



Effect of phosphorus on arsenic uptake and metabolism in rice cultivars differing in phosphorus use efficiency and response

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ABSTRACT

A hydroponic experiment was carried out to investigate the effect of phosphorus (P) nutrition on arsenic (As) uptake and translocation within the seedlings of rice cultivars. The experiment occurred in three stages: I 5 days of acclimatization (nutritive solution); II 10 days under P (0.0 and 0.09 mM) and As (0.0 and 100 µM) treatments; III 5 days under recovery. The As exposure had significant effect reducing dry weights of shoots or roots, resulted in elevated concentrations of As in shoot tissues. BR-IRGA 409 showed the highest susceptibility to As in biomass production and root system parameters regardless the P level. This cultivar showed contrasting responses of As translocation to shoot tissue dependent on P levels, with the highest As concentration under low P and lowest under normal P levels. P nutrition was most striking on plants recovery for all cultivars under As exposure. Clearer separation of cultivars for phosphorus use efficiency (PUE) occurred at lower shoot P contents, that was, at higher levels of P deficiency stress. IRGA 424 showed higher PUE as compared to the others cultivars. Our results go some way to understanding the role of P nutrition in controlling the effects of As in rice shoots.

Key words: mineral nutrition, *Oryza sativa*, phosphate, Arsenic uptake.

INTRODUCTION

Arsenic (As) is a toxic and carcinogenic element that occurs widely in soil environments around the world. Soil contamination with As occurs through natural and anthropogenic pathways (Meharg and MacNair 1992, Meharg and Rahman 2003). Arsenic is known to have many toxic effects in humans and is ranked first, in the priority list of hazardous

substances compiled by the US Environmental Protection Agency (USEPA) (ATSDR, 2011).

Rice, an important staple food, is considered to be a major source of As in the human diet (Mondal and Polya 2008, Liang et al. 2010, Carey et al. 2015, Otero et al. 2016). There is therefore an urgent need to develop mitigation measures to reduce As concentrations in rice. However, not much attention has been given to fertilizer management and its relationship with As accumulation in rice grains. Furthermore, there still lack of information

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regarding As concentration in Brazilian rice, considering the different field managements that occurs in the country and rice cultivars.

Phosphorus (P) is a critical element required for optimum plant growth, and is essential for sustainable production of food across the globe. As such, agricultural production consumes 90% of non-renewable rock phosphate reserves mined each year to supply P to crops and pastures (Cordell et al. 2009). As the world's population increases and high-grade rock phosphate resources decline, there is a growing need to improve the efficiency of P use (PUE) at the plant and whole farm scale (Simpson et al. 2011).

Moreover, to mitigate As stress, plants may modulate pathways to maintain a minimal cellular concentration (Bleeker et al. 2006); the adaptive capacity of each genotype, including mineral nutrition and As translocation and remobilization may be the key to cope As stress. Thus PUE has become topical in recent times, showing potential for a better management in agricultural systems, and in this study we aimed evaluated its relationship with As tolerance.

MATERIALS AND METHODS

PLANT MATERIALS AND GROWTH CONDITIONS

Rice seeds of the *indica* variety were obtained from IRGA (Instituto Rio Grandense do Arroz), Rio Grande do Sul, Brazil. The seeds of three rice cultivars used in Southern Brazil, BR/IRGA 409, 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 ten days.

HYDROPONIC EXPERIMENT

After ten days in distilled water, the seedlings were transferred to plastic pots containing 8 L of

nutrient solution containing the macronutrients 0.18 mM $(\text{NH}_4)_2\text{SO}_4$, 0.27 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.09 mM KNO_3 , 0.18 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, and 0.09 mM P_2O_5 and the micronutrients 20 μM NaEDTA- $\text{Fe} \cdot 3\text{H}_2\text{O}$, 6.7 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 9.4 μM H_3BO_3 , 0.015 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.15 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.16 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The pH was adjusted to 5.5, and the solution was renewed every two days in a controlled environment.

The solution culture experiment was carried out in three stages (Figure 1). At stage I, all seedlings were grown in full nutrient solution (+P) and without As (-As) during 5 days. At stage II, half of the seedlings were rinsed three times with deionized water and transferred to pots containing nutrient solution without phosphorus (-P), and the remaining seedlings were grown in +P solution. Then, half of each group seedlings had As added in the nutrient solution as $\text{Na AsO} \cdot 12 \text{H}_2\text{O}$ (+As) under the concentration of $100 \mu\text{M}$. This stage lasted 10 days. Stage III, half of +P and -P seedlings with and without As exposure were collected (25 plants per replicate, each treatment consisted of 4 replicates) randomly harvested and separated into shoots and roots. Thus, the remain plants were rinsed three times with deionized water and transferred to pots containing complete nutrient solution (+P) and without As (-As) This stage lasted 5 days. All remain plants were collected (25 plants per replicate, each treatment consisted of 4 replicates) and separated into shoots (Figure 1).

TISSUE ELEMENTS ANALYSIS

Arsenic speciation - The roots and shoot of seedlings were oven-dried at 65 °C to a constant mass for the determination of biomass and then weighed accurately to a weight of 0.1 g into 50 ml polypropylene centrifuge tubes to which 2 ml of 1% conc. Aristar nitric acid was added and allowed to sit overnight. Batches of up to 48 samples were prepared which also included 2 blanks and

2 rice CRM (NIST 1568b Rice flour) that has the arsenic species As_i and dimethylarsonic acid (DMA) concentrations certified. Samples where

then microwave digested in an CEM MARS 6 instrument for 30 min. at 95 °C using a 3 stage slow heating program: to 55 °C in 5 min. held for

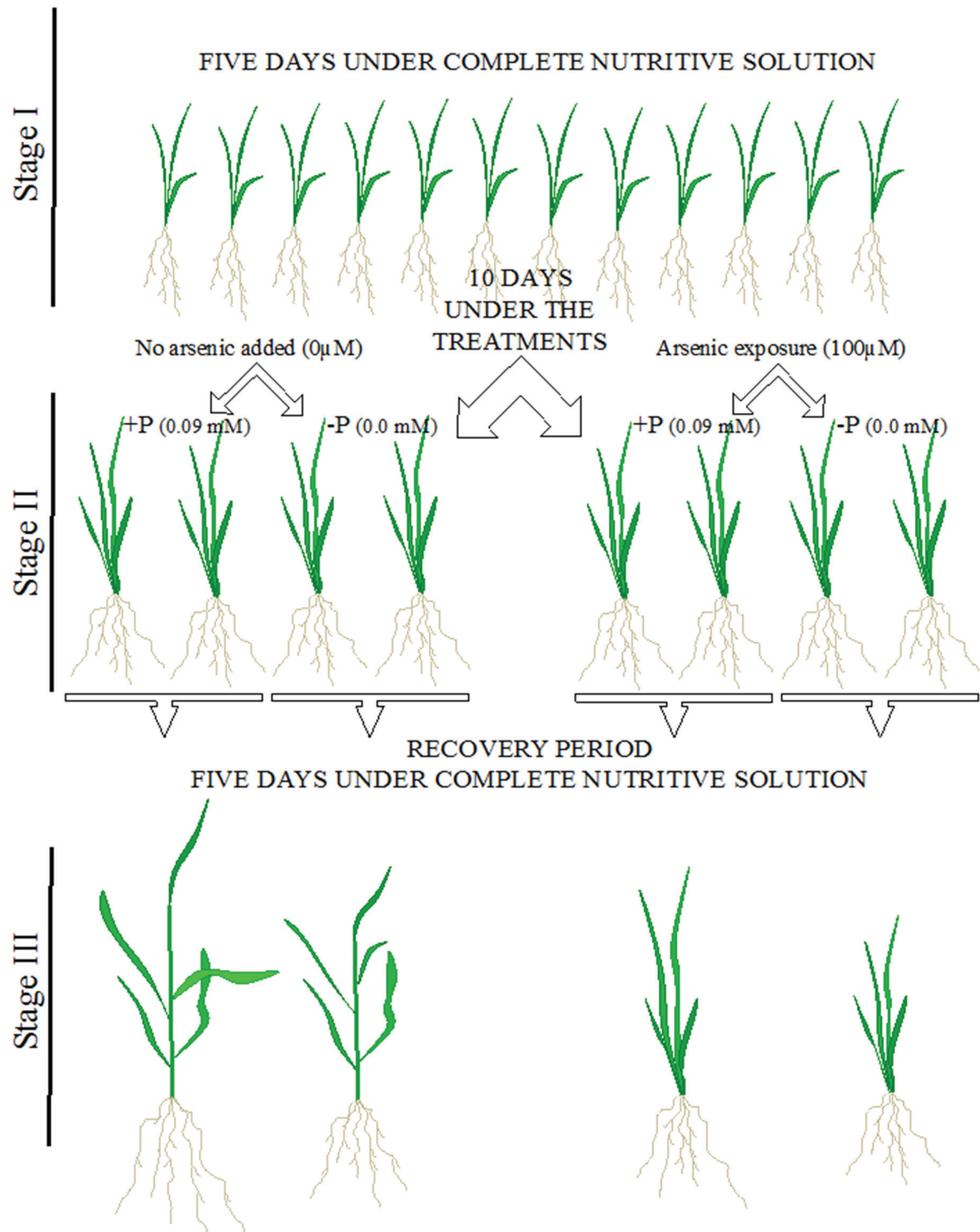


Figure 1 - Practical scheme of the experimental design used in this study (see the colors in the online version).

10 min., to 75 °C in 5 min., held for 10 min. to 95 °C in 5 min., held for 30 minutes). The digestate, on cooling, was accurately diluted to 10 ml with deionized distilled water and centrifuged at 3,500 rpm for 15 min.

A 1 ml aliquot was transferred to a 2 ml polypropylene vial and 10 µl of analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic detection. For multi-element analysis by ICP-MS, a more aggressive digestion procedure (heat to 95 °C in 5 min. hold for 10 min. to 135 °C then hold for 10 min., to 180 °C then hold for 30 min.) was employed, with 2 ml of concentrated Aristar nitric acid and left to soak overnight before microwaving. After microwaving, 2 ml of hydrogen peroxide was added. Blanks and CRM NIST 1568b, which is certified for both arsenic speciation (As_i and DMA) and for a range of trace and macro elements, were included in each batch of 48 samples analyzed.

Phosphorus quantification- Oven-dried samples were ground and digested with 4 ml of concentrated HNO₃. 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 concentration was 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.

DATA ANALYSIS

After the period of exposure, the roots were digitized with the aid of a scanner Epson 11000 XL and analysis was performed with the aid of WinRhizo Pro Software, which uses the first method proposed by Tennant (1975), for determining the total root length and root tips.

Element concentrations in, roots and shoots were calculated on the basis of dry weight. Total

P uptake (T_P); phosphorus use efficiency (PUE), according to Wissuwa et al. (2015); roots and shoots were calculated as follows:

$$T_P = T_{\text{Root-P}} + T_{\text{Shoot-P}}$$

$$T_{\text{Root-P}} = C_{\text{Root-P}} \cdot \text{Roots biomass}$$

$$T_{\text{Shoot-P}} = C_{\text{Shoot-P}} \cdot \text{Shoots biomass}$$

$$\text{PUE} = (T_{\text{Shoot-P}} / \text{Shoot biomass}) \quad (\text{Wissuwa et al. 2015}).$$

RESULTS AND DISCUSSION

The present study demonstrated that exposure to higher levels of As led to a decrease in biomass production for both shoot and root tissues of rice plants during the vegetative development stage, as was previously reported by others authors (Geng et al. 2009, Bhattacharya et al. 2010) (Figure 2).

Overall, regardless the P level or As exposure, the cultivar IRGA 424 had higher values of root and shoot dry weight as well as total root length and number of root tips as compared to BR-IRGA 409; while IRGA 423 seems to be in an intermediate condition between the other two cultivars (Figure 2, Figure 3).

The maintenance of substantial root system as well as leaf production may be the key to cope different stress situations, i.e. nutritional stress or exposure to non essential toxic elements. In the case of nutritional stress, as the lack of an essential element, the plant metabolism is directly affected; however, the effects and time of appearance of the first symptoms vary among different elements and plant species (Marschner 1995).

Although P is an essential element to all higher plants, in the present study, when evaluated without As presence, cultivars had distinct response to P levels. BR-IRGA 409 showed deficient symptoms in root system in a short period of exposure (10 days), with lower root dry weight and total root length under P starvation, while IRGA 423 and IRGA 424 were not affected (Figure 2, Figure 3). On the other hand, under As exposure, overall P

CULTIVAR	NO ARSENIC ADDED		ARSENIC EXPOSURE (100 μ M)	
	0 mM P ₂ O ₅	0.09 mM P ₂ O ₅	0 mM P ₂ O ₅	0.09 mM P ₂ O ₅
Root Dry weight (mg plant⁻¹)				
BR-IRGA 409	10.50 Ba	20.09 Ba*	8.55 Cb	11.51 Bb
IRGA 423	10.82 Ba	23.36 ABa	13.64 Bb	15.05 Ab
IRGA 424	30.14 Aa	32.25 Aa	16.55 Aa	22.95 Aa*
Shoot Dry weight (mg plant⁻¹)				
BR-IRGA 409	58.08 Aa	70.18 Ba	28.68 Bb	38.58 Cb
IRGA 423	66.88 Aa	81.32 Ba	39.01 Ab	50.47 Bb
IRGA 424	61.17 Aa	125.44 Aa*	47.64 Ab	71.06 Ab*
Total root length (cm plant⁻¹)				
BR-IRGA 409	83.50 Ba	100.35 Ba*	46.88 Bb	61.02 Bb
IRGA 423	99.38 Ba	101.23 Ba	55.00 Bb	72.80 Bb*
IRGA 424	123.37 Aa	133.24 Aa	87.00 Ab	112.30 Aa*
Number of root tips (plant⁻¹)				
BR-IRGA 409	168.00 Ba	186.50 Bb	125.75 Aa	204.25 Ba*
IRGA 423	217.25 Aa	168.50 Ba	106.50 Ab	167.00 Ca*
IRGA 424	244.25 Aa	230.50 Aa	155.50 Ab	247.75 Aa*
Phosphorus concentration in root tissue (mg kg⁻¹)				
BR-IRGA 409	243.84 Aa	1780.00 Aa*	219.45 Aa	1602.33 Aa*
IRGA 423	214.25 Aa	1564.00 Aa*	228.08 Aa	1665.50 Aa*
IRGA 424	205.07 Aa	1497.33 Ba*	202.19 Aa	1476.00 Ba*
Phosphorus concentration in shoot tissue (mg kg⁻¹)				
BR-IRGA 409	905.91 Aa	5565.00 Ba*	830.45 Ab	5910.00 Ba*
IRGA 423	926.27 Aa	6889.00 Aa*	523.50 Ab	6401.00 Aa*
IRGA 424	754.45 Aa	6300.33 Aa*	415.00 Ab	5006.33 Aa*

Figure 2 - Biomass and root system parameters of rice plants of three cultivars exposed to 100 μ M arsenic and without arsenic, with phosphorus 0.09 mM and without phosphorus in nutrient solution.

Means followed by capital letters indicate comparison between cultivars within the same phosphorus and arsenic levels, whereas lowercase letters indicate comparison of the arsenic exposure for the same cultivars and phosphorus level and asterisk indicate comparison between phosphorus levels for the same cultivar and arsenic exposure. Tukey test, $\alpha = 0.05$.

presence did not alter BR-IRGA 409 development, but the presence of P had a positive effect in all tested parameters (root and shoot dry weight, total root length and number of root tips) for IRGA 424 (Figure 2).

BR-IRGA 409 susceptibility to As was higher than its response to P, once the presence of P wasn't enough to mitigate the As effects on plant growth (Figure 2, Figure 3). Interestingly, P nutrition was

most striking on plants recovery for all cultivars under As exposure, but more pronounced in BR-IRGA 409, with only 8% of total root length increment after 5 days under recovery (complete nutritive solution without As presence) in plants with the prior treatment -P and 70% with the prior treatment +P respectively (Figure 3).

The increment on mineral nutrition also mitigate toxic effects of other elements, through

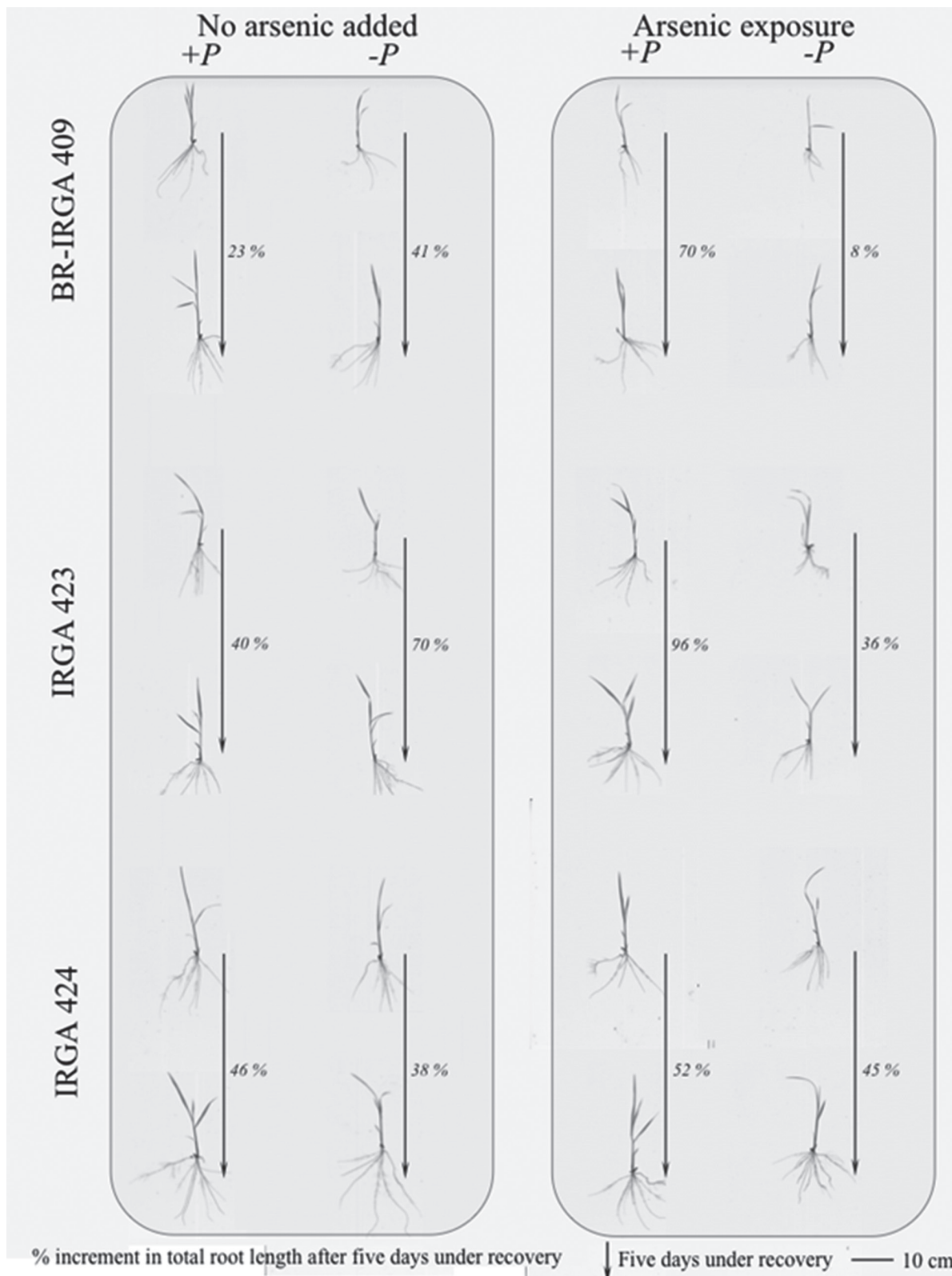


Figure 3 - Effect of phosphorus levels with or without arsenic exposure in rice plants on total root length increment (percentage inside the box) of plants after five days in control solution, recovery stage (see the colors in the online version).

complexation and tissue dilution, once the nutrition results in plant mass increase (Marschner 1995). However, P starvation combined with As exposure resulted in a very drastic biomass reduction, and this stress was almost irreversible for BR-IRGA 409 cultivar under the tested system.

The P requirement for optimal growth is in the range of 0.3-0.5% of plant dry mater during the vegetative stage of growth (Marschner 1995). In our experiment, P starvation was used to manipulate the P nutrition of rice plants grown in solution culture. Phosphorus concentrations in -P plants were significantly lower than those in +P plants, and P concentrations in -P plants were at deficiency levels while +P were at range suggested for optimal growth (Figure 2). The results of the present study indicated that rice genotypes had different nutrient partitioning requirements (Figure 2). The results also indicated that the nutrient status and distribution varied with the amount of As added.

Significant differences in nutrient concentrations among different cultivars could be the result of differences in the removal of As from the system among these genotypes, as well as development of As tolerance and adaptations to other stressful conditions (Tu and Ma 2003, Panda et al. 2010, Zheng et al. 2013, Otero et al. 2016).

Due to the diverse functional and structural roles of P in plants, P-use efficiency (PUE) is a complex trait to dissect. P-use efficiency has become topical in recent times for several reasons. There were large price increases during the last decades, and high prices are likely to continue in the future. Coincidentally, the concept of 'peak P' has gained some attention in the media, which has drawn attention to the environmental, economic, and social problems that might arise due to limited P reserves (Cordell et al. 2009, Lott et al. 2009). Unlike nitrogen (N), the amount of P available for use in agriculture is finite. Steen (1998) estimated that the depletion of current economically

exploitable reserves would occur sometime in the next 60–130 years.

The tissue P concentrations varied considerably among the genotypes under P supply, with IRGA 424 showing the lowest levels in root tissue and the highest in shoot tissue and the opposite pattern for BR-IRGA 409 (Figure 2). On the other hand, there was no difference in root and shoot P concentration among the tested cultivars under P starvation (Figure 6).

Improvements in the efficiency of P nutrition of crops will come from a variety of potential sources, including changes in fertilizer technology, improvements in exploiting soil biology, and better fertilizer management practices, as well as genetic improvement. The widespread realization that improvements in P nutrition are crucial to the future need to raise global agricultural production has resulted in several recent reviews that have explored these different opportunities (Hinsinger 2001, McNeill and Penfold 2009, Richardson et al. 2009, Ryan et al. 2009, McLaughlin et al. 2011, Simpson et al. 2011, Rowe et al. 2016).

The marked susceptibility of rice plants to As may be due to a small biomass, which results in high As concentration in plant tissue, leading to toxic As levels that drastically affect plant development (Farooq et al. 2016). Thus, BR/IRGA 409 cultivar which had a high susceptibility to As and is well known for its susceptibility to excess levels of Fe (Stein et al. 2009), had the lowest As tissue concentrations in shoots under normal P supply as compared to the other cultivars (Figure 4). On the other hand under P starvation this cultivar had over 35% higher As concentration in shoot tissue as compared to IRGA 423 and 424 (Figure 4).

Arsenite (As(III)) is the dominant As species in reducing environments such as flooded paddy soils (Marin et al. 1993, Takahashi et al. 2004, Xu et al. 2008). Thermodynamically, reduction of arsenate to arsenite can occur quite readily at intermediate redox potentials (Inskeep et al. 2002). Flooding of

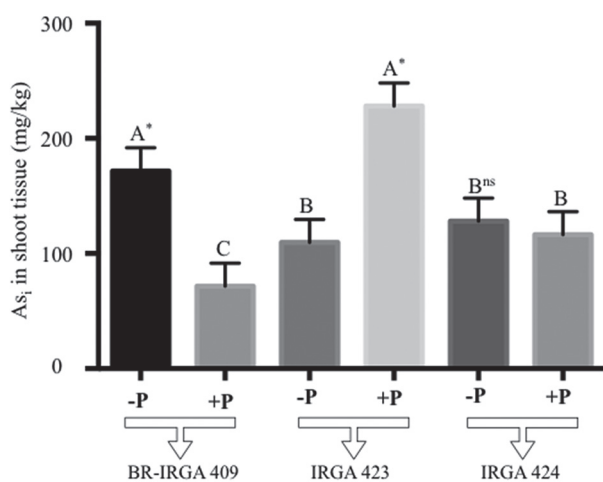


Figure 4 - Inorganic arsenic (As_i) concentration in shoot tissues of BR-IRGA 409, IRGA 423 and IRGA 424 cultivars under phosphorus starvation (-P) and normal supply (+P). Means followed by capital letters indicate comparison between cultivars within the same phosphorus and arsenic levels, whereas asterisk indicate comparison between phosphorus levels for the same cultivar and arsenic exposure. Tukey test, $\alpha = 0.05$.

paddy soils leads to mobilization of arsenite into the soil solution and enhanced As bioavailability to rice plants (Xu et al. 2008).

We also have to consider metabolic transformations of rice inside plant cell once it is absorbed by roots. In this view even though arsenite is the mainly As available form to rice plants under paddy system, in grains there is a large range of different As species (Meharg et al. 2009, Naito et al. 2015, Carey et al. 2015).

In case of exposure to non-essential toxic elements such as As, plants have both direct and secondary effects on metabolism. Direct affects include, an over production of reactive oxygen species (ROS) that lead to cell damage, lipid peroxidation and other injuries (Tripathi et al. 2012). And second effects are often related to changes in nutrient uptake (Abedin et al. 2002, Meharg and Hartley-Whitaker 2002).

Our results go some way to understanding the role of P nutrition in controlling the effects of As in rice shoots. With further work, may form the basis of management practices to alleviate As

accumulation to toxic levels. Clearer separation of genotypes for PUE occurred at lower shoot P contents, that was, at higher levels of P deficiency stress (Figure 5).

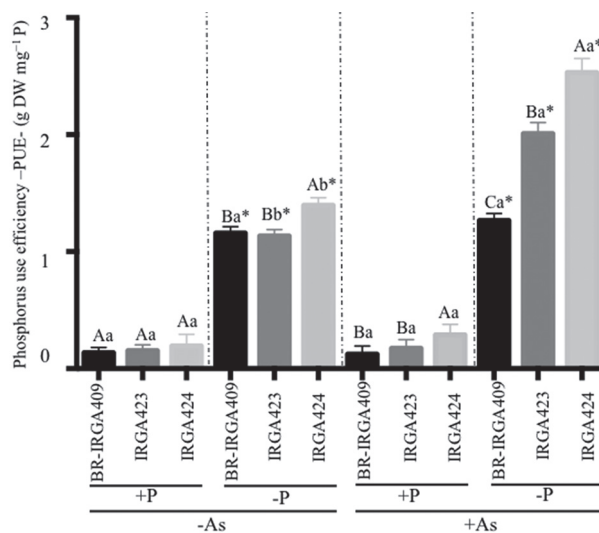


Figure 5 - Means followed by capital letters indicate comparison between cultivars within the same phosphorus and arsenic levels, whereas lowercase letters indicate comparison of the arsenic exposure for the same cultivars and phosphorus level and asterisk indicate comparison between phosphorus levels for the same cultivar and arsenic exposure. Tukey test, $\alpha = 0.05$.

Attempts to improve the P efficiency of cropping systems through plant breeding have predominantly focused on enhancing P acquisition from soils (Wissuwa et al. 2009). However, at least conceptually, it is generally agreed that concurrent improvements in vegetative stage PUE should be an important complementary trait to enhanced P uptake in any breeding approach (Wang et al. 2010, Rowe et al. 2016).

Under P starvation and P starvation combined with As exposure, IRGA 424 had higher PUE as compared to the other cultivars (Figure 5). Studies demonstrated that in general P uptake rate is very similar among rapidly growing species and even when compared to slowly growing species (Chapin et al. 1982, Chapin et al. 1989). This is not an important adaptive mechanism because in low P

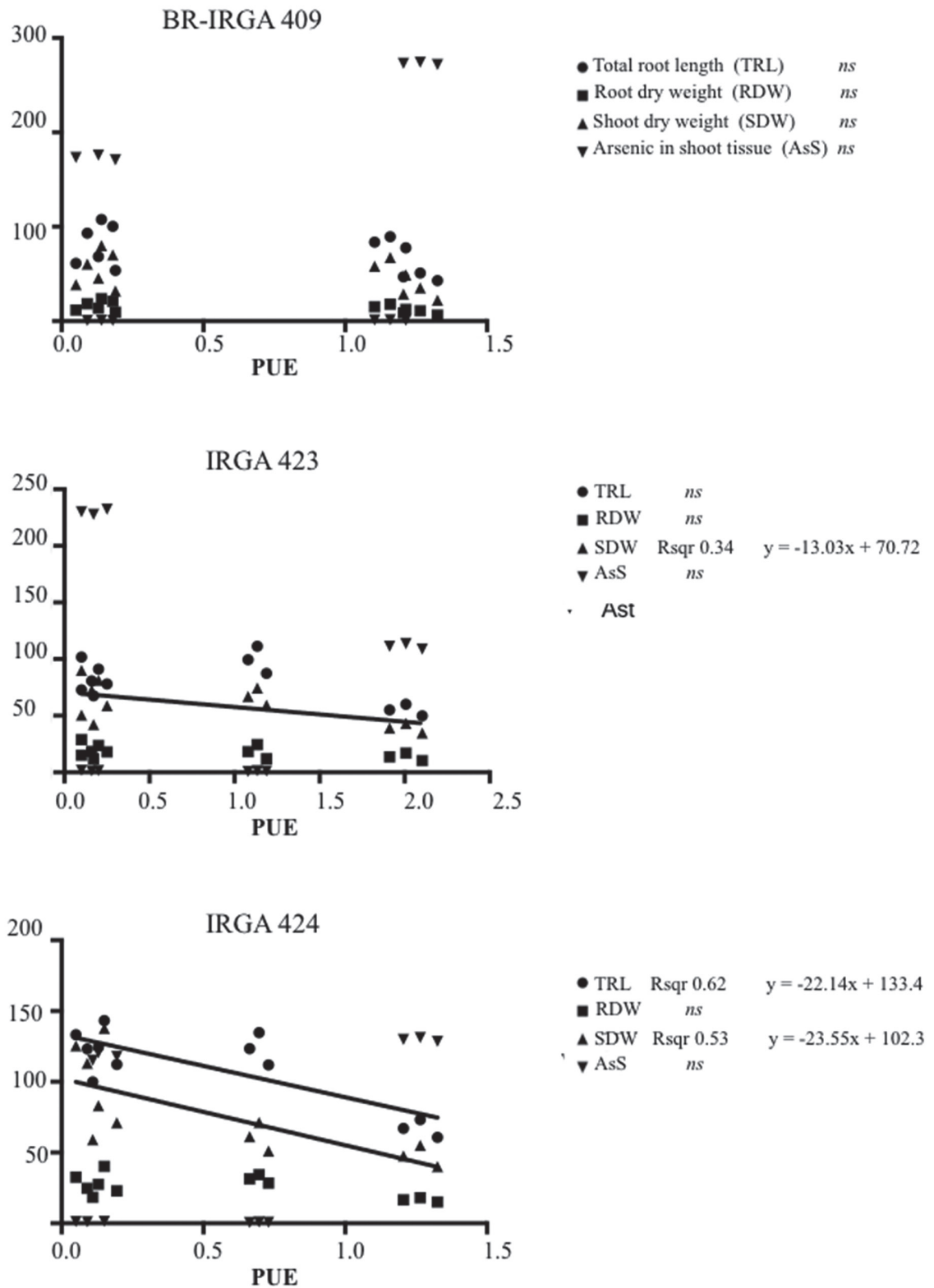


Figure 6 - Phosphorus use efficiency (PUE) relationship to sum of species of total As, shoot dry weight, root dry weight and total root length for BR-IRGA 409, IRGA 423 and IRGA 424 cultivars in shoot tissue samples.

availability absorption is limited by P diffusion to the root surface therefore, so even a low nutrient absorption capacity is adequate to absorb those nutrients that reaches the root (Aerts and Chapin 2000).

The results in our work may indicate that there are important morphologic differences in roots that directly affect shoot production among the three rice cultivars. It is therefore possible to control As impact in rice through the selection of rice genotypes with higher PUE, although this doesn't necessary is related to As uptake and accumulation (Figure 6).

When comes to PUE evaluation there are many discrepancies in the terminology, definitions and calculations (Siddiqi and Glass 1981, Ozturk et al. 2005, Wissuwa et al. 2015). Siddiqi and Glass (1981) use total plant biomass, Wissuwa et al. (2015) focused on shoot biomass and agronomic PUE refers to the increase in yield of a variety following the addition of P fertilizer. Assessed as the difference in yield between fertilized and unfertilized treatments, divided by the difference in nutrient supplied in each of the treatments (Hammond et al. 2008), agronomic PUE is a measurement of the level of responsiveness to P.

High PUE cultivar IRGA 424 showed the highest tolerance to As exposure under the tested system, with an interestingly potential for further studies. Thus, for this cultivar PUE was related with total root length and shoot dry weight (Figure 6), and this cultivar would still have the highest PUE even with we considered total biomass (Siddiqi and Glass 1981), however this cultivar hasn't showed high responsiveness to P increment (Figure 2,4, Figure 3, 6).

CONCLUSIONS

The As exposure had significant effects on reducing dry weights of shoots and roots, thus resulted in elevated concentrations of As in shoot tissues. The

As effect was cultivar-dependent, and BR-IRGA 409 showed the highest susceptibility to As in biomass production and root system parameters regardless of the P level. The response to P levels was also distinct among the cultivars as well as phosphorus use efficiency (PUE). Under P starvation and P starvation combined with As exposure, overall, PUE increased, being higher for IRGA 424 cultivars. This cultivar was the only one with significant correlation for both shoot dry weight and total root length with PUE; while BR-IRGA 409 had no correlation and IRGA 423 only had for shoot dry weight. Even though there was no evidence of relation between PUE and As translocation in early stage in rice plants, however it was related to As tolerance. Thus, our results go some way to understanding the role of P nutrition in controlling the effects of As in rice growth.

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