MINERAL AND BIOCHEMICAL RESPONSE TO ARSENIC-INDUCED STRESS IN INDICA RICE CULTIVARS

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INTRODUCTION

Environmental stress is a driving force in evolution. In this view, arsenic (As) exposure over billions years has led organisms to different adaptive processes (Oremland et al., 2009). Plants have developed sophisticated mechanisms to perceive different environmental stresses and activate specific tolerance mechanisms. However, anthropogenic activities have released large amounts of As into the environment during a short time, which has resulted in food chain contamination and plant phytointoxication (Tripathi et al., 2007).

To mitigate As stress, plants may modulate pathways to maintain a minimal cellular concentration of free metalloid ions via thiol-mediated complexation. In particular, S-rich metal-binding peptides, such as glutathione (GSH) and phytochelatins (PCs), are synthesized in response to As stress and provide tolerance to plants via the effective complexation of As (Gupta et al., 2013).

Plant responses to As toxic levels and the As accumulation profile vary among plant species as well as among genotypes of the same species. Most studies on factors contributing to As accumulation and tolerance in plants have focused on soil conditions, especially pH (Carbonell-Barrachina et al., 1999) and mineral elements, such as nitrogen, phosphorus (Signes-Pastor et al., 2007), silicon (Guo et al., 2005), and sulfur (Hu et al., 2007). The contribution of the adaptive responses of plant root system to mitigate the stress imposed by excess As has no received limited attention. Plant roots are able to respond to the heterogeneous soil environment by improving root growth in more favorable zones, and they may do this to avoid patches of toxic elements.

Another important fact to be considered is the adaptive capacity of each genotype, including mineral nutrition and As translocation and remobilization. In this context, the present work aimed to characterize the distribution of As and S through translocation and remobilization and the morpho-physiological plasticity of the root system of indica rice (*Oryza sativa* L.) cultivars grown with their roots under two different system, i.e., with or without split root, under increasing As levels.

MATERIAL AND METHODS

Rice seedlings of the *indica* variety were obtained from IRGA **(**Instituto Rio Grandense do Arroz**)**, RS, Brazil. The seeds of five rice cultivars used in Southern Brazil, BR/IRGA 409, BR/IRGA 410, IRGA 420, IRGA 423 and IRGA 424, were used in this study. The seeds were soaked in distilled water at 25 °C in the dark for 24 hours. The pre-germinated seeds were transferred to plastic pots lined with filter paper placed in partially enclosed growth chambers; these pots were then irrigated with distilled water for five days. After five days in distilled water, the seminal roots of half of the seedlings were removed, and the seedlings were transferred to plastic pots containing 180 ml of one-half strength Kimura B nutrient solution (Ma et al., 2001). The pH was adjusted to 5.5, and the solution was renewed every two days in a controlled environment. After seven days of acclimation, seedlings were submitted to three As levels (0, 20 and 50 µM) in the nutrient solution. After ten days, 5 plants per replicate (each treatment consisted of 15 replicates) were randomly harvested and separated into shoots and roots.

Hydroponic experiment with a split-root system

To evaluate the effect of local and systemic As levels on the seedlings, a third experiment with split roots was carried out. After 5 days in distilled water, the seminal roots of all of the rice seedlings were removed, and uniform plants were selected and transferred to a split-root system, in which the two halves of the root system, each in a pot of 180 ml, were exposed to one-half strength Kimura B nutrient solution. After approximately 2 weeks of acclimation, these seedlings with split roots were cultivated for 10 d with seven treatments of varying concentrations and locations of As as follows: treatment 0*0 [0/0 µM As, with both root halves without As exposure]; 0*20 [0/20 µM As, with half of the root system being exposed to 0 µM As and the other half being exposed to 20 µM As]; 0*50 [0/50 µM As, with half of the root system being exposed to 0 µM As and the other half of the root system being exposed to 50 µM As]; 10*10 [10/10 µM, with both halves being exposed to the same concentration of 10 µM As]; and 25*25 [25/25 µM, with both halves being exposed to the same concentration of 25 µM As].

Tissue As and S concentration

The roots and shoot of seedlings were oven-dried at 65 °C to a constant mass for the determination of biomass as well as total arsenic As and sulfur (S) concentrations. The dried plant tissues (0.01–0.1 g) were ground and digested with 4 ml of concentrated HNO_3. Sample decomposition was performed using a heating block Velp Scientifica (Milano, Italy) at 130 °C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The S and As contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a PerkinElmer Optima 4300 DV (SHELTON, USA) equipped with a cyclonic spray chamber and a concentric nebulizer.

Non-protein thiol groups (NPSH) concentration

Frozen root and leaf samples were homogenized in a solution containing 50 mM Tris–HCl and 10% Triton X-100 (pH 7.5) and centrifuged at 6,800 x *g* for 10 min. To the supernatant, 10% TCA was added in a 1:1 (v/v) proportion followed by centrifugation (6,800 x *g* for 10 min) to remove the proteins. The supernatant was used to determine the NPSH concentration.

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An aliquot of the extract sample (400 μl) was added in a medium containing 550 μl 1 M Tris–HCl (pH 7.4). The reaction was read at 412 nm after the addition of 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (5 μl). A standard curve using cysteine was used to calculate the thiol group content of the samples.

RESULTS AND DISCUSSION

Once As was taken up and translocated into the shoots, an adaptive strategy against As toxicity is the remobilization of As. It was shown that rice plants showed large amounts of As in the phloem (Carey et al., 2011). In terms of As remobilization, both cultivars BR/IRGA 409 and IRGA 423 were distinctive (Table 1). In the 0/20 μM As treatment, BR/IRGA 409 exhibited major remobilization in the root tissue and lower translocation in shoot when compared to the other cultivars (Table 2).

Notably, the IRGA 423 cultivar showed the highest remobilization in the root system for the 0/50 μM As treatment and greater translocation in shoot for the 0/20 μM As treatment (Table 1 and 2). This cultivar was the only one where the tissue As concentration at 0/20 μM As treatment did not differ of that at 10/10 μM As treatment (Table 1 and 2).

Table 1: Arsenic concentration and sulfur concentration in root and shoot for five indica rice cultivars exposed to As concentration in a split system roots exposed to treatments: 0*0 [0/0 μM As]; 0*20 [0/20 μM As]; 0*50 [0/50 μM As]; 10*10 [10/10 μM As] and 25*25 [25/25 μM As].

Cultivar	Treatment	As concentration for the evaluated roots	Arsenic concentration		Sulfur concentration	
			root	shoot	root	shoot
BR/IRGA 409	0*0	0 μM	<3,1	<3,1	3.541,0BCb	3.609,0 Bb
	0*20	0 μM	20,0Ce	3,5 Cd	2.315,0Cd	3.026,0 Cd
	0*20	20 μM	397,0Aa		3.564,0Cb	
	10*10	10 μM	310,0Ab	11,3Cc	4.450,0Ba	6.168,0 Aa
	0*50	0 μM	68,6Ad	20,7 Cb	2.527,0Ad	3.463,0 Ac
	0*50	50 μM	226,0Cc		3.050,0Cc	
	25*25	25 μM	407,0Ba	43,8Ba	4.132,0Aa	3.729,0 Bb
BR/IRGA 410	0*0	0 μM	<3,1	<3,1	3.371,0Cb	4.066,0 Ab
	0*20	0 μM	30,1Bc	6,9 Bc	2.337,0Cc	3.301,0 Bd
	0*20	20 μM	286,0Cb		3.644,0Cab	
	10*10	10 μM	326,0Aa	15,1 Bb	3.836,0 Ca	4.475,0 0Ca
	0*50	0 μM	35,4Cc	50,7 Aa	2.357,0Cc	3.699,0 Ac
	0*50	50 μM	312,0Bab		3.633,0Aa	
	25*25	25 μM	343,0Ca	49,3 Ba	3.484,0Bb	3.589,0 Cc
IRGA 420	0*0	0 μM	<3,1	<3,1	3.786,0Bb	2.584,0 Cd
	0*20	0 μM	28,9Bd	5,5 Bd	2.545,0Bd	2.927,0 Cc
	0*20	20 μM	254,0Cb		3.636,0Cb	
	10*10	10 μM	295,0Ab	26,9 Ab	3.953,0Cab	4.892,0 Ba
	0*50	0 μM	44,3Bc	12,5 Cc	2.434,0Bd	2.901,0 Cc
	0*50	50 μM	245,0Cb		3.039,0Cc	
	25*25	25 μM	351,0Ca	60,9 Aa	4.089,0Aa	3.897,0 Ab
IRGA 423	0*0	0 μM	<3,1	<3,1	4.025,0Aa	4.189,0 Aa
	0*20	0 μM	68,8Ad	12,1 Ac	2.627,0Ad	3.344,0 Bc
	0*20	20 μM	356,0Bb		4.141,0Aa	
	10*10	10 μM	294,0Ac	11,2 Cc	3.855,0Cb	4.013,0 Da
	0*50	0 μM	47,5Bd	40,9 Ba	2.517,0Ad	3.343,0 Bc
	0*50	50 μM	369,0Ab		3.431,0Bc	
	25*25	25 μM	439,0Aa	19,9 Cb	4.041,0Aa	3.502,0 Cb
IRGA 424	0*0	0 μM	<3,1	<3,1	2.932,0Dd	3.665,0 Bc
	0*20	0 μM	35,3Abd	11,9 Ad	2.427,0Be	3.929,0 Ab
	0*20	20 μM	324,3Bb		3.846,0Bb	
	10*10	10 μM	255,1Bc	30,0 Ab	6.436,0Aa	4.243,0 Ca
	0*50	0 μM	43,4Bd	18,8Cc	2.646,0Ae	3.484,0 Acd
	0*50	50 μM	320,1Bb		3.463,0Bc	
	25*25	25 μM	401,9Ba	41,0 Ba	3.642,0Bc	3.348,0 Dd

Different capital letters indicate significant difference among rice cultivars; different lower case letters indicate significant difference among arsenic level (P<0,05).

In higher plants, the nodulin 26-like intrinsic proteins (NIPs) are the structural and functional equivalents of the microbial and mammalian aquaglyceroporins (Wallace et al. 2006) used for absorption of As. NIPs are a subfamily of the plant major intrinsic proteins (MIPs), collectively known as aquaporins or water channels (Maruel et al. 2008). In most plant species, arsenite dominates in the xylem sap, suggesting that arsenite is the main form loaded into the xylem (Zhao et al. 2009). This pattern applies even when arsenate is supplied to plant roots and is consistent with the fact that roots have a high capacity for arsenate reduction (Zhao et al. 2010).

Little is known about the transport of As in the phloem, such as the form in which As is transported and the transporters that are involved in phloem loading and unloading. In a recent study using rice panicles that were excised below the flag leaf node, Carey et al. (2010) found that dimethylarsinic acid (DMA) was transported to the immature grain approximately 30 times more efficiently than arsenite. Carey et al. (2011) reported that arsenite is delivered to the rice grain mainly through the phloem, whereas both the phloem and xylem pathways make an equal contribution to the transport of DMA to the grain.

Table 2: Arsenic concentration and sulfur concentration in root and shoot for five indica rice cultivars exposed to three As concentration.

Cultivar	As concentration µM	Arsenic concentration µg g $^{-1}$		Sulfur concentration µg g $^{-1}$	
		root	shoot	root	shoot
BR/IRGA 409	0	<8,00	<8,00	1.495,0 Ab	2.778,5 Ba
	20	384,3 Ab	55,0 Bb	2.032,5 ABa	2.269,0 Cb
	50	415,9 Bc	67,2 Bbc	2.188,7 ABa	1.839,1 Bc
BR/IRGA 410	0	<8,00	<8,00	1.216,7 Bb	3.409,6 Aa
	20	382,2 Ab	72,0 Ab	2.254,1 Aa	2.587,3 Cb
	50	483,0 Bc	80,1 Ab	2.142,4 Aba	2.009,6 Bc
IRGA 420	0	<8,00	<8,00	1.411,2 Ab	2.888,3 Bab
	20	480,5 Ab	55,7 Bb	2.240,6 Aa	3.213,1 Aa
	50	493,0 ABc	82,4 Bc	2.316,7 ABc	2.460,7 Ab
IRGA 423	0	<8,00	<8,00	1.348,3 ABb	2.964,2 Ba
	20	355,2 Ab	53,0 Aab	2.152,3 Aa	3.216,9 Aa
	50	506,2 Ac	72,5 Ab	2.293,4 Aa	1.966,7 Bb
IRGA 424	0	<8,00	<8,00	1.172,6 Bc	3.044,8 ABa
	20	673,3 Ab	62,2 Ab	1.759,1 Bb	2.918,9 Ba
	50	680,5 Abc	70,5 Abc	2.076,1 Ba	1.998,2 Bb

Different capital letters indicate significant difference among rice cultivars; different lower case letters indicate significant difference among arsenic level (P<0,05).

In seedlings grown with the split root system IRGA 424 showed an increase in the S concentration in both root halves directly exposed to As compared to the control, as well as plants without Split roots exposed to As (Tables 1 and 2). This cultivar showed reduced concentrations of S in the root system halves not directly exposed to As (0/20 and 0/50), which reinforces the idea that S content is higher in tissues with greater requirements. In plants, the assimilation of S provides amino acids and proteins that are important for the nutritional value of food, and crop feed yields specialized sulfur-containing metabolites, such as glucosinolates and allylsulfur compounds, for protection from herbivory and microbial infection; the synthesis of specialized peptides (i.e., glutathione and phytochelatins) which provides protection against various oxidative stresses (Gupta et al., 2013).

The multi-faceted role of S in plant metabolism requires an integrated network of pathways involving both primary and specialized metabolisms, as the amino acid cysteine is required for the synthesis of proteins but is also a critical component for multiple peptides found in plants (Ravilious & Jez, 2012). Although many papers have described the importance of non-protein thiol groups (NPSH) for plants, few studies have reported differences in the genotypic concentrations of these compounds. In the present study, we observed two distinct situations in response to the toxicity of As in terms of NPSH: the first is the difference observed between shoots and roots, and the other is a large discrepancy between the cultivars in relation to the root system (Figure 1).

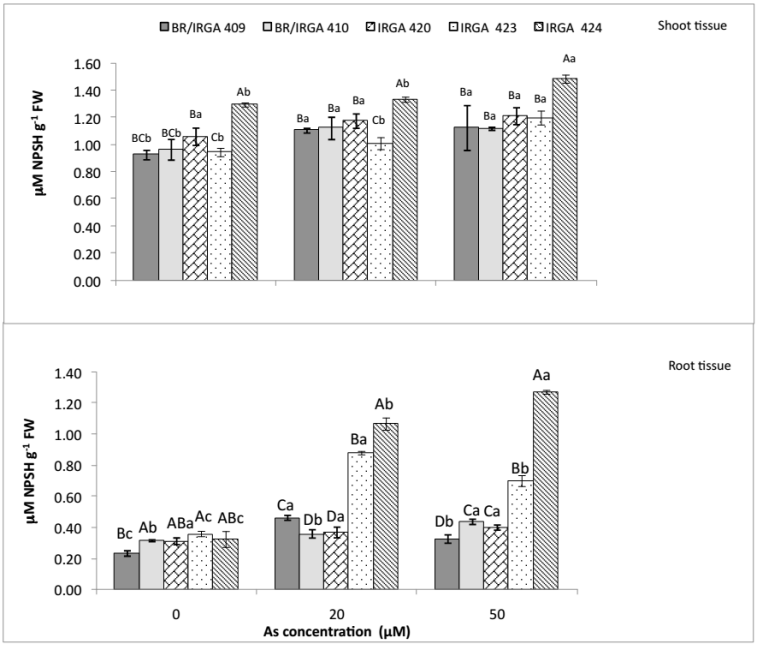

Figure 1: Concentration of non-protein thiol groups (NPSH) in root and shoot of five rice cultivars exposed to three As concentration.

Different capital letters indicate significant difference among rice cultivars, different lower case letters indicate significant difference among arsenic level (P<0,05)

The alterations in tissue NPSH concentration were much more pronounced in the roots than in shoots of seedlings upon addition of As in nutrient solution. In tissues of both shoot and root, IRGA 424 showed a higher increase in the NPSH concentration compared to the other cultivars. Interestingly, under control conditions without the addition of As, the concentration of NPSH was similar among the cultivars, with the exception of the IRGA 424, in which the NPSH concentration in the shoot was higher compared to the other cultivars, and BR/IRGA 409 cultivar,

which showed the lowest root NPSH concentration.

Phytochelatins (PCs) are thiol (SH)-rich peptides and are induced by a range of heavy metals, including Cd, As, Cu, and Zn (Grill et al., 1985). In this view, non-protein thiols (NPSH) could indicate the PC levels (Metwally et al. 2005). Gupta et al. (2013) has studied the effects of various metals/metalloids and found that only As induces both PCs and GSH in *Pfaffia glomerata*, with the GSH occurring in both the roots and shoots and the PCS occuring only in the roots; whereas mercury (Hg) and lead (Pb) only induced GSH in the tissues. These results show the importance of S and NPSH to As detoxification and that the roots are the main organ involved in this process.

CONCLUSION

A common response to As increase was an increase root S concentration and a decrease in shoot S concentration. However, distinct patterns were also found, such as contrasting production of NPSH. The genotype BR/IRGA 409 showed lower NPSH concentration upon As exposure, whereas IRGA 423 and 424 showed the highest. The genotype IRGA 423 showed the highest remobilization capacity and BR/IRGA 409 showed the lowest under 0/20 µM As treatment. However at 0/50 µM As treatment an opposite response was noticed. This work suggests that cultivars IRGA 423 and 424 have higher As tolerance.

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