enzymes act in a cooperative or synergistic way, it is more likely that CAT effectively eliminates H_2O_2, thereby regulates the activity of APX [14]. The latter can scavenge H_2O_2 that is not removed by catalase and requires a specific reductant with a higher affinity for H_2O_2 (ascorbate), allowing for the scavenging of small amounts of H_2O_2, in more specific locations (shoots). This explains why the most elevatedrate of CAT activity was observed in roots, while of APX was detected in shoots of As^{III}- treated plants. Glutathione reductase is another H_2O_2 removing enzyme which functions in ascorbate-glutathione cycle using GSH as a specific substrate. Besides, the enzyme catalyzes the reduction of GSSG to GSH. Glutathione has important functions as a scavenger of O_2^{-}, H_2O_2 and OH• to maintain the reduced state of plant cell [26] . Glutathion also contributes to As detoxification since it is a building block of PC and since GSH can directly bind to As^{III} i to form the as(GS)3 complex it is well-established that not only the pool of GSH but also the GSH/GSSG ratio (i.e., high levels of GSH but lower level of GSSG) are important to maintain the redox status of the cell, mean while may modulate some pathways including synthesis of glutathione itself. A comparison between the two examined rice cultivars clearly showed higher GSH and GSH/GSSG in amber. Altogether, the results reveal the high potential of the amber cultivar to reduce As^{III} uptake, minimize oxidative damage, and maintain redox homeostasis.

It is note worthy however that not only transporters and redox enzymes, but alsobiotrans formations and biosynthetic pathways for conjugation of arsenic are critical determinants of As resistance [17, 6]. Among arsenic species, As^{III} has a high affinity to the thiol (-SH) group of peptides and proteins. Plants form As^{III} complex with S-containing ligands to diminish the free As^{III} concentration and transport the As^{III}-thiol complexes into vacuoles through ABC transporters [7].

The most prominent conjugated as forms include as $(GS)_3$, (GS) As^{III}-PC_2, As^{III} -PC_3, and As^{III}-$(PC2)_2$ [13]. A positive correlation was observed between the duration of exposure of rice cultivars to As^{III} and the amount of NPT, GSH and PCs. It is accepted that despite the sequestration of as in the vacuoles within root cell, still an appreciable amount of arsenic can be transported to rice shoots [23]. Remarkably high amounts of PC were found in shoots of amber during as treatment, emphasizing again that besides lower as uptake, this cultivar has the potential to efficiently detoxify a sin shoots and rescue photosynthetic apparatus.

Another positive correlation was observed between GSH, PC, and their amino acid precursorsi.e., Glutamic acid (Glu), Glycine (Gly), and Cysteine (Cys). Glutamic acid is also used to produce Proline (Pro) via a phosphorylation reaction catalyzed by glutamate kinase. In agreementwith previous reports [22], the content of Pro significantly increased after exposure of rice plants to As^{III}. Different functions have been attributed to Pro accumulated under various abiotic stresses i.e., regulation of osmotic potential and redox state, and scavenging of ROS [33]. From the results presented here, it seems that under As^{III} stress glutamate kinase activity is regulated through a feedback mechanism by the content of Pro. An increased level of free Pro causes allosteric regulation of glutamate kinase activity to inhibit excessive production of Pro and the preferred utilization of Glu for GSH and PC synthesis [12].

7. Conclusion

Altogether, the results presented here show that or chestrated functions of minimizing As^{III} uptake via downregulated expression of transporter genes in addition to the enhanced redox enzymatic activities, and improved conjugating metabolites are employed by the amber cultivar to overcome As^{III} toxicity for the plant. The high ability to sequester As^{III} provides the plant with reduced As^{III} translocation to grains, there by decreases the risk associated with rice consumption as a staple food. Amber is cultivated in habitats with very warm, dry, and long summers which arethe characteristics of semiarid and arid lands. The results presented here suggest that long-term adaptation of amber cultivar to hot climate has driven genomic changes resulting in As^{III} sequestration and other protective strategies against As^{III} toxicity. Amber cultivar can be therefore suggested not only as a good candidate for further molecular investigations on As^{III} transport system, but also for sustainable development of agricultural programs in As-contaminated soils.

8. Acknowledgments

The authors would like to thank Tarbiat Modares University

(TMU) for providing the laboratory facilities and financial support for this project.

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