UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
CURSO DE PÓS-GRADUAÇÃO EM AGRONOMIA

Júlia Gomes Farias

DINÂMICA DO ARSÊNIO NO SISTEMA SOLO - ÁGUA - ARROZ NA AMÉRICA DO SUL: DISTINÇÕES FISIOLÓGICAS ENTRE CULTIVARES E OCORRÊNCIA EM GRÃOS COMERCIAIS.

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Tese apresentada ao Curso de Pós-Graduação em Agronomia da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Agronomia.**

Orientador: Prof. Dr. Fernando Teixeira Nicoloso

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Aprovado em 29 de fevereiro de 2016

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Santa Maria, RS
2016

DEDICATÓRIA

A maior de todas as minhas inspirações, minha grande amiga, meu grande amor, meu porto seguro e para sempre meu norte, minha *mamis* Iria Luiza Gomes Farias, dedico. Te amo hoje, ontem e sempre.

AGRADECIMENTOS

O universo sempre foi um grande mistério para mim, onde começa e quando termina? O finito e o infinito sempre me deixaram muito confusa, ainda sim sempre tive a sensação de algo maior que todos nós. Alguns chamam de Deus, outros de Alá, Shiva Ganesha... outros de destino e há quem não dê "nome aos bois". Para mim tudo isso é energia. Gostaria então de começar agradecendo esta energia maravilhosa, que vem me acompanhando a vida inteira e, claro, super presente em meu momento filósofa, escrevendo a primeira versão da tese.

Bem dito isso, agradeço a minha família maravilhosa, que sempre me apoiou, mesmo quando apoiar significava olhar para o lado e deixar eu levar um tombo, pois levantar e persistir é uma das mais importantes lições da vida. Além do apoio e é claro amor incondicional, minha família sempre me estimulou, questionando e sempre me instigando ao aprimoramento. Meu agradecimento especial aos meus pais e a minha irmã, amores da minha vida!

Agradeço também a oportunidade de desde muito cedo conviver com professores apaixonados por esta linda profissão que é lecionar, partilhar, transmitir o saber. Também sou grata pela oportunidade que me foi dada tanto em termos de infraestrutura quanto de profissionais competentes na UFSM além é claro dos órgãos financiadores CAPES, CNPq e FAPERGS. Este imenso investimento do governo nos dá a oportunidade de aprender e desbravar novos caminhos, no meu caso pude ainda realizar um sonho de trabalhar com um pesquisador que admiro muito, prof. Andy Meharg no Reino Unido.

Também tive a grande alegria de contar sempre com um time maravilhoso. Sim porque o nosso grupo de pesquisa é um grande time onde crescemos juntos e com um objetivo em comum, o saber. Para estes faço um agradecimento especial (em ordem cronológica) para meus queridos Sibila, Jader, Pedro, Bianca, Raissa, Katieli e Anderson pelo profissionalismo, amizade e confiança sempre.

Eu contei ainda com um outro time, de amigos dispostos às mais diversas "indiadas", as quais em geral estavam relacionadas com coleta de arroz, solo, água ou as três alternativas anteriores, agradeço por tudo meus queridos Heuri, Roberto, Roberta e Glaucia.

Finalizo agradecendo o grande responsável por tudo isso, meu grande mestre prof. Nicoloso, que no decorrer destes quase 8 anos sempre me respeitou, incentivou e acreditou em mim, tantas vezes mais do que eu. Professor que bom poder trabalhar contigo! Que nunca nos faltem sonhos nem vontade de lutar. Como o senhor me disse uma vez, ser sua orientada foi o meu Everest. Que bom que tive um grande companheiro de jornada.

A todos vocês, sou grata!

Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning.

Albert Einstein

RESUMO

DINÂMICA DO ARSÊNIO NO SISTEMA SOLO – ÁGUA - ARROZ NA AMERICA DO SUL: DISTINÇÕES FISIOLÓGICAS ENTRE CULTIVARES E OCORRÊNCIA EM GRÃOS COMERCIAIS

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O arroz é a principal fonte de arsênio inorgânico (As_i), uma substância cancerígena, na dieta humana. Neste sentido, existe um grande interesse em reduzir o acúmulo do mesmo em grãos. Para tal, a gestão a campo, criação/modificação no processamento de arroz e estudo com diferentes cultivares vêm sendo explorados. A nível global, muitos países que consomem arroz não produzem o mesmo, ou mesmo regiões dentro de um país produtor. Visto que para manter o consumo tais regiões importam arroz, a identificação de regiões com baixa concentração de As é uma importante opção para a salubridade dos consumidores. Entretanto, a localização de pequenas áreas livres ou com baixas concentrações de As não parece ser a solução completa para a contaminação observada em alimentos; visto a grande demanda de arroz e o número já considerável de áreas descritas como contaminadas. Desta forma, faz-se necessário o uso conjunto de informações como a descrição dos níveis de As em diferentes áreas, comparação entre cultivares, incluindo mecanismos específicos de tolerância e baixa translocação, além da descrição de efeitos secundários como nível dos demais minerais presentes no grão e efeito do manejo da cultura na qualidade de grãos. Visto o exposto, o presente estudo teve por objetivos avaliar amostras comerciais de arroz da América Latina, bem como amostras coletadas a campo sob diferentes manejos de água, fertilizantes e uso do solo; além de utilizar experimentos de curta duração em casa de vegetação para avaliar parâmetros morfofisiológicos em diferentes cultivares. Existe uma grande discrepância na concentração de As nas diferentes regiões testadas. Embora o fator genético (cultivar) tenha efeito sobre a concentração de As em grãos, os fatores ambiente e manejo são determinantes. Aparentemente, a cultivar BR-IRGA 409 apresenta uma maior suscetibilidade ao As, apresentando também uma menor translocação para os grãos, enquanto que cultivares com comportamentos tolerantes apresentaram um maior acúmulo deste elemento. A eficiência de uso de fósforo e a concentração de tióis não proteicos parecem estar relacionadas à tolerância ao As, sendo altamente pronunciadas em cultivares tolerantes. Finalmente, o As apresenta alto potencial genotóxico e oxidativo, sendo a principal anormalia observada a presença de micro-núcleos.

Palavras chave: Arsenito. Contaminação. Metaloide. Nutrição mineral. *Oryza sativa*.

ABSTRACT

ARSENIC DYNAMICS IN THE SYSTEM SOIL - WATER - RICE IN SOUTH AMERICA: PHYSIOLOGICAL DISTINCTIONS BETWEEN CULTIVARS AND OCCURRENCE IN COMMERCIAL GRAINS

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Rice is the main source of inorganic arsenic (As_i), a carcinogenic substance, in the human diet. In this sense, there is great interest in reducing the accumulation of As in rice grains. To achive this, field management / rice processing and study of different cultivars have been explored. Globally, many countries that consume rice don't produce it, or even specifics regions of a producer country. As to maintain the rice consumption these regions have to import rice, the identifycation of regions with low As is an important option to ensure food security for the consumers. However, the location of As free areas seems to be an incomplete solution; as there is a large demand for rice and the already considerable number of areas described as contaminated. Thus, it is necessary to use a set of information able to describe As levels in different areas, comparing cultivars, including specific mechanisms of tolerance and low translocation, besides the description of side effects such as levels of other minerals present in the grain and effect of soil management on grain quality. Given the above, this study aimed to evaluate commercial samples of rice in Latin America, as well as samples collected in the field under different managements of water, fertilizer and land use; in addition it was used short-term experiments in greenhouse were performed to evaluate morphophysiological parameters in different cultivars. There is a large discrepancy regarding As concentration among the different regions tested. Although the genetic factor (cultivar) has an effect on As concentration in grain, environmental and management factors are decisive. Apparently the cultivar BR-IRGA 409 presents a large susceptibility to As, also presenting a lower translocation to grains while cultivars described as tolerant had a higher accumulation of this element. The phosphorus use efficiency and the concentration of non-protein thiols appear to be related to tolerance, being highly pronounced in tolerant cultivars. Finally, As has a high potential to genotoxic and oxidative stress, and the main abnormality observed was the presence of micro-nuclei.

Keywords: Arsenite. Contamination. Metalloid. Mineral nutrition. *Oryza sativa*.

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SUMÁRIO

1. APRESENTAÇÃO

Neste estudo buscou-se o esclarecimento e a caracterização da relação entre arsênio (As) e planta, sob diferentes módulos experimentais, e predição de possíveis efeitos para a saúde humana. Através de um enfoque acadêmico, uma série de experimentos foi realizada. O trabalho iniciou com um quadro global, onde avaliou-se amostras de arroz comerciais em diferentes países da América Latina quanto aos níveis de As ocorrente. Posteriormente, avaliando-se experimentos em casa de vegetação, com experimentos em hidroponia *floating* e com areia, a fim de determinar diferenças específicas entre cultivares e sistemas. Finalmente partimos para experimentos a campo, onde manejo, solo e cultivares foram avaliados juntos, sendo consideradas suas inter-relações para o acúmulo de As em grãos. No manuscrito final, esta tese demonstra o efeito do As a nível celular, mais especificamente na avaliação de danos à cromossomos.

Este trabalho buscou não somente caracterizar o problema, mas também, ainda que de uma forma sutil, mostrar alternativas ao mesmo: seja no manejo do solo, na escolha de cultivares ou ainda com mudanças sutis em nossa alimentação.

Existem dois fatores fundamentais que sustentam a pesquisa científica: I) O problema, pois só se faz pesquisa frente a algo que deva ser melhorado, incorporado, modificado, ou que seu contexto atual não seja adequado, satisfatório ou ainda que represente algo prejudicial; II) A curiosidade do pesquisador. Uma vez identificado o problema, o fator curiosidade torna-se fundamental para um desenvolvimento satisfatório e que culmine em uma descoberta ou inovação de sucesso.

O As têm recebido atenção crescente principalmente em produtos a base de arroz ou mesmo grãos de arroz in natura; especialmente pela comunidade europeia e norte americana. Sendo a América do Sul , em especial o Brasil, um grande produtor de arroz, e tendo em vista a carência de trabalhos caracterizando amostras produzidas nesta região, justifica-se uma abordagem detalhada a qual inclui desde experimentos em casa de vegetação, à coletas a campo e análise de amostras comerciais. Este trabalho tem como hipótese: “existe ocorrência de arsênio em grãos de arroz produzidos na América do Sul, com alta variabilidade de concentração, sendo esta dependente do material genético, local de produção e manejo agrícola”.

1.2 REFERENCIAL TEÓRICO

A contaminação ambiental por metais pesados tem despertado crescente interesse de órgãos vinculados à pesquisa. Isto devido a problemática ambiental e eminente risco à saúde humana, bem como pelos resultados de estudos mais específicos. Este fato tem resultado em um significativo avanço em diferentes linhas de pesquisa, como fitoremediação, fitointoxicação e contaminação alimentar. Neste cenário, a contaminação por As tem recebido atenção especial, devido à extensa área agricultável contaminada já descrita e pela significativa entrada deste elemento na cadeia alimentar, via água, animais e vegetais contaminados (IARC, 2004; ZHAO et al., 2010; ZHAO et al., 2013, CAREY et al., 2015; SIGNES-PASTOR et al., 2016).

Em humanos, o As ingerido a partir do consumo do arroz pode ser substancial, pois o arroz (*Oryza sativa)* é particularmente eficiente em absorver As, podendo também translocá-lo para os grãos (MA et al., 2008). Além de fatores relacionados à entrada e ao acúmulo de As na cadeia alimentar humana, é importante também avaliar o efeito do As na produção agrícola, bem como na qualidade nutricional dos grãos. A contaminação por As pode diminuir sensivelmente a altura de plantas, o rendimento da produção de grãos, raiz e biomassa total (ROBERTS et al., 2012), sendo também responsável por alterações na concentração de nutrientes dos grãos (LOMBI et al., 2009).

Notavelmente, fatores referentes ao solo (pH, óxidos, fósforo e enxofre) juntamente com clima e condições hídricas (solo drenado ou inundado) são determinantes para ocorrência de diferentes espécies de As, bem como de formas de absorção das mesmas por vegetais. Dentre estes, o ferro (Fe) tem um papel fundamental no ciclo do As, atuando tanto como agente de adsorção como agente redutor, de acordo com o nível de umidade do solo. Assim, o As apresenta comportamento divergente em solos bem drenados em relação a solos saturados de água. Adicionado a esta equação, temos o fator genético vegetal como outro determinante na cinética de absorção e acúmulo deste metal (YAMILY et al., 2008; ZIA et al., 2010).

O fluxo de nutrientes no sistema solo-planta envolve mecanismos complexos, muitos deles relacionados à transformação e à mobilização dos nutrientes pela biota do solo (RHEINHEIMER et al., 1999). Adicionalmente, este fluxo é diretamente afetado pela combinação de nutrientes coexistente em um dado momento. Nesse sentido, o fósforo (P), que é um elemento essencial para o crescimento e desenvolvimento das plantas, também pode atenuar o estresse por metais pesados, através processos de complexação e aumento na

produção de biomassa e, consequentemente, diluição de metal nos tecidos (MARSCHNER, 1995).

Embora em solos com elevado nível de inteperização, percentual de argila e pH ácido o conteúdo total de P do solo esteja geralmente elevado, a disponibilidade de P é frequentemente um fator limitante para o crescimento das plantas e sua produtividade. Este paradoxo surge porque a concentração de Pi disponível na solução do solo é cerca de 1 μM e raramente excede 10 μM (BIELESKI, 1973). Nos solos que sofrem ciclos de umedecimento e drenagem, como os solos de várzea, a disponibilidade de P mostra-se mais complexa que em solos bem drenados, visto que as reações de redução, hidrólise e solubilização de diversos compostos influem no teor de P disponível.

Atualmente existe um banco de dados considerável em relação à absorção, especiação e acúmulo de As em plantas de arroz, bem como ocorrência de As em regiões diversas do mundo (MA et al., 2006, MA et al., 2008). Entretanto, são poucas as informações sobre os mecanismos de translocação do As entre colmo principal e perfilhos de arroz, bem como a interação da fitointoxicação com os parâmetros nutricionais da planta e do solo, além da qualidade de grãos comerciais e o impacto de diferentes manejos nos aspectos anteriormente citados.

O histórico de rizicultura e a proximidade com regiões onde foram descritas contaminações por As além do potencial produtivo da América Latina, a qual exporta arroz para um considerável número de países da África e Europa, justificam a investigação e a caracterização de diferentes áreas da América do Sul. Sendo o Brasil o principal produtor de arroz, uma descrição mais detalhada das diferentes regiões produtoras foi proposta, de forma que o conhecimento do mecanismo da aquisição do As pelo arroz e suas relações com manejo e cultivares permita estudar estratégias para a redução do acúmulo de As nos grãos, reforçando a segurança dos alimentos, através do uso de diferentes genótipos, manejo de água e fertilização.

1.2.1 - Arsênio: características, abundância e quantificação

O arsênio (do latim *arsenium*; do grego *arsenikos* = potente), ou também arsênico, é um elemento químico, de símbolo As, com número atômico 33 e massa atômica de 75 g mol^{-1}. É um semimetal (ou metaloide) encontrado no grupo 15 (5A) da Classificação Periódica dos Elementos. Distribui-se de maneira relativamente uniforme nos principais tipos de rochas e sua concentração média está entre 0,5-2,5 mg kg^{-1}. Apenas em sedimentos argilosos a média

pode atingir valores mais expressivos, cerca de 13 mg kg^{-1} (KABATA PENDIAS, 2001; FAURE, 1991).

Desde sua descoberta pelo alquimista alemão Albertus Magnus (1193-1280), este elemento foi extensamente explorado. Embora hoje o As seja um elemento notavelmente tóxico, ele já foi muito utilizado como defensivo agrícola e também no tratamento de doenças de pele e sífilis em humanos (MORENO-JIMÉNEZ et al., 2012).

O As está associado a depósitos minerais, sendo frequentemente encontrado junto com S, Se e Te. Numerosos minerais de As são o resultado da oxidação de depósitos de sulfetos. Estes são os arsenatos e arsenitos, em que o As é combinado com algum metal (por exemplo, Fe, Pb e Cu). O mais comum dos arsenominerais, porém, é um sulfeto, a arsenopirita (FeAsS) (KABATA PENDIAS, 2001; FRANKENBERGER Jr, 2002). A arsenopirita é o mineral de As mais comumente encontrado no Brasil, associado a outros minerais originados por hidrotermalismo (MATSCHULLAT, 2007).

A exposição de minerais sulfatados a condições oxidantes pode desencadear reações irreversíveis que causam severa acidificação do meio, processo conhecido por drenagem ácida. Além da severa acidificação causada pelo intemperismo rápido destes minerais, quando expostos a condições oxidantes, a dispersão do As no ambiente e contaminação de solos e águas subterrâneas são os principais problemas ambientais gerados pela atividade de mineração.

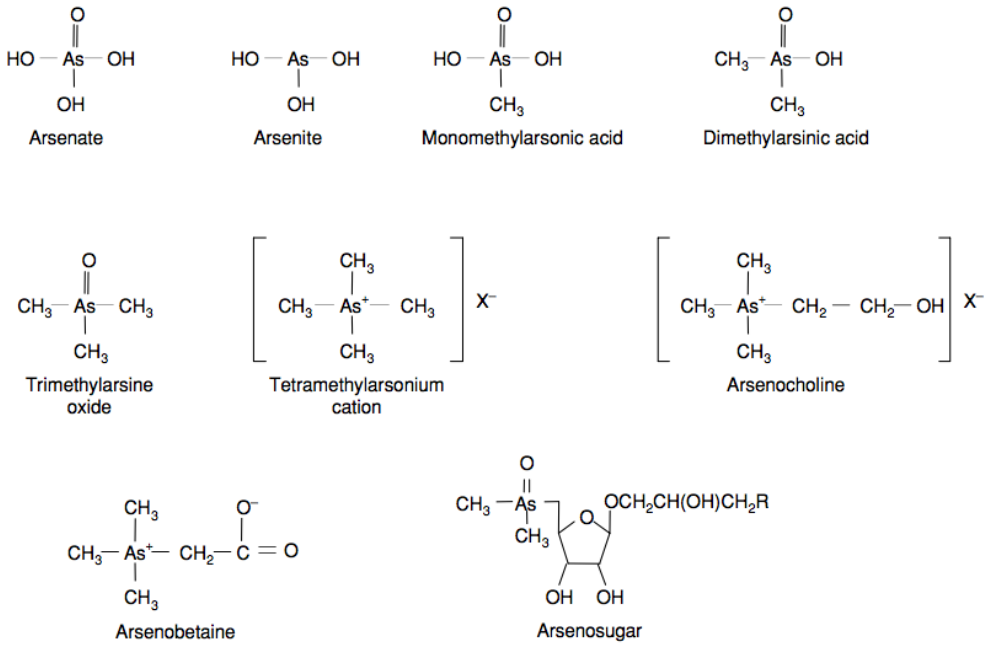
Embora os arseno-minerais e compostos de As sejam facilmente solúveis, a migração de As é limitada, devido à forte adsorção por argilas, óxidos e matéria orgânica. O enriquecimento de As em solos e sedimentos argilosos, bem como em solos superficiais, em relação às concentrações em rochas ígneas, aparentemente refletem algumas fontes externas de As, tais como erupções vulcânicas e poluição, como também o enriquecimento supergênico natural dos elementos pouco móveis, durante o intemperismo das rochas e a formação do solo. No solo, o As também está presente na fração mineral pesada do solo, mas sua contribuição é pequena (geralmente cerca de 1%). A maior proporção do total de As (27-90%) no solo está associada com a fração argila (<0,002mm) (MOTUZOVA, 1999, apud KABATA PENDIAS, 2001).

Os estados de oxidação de As são -3, 0, +3 e +5, dos quais As^0 e As^{3+} são característicos de ambientes redutores. Os ânions complexos AsO_2^-, AsO_4^{3-}, $H_2AsO^{3-,}$ $HAsO_4^{2-}$ são as mais comuns formas móveis de As, sendo adsorvidos na faixa de pH entre 7 e 9 (Figura1) (FRANKENBERGER Jr e WILLIAM, 2002; MATSCHULLAT, 2007).

O comportamento do ânion arsenato (AsO_4^{3-}) é semelhante ao dos fosfatos e

vanadatos. Na maioria das condições ambientais o As^{5+} está presente como $H_2AsO_4^-$, enquanto o As^{3+}como $H_3AsO_3^0$, sendo este dominante em ambientes com baixo Eh e pH < 9,3. (CRECELIUS et al., 1986; FRANKENBERGER Jr e WILLIAM, 2002; MATSCHULLAT, 2007).

Figura 1- Representação em diagramas das espécies de arsênio detectadas em plantas terrestres



Fonte: (Meharg e Hartley-Whitaker, 2002).

As reações de As em solos são altamente reguladas pelo seu estado de oxidação. Os íons arsenato são conhecidos por serem facilmente fixados pelos componentes do solo, tais como argilas, húmus e cálcio, sendo mais ativos na retenção de As os óxidos hidratados de Fe e Al. Hidróxidos de Al na superfície externa de minerais micáceos são considerados especialmente significantes na retenção de As (HUANG, 1975). A forte associação de As_i tanto natural quanto adicionado, com Fe (principalmente goethita) em solos foi relatada por Norrish (1975).

É pouco provável que o As fortemente adsorvido no solo seja novamente dissolvido e geralmente a retenção de As adicionado ao solo aumenta com o passar dos anos (EL-BASSAM et al., 1975). No entanto, As combinado com Fe e com os óxidos de Al pode ser liberado após hidrólise. Apesar da existência de alguns estudos, ainda se conhece relativamente pouco sobre a especiação e a dinâmica do As no solo, porém a grande presença de arsenatos indica um comportamento semelhante aos fosfatos, sendo os arsenatos ligados a

Fe ou Al provavelmente as formas mais comuns do elemento em solos, posto que a maioria das condições ambientais é oxidante (FRANKENBERGER Jr e WILLIAM, 2002; MELLO et al., 2007; MATSCHULLAT, 2007).

Elkhatib et al. (1984) afirmaram que a concentração de óxidos e o Eh são as principais variáveis do solo que controlam a taxa de sorção do arsenito e que o pH do sistema influencia a quantidade de As adsorvido na superfície dos óxidos.

Silva (2008) estudando a adsorção de arsênio em óxidos sintéticos de Fe e Al verificou que, sob condições redutoras, o As é mais fortemente adsorvido em $Al(OH)_3$ pobremente cristalizado, seguido de ferrihidrita, goethita com substituição isomórfica de Fe por Al, gibbsita e, por último, hematita e goethita, sendo que a máxima adsorção de As ocorreu em condições levemente ácidas.

A determinação da capacidade máxima de adsorção é uma prática comumente utilizada para o estudo do comportamento de certos ânions, ou mesmo cátions; que em condições ambientais se ligam principalmente ao oxigênio e passam a ter comportamento químico de ânion. A capacidade de adsorção fornece indícios do poder-tampão do sistema, visto que muitos atributos do solo (como textura, teor de óxidos de Fe e Al, entre outros) interferem na capacidade-tampão e, por consequência, influenciam a perda por lixiviação (CAMPOS et al., 2007). A adsorção química do arsenato ocorre principalmente em óxidos de Fe e Al, aluminossilicatos amorfos e, em menor extensão, nos argilossilicatos (SMITH et al., 1999). Livesey e Huang (1981) encontraram correlação significativa entre a capacidade máxima de adsorção de arsênio (CMA-As) e teores de Al e Fe extraíveis com oxalato e com o teor de argila, não encontrando correlação significativa com o pH. É importante ressaltar que há diferenciação na capacidade de adsorção para As(III) e As(V) em função do pH, visto que o aumento do pH aumenta a disponibilidade de As(V) e diminui a do As(III) (Goldberg, 1986; Jain e Loeppert, 2000).

A adsorção de As(V) em diferentes adsorventes tem sido avaliada por isotermas de Freundlich e Langmuir (SINGH et al., 1996; NAMASIVAYAM e SENTHILKUMAR, 1998; ASSIS, 2010). O ajuste dos dados de adsorção utilizando as isotermas de Langmuir, além de fornecer a CMA-As, fornece também outra importante variável, que é a energia de ligação ou, mais exatamente, a constante relacionada à energia de ligação (EL) (SMYTH e NOVAIS, 1999). Diferentes estados de oxidação do As apresentam diferentes constantes relacionadas à energia de ligação com as micelas do solo (SUN e DONER, 1998).

Conforme a Deliberação Normativa COPAM No 166, de 29 de Junho de 2011 (MINAS GERAIS, 2011), em consonância com a Resolução N°420 de 28/12/2009 do

Conselho Nacional do Meio Ambiente – CONAMA, as amostras de solo a serem estudadas a fim de se saber o seu grau de contaminação por determinado elemento ou composto tóxico devem ser submetidas ao método de extração EPA 3050b ou 3051a para que possam ser comparados com os VRQ.

O método EPA 3050b (digestão semi-total) fornece medidas de concentrações de metais relacionadas não só aos compartimentos lábeis como também a outras formas passíveis de liberação, incluindo os metais ligados à matéria orgânica, óxidos, trocáveis, adsorvidos em argila e precipitados, acessando de forma mais ampla as diferentes espécies de elementos traço. Segundo a EPA (1994), os métodos 3050b e 3051 são comparáveis, sendo aplicáveis para extrair metais e semi metais de amostras de solo, sedimento, e lodo. Atualmente, na maioria dos laboratórios, o método 3051 é preferido, visto que é realizado em tubos de teflon fechados, evitando a volatilização dos ácidos utilizados, melhorando as condições de trabalho e reduzindo o risco de acidentes e intoxicações.

Conforme já discutido anteriormente, o As pode assumir diversas formas (íons complexos) na natureza, bem como interagir (adsorção) com diversos minerais do solo. Uma ferramenta utilizada para a avaliação da biodisponibilidade de arsênio em solos é a extração sequencial, proposta por Wensel (2001), que consiste em uma marcha utilizando diversos extratores.

1.2.2 – Solo, o grande receptor de resíduos e seu valor de preservação

O solo tradicionalmente figura como receptor final de resíduos antrópicos, porém recentemente deixou de ser visto como depósito passivo, sendo entendido como um reservatório depurador de diferentes materiais, e também, muitos solos possuem grande potencial de uso como insumo em diferentes cadeias produtivas.

O desenvolvimento da política ambiental no Brasil, durante os últimos 30 anos, gerou o aprimoramento da legislação em vários aspectos. Como resultado foram criadas diversas regulamentações por parte dos órgãos governamentais integrantes do Sistema Nacional de Meio Ambiente (CONAMA 2009). Nesse contexto, a regulamentação da destinação final de resíduos urbanos, agrícolas e industriais sempre representou grande desafio.

A disposição inadequada de resíduos perigosos ou sua liberação acidental acarreta graves danos aos diferentes ecossistemas de forma individualizada. A agricultura é hoje grande gerador/receptor de resíduos via corretivos e fertilizantes, que frequentemente resultam no aumento de elementos-traço no solo. Atividades de mineração e industrial

também têm recebido grande atenção neste sentido e cada vez mais a busca por soluções para a deposição de rejeitos e resíduos se torna um desafio. Ao mesmo tempo, as grandes cidades investigam áreas para a implantação de novos aterros e deparam com diversas formas de contaminação do solo e de águas subterrâneas, o que se torna uma questão de saúde pública.

Para o elemento As, os ensaios ecotoxicológicos considerados para a definição da Máxima Adição Permitida (MAP) na legislação Holandesa foram conduzidos com plantas superiores (soja) e minhocas terrestres (CROMMENTUIJN et al., 1997), onde a MAP determinada foi igual a 4,5 mg kg^{-1}, considerando-se um solo padronizado contendo 10 % de matéria orgânica e 25 % de argila. Para a definição deste valor, foi utilizado o menor NOEC (concentração teste sem efeito observado), obtido em experimento de toxidez crônica com duração de oito semanas utilizando-se a minhoca terrestre *Eisenia fetida* (NOEC = 45 mg kg^{-1}), dividido por um fator de segurança igual a 10.

Existem na literatura nacional e internacional, diversos estudos de caso relacionados à bioacumulação, ecotoxicidade e fitotoxicidade de metais e metaloides para micro-organismos, plantas e mesofauna do solo, porém sem nenhuma padronização metodológica voltada para a definição de valores de prevenção.

1.2.3 Arsênio e o meio ambiente

A contaminação por As no meio ambiente tem recebido grande atenção devido aos seus potenciais riscos toxicológicos à saúde humana, sendo precursor de cânceres, diabetes além de ocasionar doenças de pele, distúrbios hepáticos, de visão e / ou audição, entre outros, mesmo em níveis baixos de exposição (ABERNATHY et al., 1999 apud MATSCHULLAT, 2006).

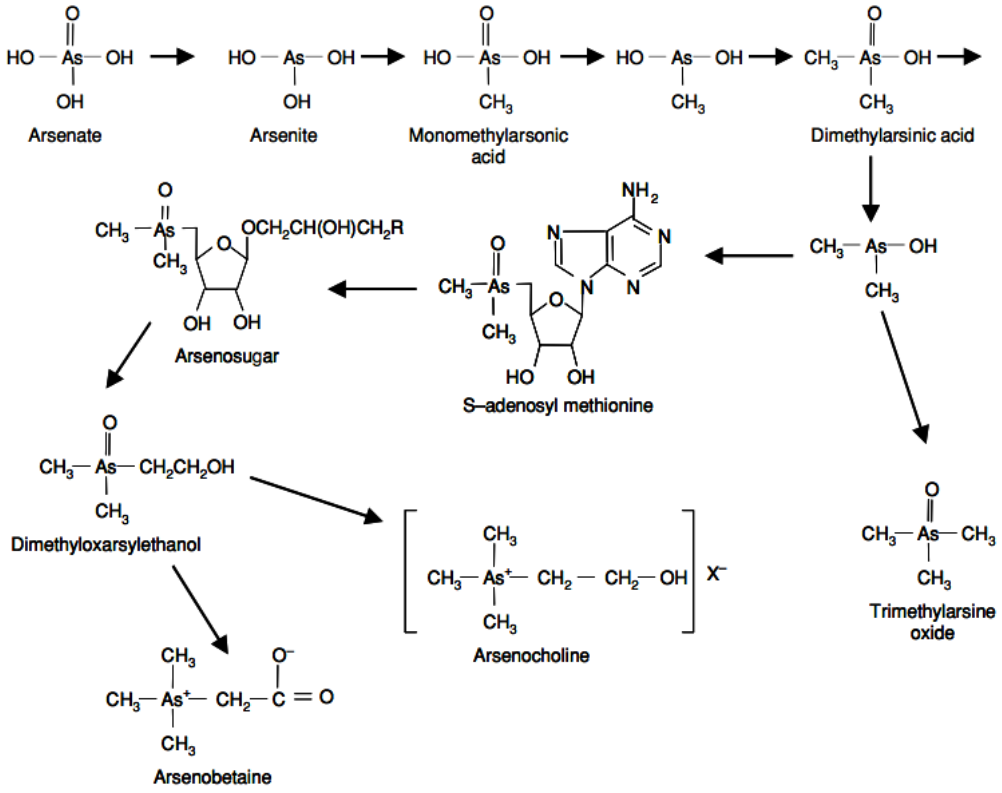
A presença de As em elevadas concentrações no meio ambiente deve-se às ações antropogênicas que incluem mineração, produção/uso de herbicidas e fungicidas, produção/uso de preservadores de madeira e corantes, fabricação de vidros dentre outras. Adicionalmente podem ocorrer acréscimos decorrentes de processos naturais oriundos de fontes geoquímicas e atividades vulcânicas (SANTOS, 2006; MATSCHULLAT et al., 2006).

Águas e solos contaminados têm sido encontrados em diversos países, incluindo o Brasil. Em estudo conduzido em Minas Gerais, foi constatada elevada contaminação do ambiente, atingindo a população. Neste estudo, avaliando a presença de metais pesados na urina de crianças, foi observado que 44,8% das crianças apresentavam níveis elevados de As e 19,2% apresentavam valores muito elevados, onde os efeitos adversos à saúde em longo prazo

não podem ser excluídos (MATSCHULLAT et al., 2000). Neste estudo, em princípio, duas grandes vias podem ser assumidas a priori para a dispersão de As no meios ambientais: i) através do intemperismo de resíduos de minas de minério, incluindo a libertação de arsenato por erosão e dissolução dos solos contaminados por As e de rejeitos de materiais em águas de superfície e sedimentos; e ii) através de atividades de fundição que libera As para a atmosfera.

O As pode ser encontrado tanto na forma de compostos inorgânicos como em orgânicos. Portanto, o As é libertado para o ambiente, tanto nas formas inorgânicas como nas formas orgânicas. Arsenato [As (V)] e arsenito [As (III)] são as formas inorgânicas de arsênio fitodisponíveis em solução no solo. No entanto, micróbios, que podem metilar e de-metilar As nos solos, podem transformar o As das formas inorgânicas em formas orgânicas e vice-versa. Além disso, invertebrados e mamíferos metabolizam formas de As inorgânicos para formas orgânicas (MEHARG and HARTLEY-WHITAKER,2002). Um esquema dessas biotransformações pode ser visto na figura 2.

Figura 2- Possíveis rotas para a redução e metilação de arsênio por plantas terrestres com base em rotas para os organismos aquáticos e fungos



Fonte: (Philips, 1990; Tmaki e Frankenberger, 1992).

Sob condições oxidantes, arsenato [AsO_4^{5-}] é a mais provável em forma de As, enquanto a sua forma trivalente, arsenito [AsO_3^{3-}] domina sob condições redutoras. Tendo em vista que há abundância de As nas águas de superfície e na atmosfera, como resultado da atividade antropogênica, verifica-se crescente preocupação com essas questões ambientais em países como o Brasil. (MATSCHULLAT et al., 2000).

Nos países europeus, a necessidade de equilíbrio entre o pró-desenvolvimento econômico, e orientação para minimização de seus potenciais impactos ambientais negativos, é visto cada vez mais como importante ferramenta para avanços tecnológicos, crescentemente inserida na educação (MATSCHULLAT et al., 2000).

O ácido dimetil arsênico (DMA) e seus sais de sódio são ainda registrados como herbicidas nos EUA (EPA 2006), de contato não seletivos, para desfolhar ou dessecar uma grande variedade de espécies vegetais. DMA e seus sais de sódio são usados em combinação, principalmente, como desfolhantes na cultura do algodão, para controle das ervas daninhas em torno de pomares de citros, renovação de gramado, controle de plantas daninhas ao redor prédios, calçadas etc. (EPA 2006). A meia-vida de DMA é de cerca de 20 dias em solos não tratados e é a principal fonte de grandes áreas contaminadas como o Arkansas (MEHARG, 2008).

1.2.4 Arsênio: toxicidade para plantas e animais

Uma vez que o As não é um elemento essencial pra o metabolismo vegetal, ou tão pouco tem função benéfica conhecida (NIES, 1999), o As pode causar toxidez em plantas e animais mesmo em baixas concentrações, na faixa de $\mu g\ kg^{-1}$ (WELCH et al., 1988; BERG et al., 2001; SMEDLEY e KINNIBURGH 2002; BUSCHMANN et al., 2008). A acumulação de As em plantas não afeta somente o seu crescimento, também causa a entrada deste elemento na cadeia trófica, o que pode causar riscos potenciais ao ecossistema, bem como à saúde humana, como por exemplo, o risco de desenvolvimento de câncer de pele, dentre outros.

Devido às semelhanças entre os elementos P e As, ambos comportam-se quimicamente de forma parecida no ambiente, formando ânions complexos em combinação com oxigênio e hidrogênio na maioria das condições de Eh e pH encontradas no meio ambiente, fato já discutido anteriormente. Tal semelhança estende-se ao comportamento destes elementos quando interagem com as células vivas, tanto animais quanto vegetais.

De maneira geral, quando dentro das células, o íon arsenato ($H_2AsO_4^-$) atua de forma semelhante a do íon fosfato ($H_2PO_4^-$), e compete por sítios de ligação. Mais especificamente,

dentro do ciclo do ácido cítrico (respiração celular), o As inibe a ação da enzima piruvato desidrogenase, ligando-se às moléculas de adenosina di-fosfato (ADP) e formando a molécula As-ADP, desacoplando assim a fosforilação oxidativa e impedindo a respiração mitocondrial e a síntese de ATP (PANDA et al., 2009).

Em suma, temos que o As, na sua forma penta valente, que é predominante na maioria dos solos de mineralogia oxídico-caulinítica de regiões tropicais, bem drenados, moderadamente ácidos e com condições oxidantes (elevado Eh), quando adicionado a estes solos, que possuem naturalmente baixos teores deste elemento, reage de forma reversível com a fração argila, adsorvendo-se preferencialmente nas superfícies carregadas positivamente dos óxidos de Fe e Al, como goethita, hematita e gibbsita. Tal reação é específica e compete por sítios de ligação com o P na sua forma iônica $H_2PO_4^-$, nas faixas normais de pH levemente ácido e Eh elevado, tendendo a aumentar essa ligação, sendo esta reversível com a adição de fosfatos. A reversibilidade da reação de adsorção de As também pode ser causada pela redução do Eh (ambiente redutor) e elevação do pH do solo alagado (saturado por água), devido à dissolução dos minerais de Fe e Al, o que também deve ser considerado em casos de suspeita de contaminação.

1.2.5 Dinâmica do arsênio em áreas de rizicultura

A concentração de As no arroz é um assunto particularmente problemático, dado que o arroz é a única cultura de grande escala para consumo cultivada anaerobicamente, ou seja, sob condições de alagamento. Este fato, em conjuntamente com a própria fisiologia da planta, cria as condições para que o arroz seja eficiente na absorção, translocação e assimilação de algumas formas de As (Williams et al., 2007).

Os processos químicos que ocorrem nos arrozais, quer a nível das águas, quer do solo, facilitam uma excessiva mobilização de As e subsequente absorção deste elemento químico pelo arroz. No entanto, para que ocorram esses processos, o primeiro fator a considerar é a quantidade de As presente. O As presente naturalmente no solo sem pressão antrópica resulta essencialmente do As constituinte das fases sólidas que constituem a rocha-mãe do solo. Os níveis de As na crosta terrestre situam-se abaixo dos 2 mg/kg, podendo no entanto ser bastante superiores a esse valor em rochas ígneas e metamórficas (Smedley & Kinniburgh, 2002). De fato, um grande número de minerais (mais de 200) contem As, sendo a sua inteperização uma fonte importante de As.

As adições de As devidas a resíduos industriais não são normalmente consideradas, mas os seus efeitos não podem ser subestimados, pois em algumas regiões de produção de arroz situam- se a jusante de centros industriais e urbanos importantes e o arroz aí cultivado apresenta elevadas concentrações de As (caso da Camarga, França, com uma média de 0,28 mg As/kg; Meharg et al., 2009).

As espécies inorgânicas, arsenato [As(V)] e arsenito [As(III)], são sensíveis às condições redox: o arsenato predomina geralmente em condições oxidantes e o arsenito em condições redutoras (Zhao et al., 2010). As alterações de pH e de Eh (potencial de redução) são determinantes no balanço entre estas duas espécies, bem como alguns compostos químicos oxidantes ou redutores.

Nos arrozais, o pH dos solos tende a centrar-se em valores próximos da neutralidade após o alagamento, dando-se a redução dos arsenatos antes da redução dos sulfatos. Quando a água dos arrozais é drenada, como é normal na fase de enchimento do grão, as reações anteriormente descritas são invertidas, registando-se a oxidação de Fe(II) e arsenito, com uma acentuada redução da concentração de arsénio dissolvido na água (Arao et al., 2009). Dessa forma, o alagamento e a consequente alteração das condições redox têm uma importância determinante na disponibilidade de arsénio no meio para a planta.

As espécies arsenato e arsenito, em condições propícias, apresentam capacidades distintas para reagir com várias fases sólidas presentes no solo. Sob condições oxidantes, o arsenato tem uma grande capacidade para reagir com os óxidos/oxihidróxidos de ferro e óxidos de manganês (Chen et al., 2005), dependendo do valor de pH do solo, o que o torna relativamente imóvel no solo pois pode ser adsorvido na superfície daquelas fases sólidas; o arsenito, por sua vez, apresenta menor capacidade de reação com os referidos compostos e é, geralmente, mais móvel. A mobilização de arsénio em condições de alagamento é geralmente acompanhada pela mobilização paralela de ferro (Takahashi et al., 2004).

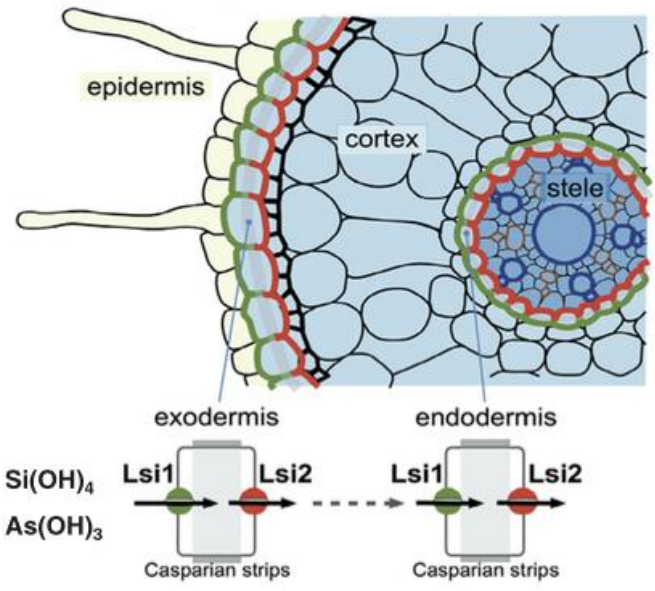
A escassez de oxigênio ao nível do solo é compensada pelas próprias raízes da planta, que provocam a aeração da sua rizosfera – libertando oxigénio molecular através do aerênquima – para sobreviverem em condições altamente redutoras, criando gradientes redox desde a superfície da raíz precipitação de óxidos e hidróxidos de ferro na superfície da raíz.

Elevados níveis de As no solo provocam grande preocupação com relação a captação pelas plantas e posterior entrada em cadeias alimentares humanas. O As existe tanto em formas inorgânicas como orgânicas, com a interconversão entre elas regulada pelos processos bióticos e abióticos. Para compreender e gerir os riscos colocados pelo As no

solo é essencial conhecer como o As é absorvido pelas raízes e metabolizado dentro das plantas (Figura 3).

ABEDIN et al (2002) avaliaram os efeitos da água de irrigação contaminada com As no crescimento do arroz. O aumento da concentração de As na água de irrigação diminuiu sensivelmente a altura das plantas, rendimento de grãos, o número de grãos cheios, o peso de grãos, raiz e biomassa, enquanto as concentrações de As na raiz, palha e casca de arroz aumentou significativamente. As concentrações de As na palha de arroz (até 91,8 mg kg^{-1}) eram da mesma ordem de grandeza que as concentrações de As na raiz (até 107,5 mg kg^{-1}), sugerindo que o As pode ser facilmente translocado. Embora não abrangida por regulamentos de segurança alimentar, a palha de arroz é utilizada como alimento para bovinos em muitos países. As elevadas concentrações de As podem ter efeitos adversos para a saúde do gado e um aumento da exposição ao As pode ocorrer nas pessoas pelo consumo de carne e leite. A concentração de As nas várias partes da planta do arroz irrigado não foi afetada pela aplicação de P, com exceção da casca (ABEDIN et al., 2002). Esses dados são semelhantes aos encontrados no milho por Abbas e Meharg (2008).

Figura 3 - Absorção de arsenito via raízes de arroz.



Fonte: Modificado de Ma et al. (2007). Lsi1 e LSI2 são transportadores de influxo e de efluxo de Si respectivamente, respectivamente.

MARIN et al (2002) também avaliaram a absorção do arsênio pelo arroz em relação à forma química (arsenito [As (III)]; arsenato [As (V)]; monometílico ácido arsênico (MMAA); e dimetil ácido arsênico, (DMA) e duas cultivares (Lemont e Mercúrio), com um diferente grau de suscetibilidade a uma doença fisiológica atribuída a toxicidade do arsênio. As(V) não afetaram o crescimento de plantas, As (III) e MMAA foram fitotóxicos de arroz. Disponibilidade de arsênio ao arroz acompanhou a tendência: DMA <As (V) <MMAA <As (III). Após a absorção, DMA foi prontamente translocado para a parte aérea. Arsênio (III), As (V), e MMAA acumulada nas raízes. (MARIN et al., 2002).

As interações do arsênio com biomoléculas auxilia na compreensão do metabolismo e da resistência de algumas espécies. Enquanto As (V), sob a forma de arsenato liga-se a sítios de ligação de fosfato, As (III) é conhecido por ter uma elevada afinidade para sítios ricos em tiolato. Bactérias como *Escherichia coli* desenvolveram resistência ao As utilizando o Ars operon, que é regulado por ArsR, um repressor de proteína que se dissocia do DNA quando As (III) se liga. Esta proteína sofre uma mudança conformacional crítica sobre a ligação do As (III) com três resíduos cisteína (TOUW et al., 2007).

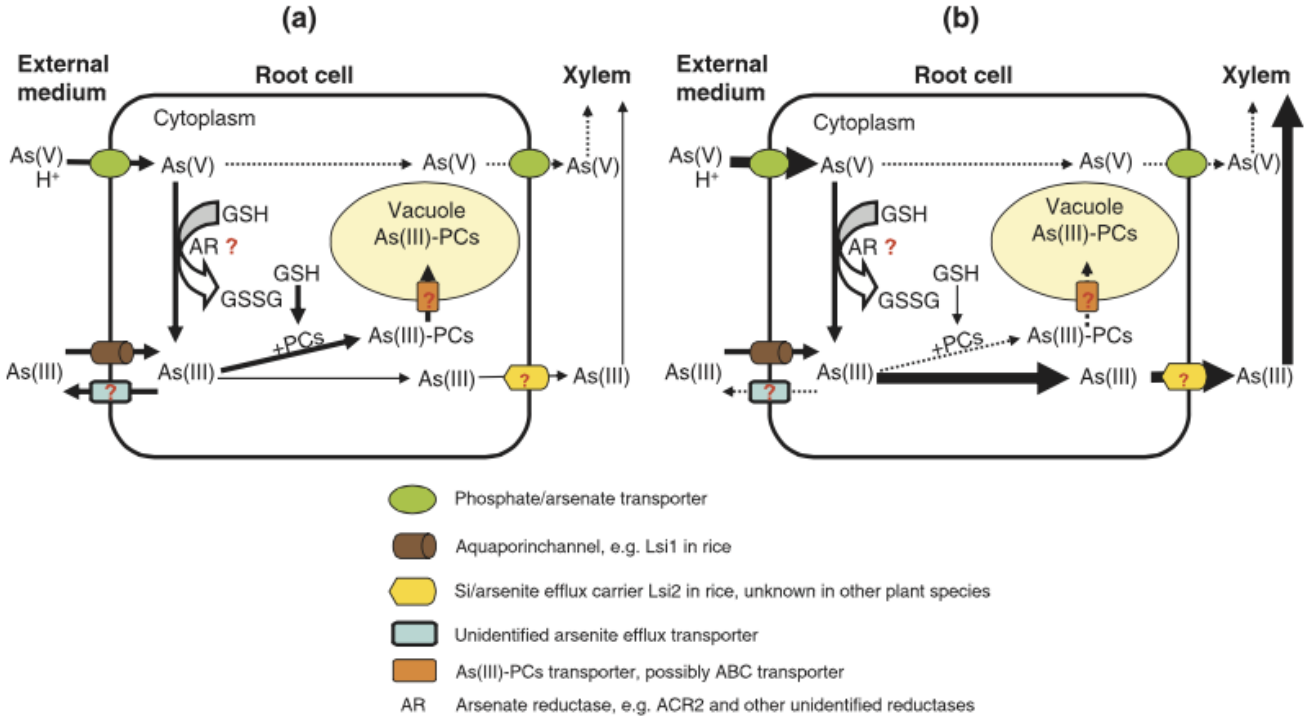
1.2.6 Variedades resistentes e não resistentes ao arsênio

Algumas espécies de plantas apresentam variação fenotípica em resposta ao As, o que nos ajuda a compreender a toxicidade do As e a forma com que plantas têm desenvolvido resistência ao As (Figura 4) (MEHARG and HARTLEY-WHITAKER, 2002).

Resistência ao As tem sido identificada em uma série de espécies em solos contaminados com As, incluindo: *Andropogon scoparius* (ROCOVICH e WEST, 1975), *Agrostis castellana, A. delicatula* (De KOE e JACQUES, 1993), *A. capillaris, Deschampsia cespitosa* (MEHARG e MACNAIR, 1991), *H. lanatus* (MACNAIR e CUMBES, 1987), *S. vulgaris* (PALIOURIS e HUTCHINSON, 1991), *Plantago lanceolata* (POLLARD, 1980), e *C. vulgaris* (SHARPLES et al., 2000) (MEHARG; HARTLEY-WHITAKER, 2002).

Plantas não resistentes ao arsenato podem tornar-se mais resistentes elevando seus status de P, que por sua vez contribui para reduzir os níveis de acumulação através da supressão do sistema de captação fosfato/arsenato, isto para ambiente aerados (MEHARG; HARTLEY-WHITAKER, 2002).

Figura 4. Diagrama esquemático da absorção e metabolismo do arsênio em raízes de não hiperacumuladoras (a) e (b) hiperacumuladoras.



Espessura da linha refere-se a taxa de fluxo, a linha pontilhada indica o ritmo mais lento. Pontos de interrogação indicam grandes lacunas de conhecimento.

Grandes variações na concentração de As em grãos de diferentes variedades de arroz têm sido observada em áreas consideradas de alta contaminação como Bangladesh (DUXBURY et al., 2003;. ZAVALA e DUXBURY 2008). Pesquisas com amostras comerciais também revelou variabilidade semelhante (WILLIAMS et al., 2006). Parte dessa variabilidade pode ser explicada por diferenças nos níveis de irrigação de águas subterrâneas com As (WILLIAMS et al., 2006) e do tipo de solo (LU et al., 2009). Entretanto, um estudo recente indicou que a variação de As no grão de arroz é controlada em grande parte pela genética do arroz (NORTON et al., 2009).

1.2.7 Exposição ao arsênio e estresse oxidativo de plantas

O As assim como os metais pesados, estimula a formação de intermediários reativos de oxigênio (EROS). As EROs são formas parcialmente reduzidas do oxigênio atmosférico, resultantes da excitação do O_2 para formar oxigênio singleto (O_2^1) ou da transferência de um, dois ou três elétrons para o O_2 para formar, respectivamente, o radical superóxido (O_2^-), peróxido de hidrogênio (H_2O_2) ou o radical hidroxila (OH^-) (MITTLER, 2002). Elas são

geradas endogenamente durante transições do desenvolvimento, tais como na maturação de sementes, e como um resultado normal do metabolismo respiratório e do processo fotossintético.

No entanto, uma ampla variedade de estresses ambientais (tais como temperaturas extremas, seca, salinidade, UV, metais pesados e infecção por patógenos) são potencialmente danosa às plantas (VAN BREUSEGEM et al., 2001), uma vez que, sob tais condições ambientais adversas, a homeostase redox da célula é interrompida (FOYER et al., 1994), porque cessam o transporte de elétrons na cadeia transportadora de elétrons (CTE) (VAN BREUSEGEM et al., 2001). Com o rompimento da homeostase celular, ocorre a aceleração da produção de EROs (LAMB e DIXON, 1997), o que pode causar estresse oxidativo (ASADA, 1994). A produção aumentada de EROs durante o estresse pode ser uma ameaça às células, mas sua produção pode ter função de sinalização para a ativação dos processos de defesa e resposta ao estresse (DESIKIN et al., 2001; KNIGHT e KNIGHT, 2001). A ação das EROs como danosas ou como sinalizadoras e protetoras depende do equilíbrio delicado entre a produção de EROs e sua limpeza em local e tempo apropriado, através da ação do sistema de defesa antioxidante (Fig. 4). A toxicidade do oxigênio pode resultar em dano tecidual e até morte celular se houver produção descontrolada ou limpeza ineficiente das EROs (EDREVA, 2005).

As defesas antioxidantes em plantas incluem a participação de antioxidantes enzimáticos (SOD, CAT, POX, APX,GR) e antioxidantes não enzimáticos (glutationa, antocianinas), com a finalidade de diminuir os efeitos tóxicos dos EROs (VALKO et al., 2006) .

A toxicidade do As para as plantas pode ser induzida através da geração de EROs. Estudo com trigo mostrou que a atividade arsenato redutase (AR) aumentou em 108% com exposição ao As sob condição P-deficiente e 130% sob condição P- suficiente, mas não exibiu atividade AR em resposta ao tratamento com P. Adicionalmente a concentração de H_2O_2 e malondialdeído (MDA) nos tecidos aumentou significativamente sob exposição ao As, sobretudo na situação P-deficiente, aumentando em 180% de H_2O_2 e 51% para o MDA. Atividade da Superóxido Dismutase (SOD) e Peroxidase (POD) diminuíram significativamente; atividade da Catalase (CAT) diminuiu em condições P-deficientes e aumentou sob condições P-suficientes; Glutationa (GSH), Ascorbato (ASA) e tióis não proteicos (NPSH) aumentaram suas concentrações quando expostos ao As sob condição P-deficiente (WANG et al., 2002)

Estudos com milho sugerem fortemente que a indução de estresse oxidativo é um processo principal subjacente a toxicidade do As em plantas (REQUEJO and TENA, 2005). Os efeitos do As na expressão dos genes que codificam SOD, CAT e glutationa S-transferase, foram examinados em diferentes fases de desenvolvimento e em diferentes tecidos de milho. Os resultados indicam que o As aciona genes relacionados respostas de defesa antioxidante e desintoxicação específico, conforme tecidos e estádio de desenvolvimento de milho (MYLONA et al., 1998).

Espécies de plantas capazes de acumular metais pesados são de grande interesse para fitorremediação, e diferem em sua capacidade de acumular metais a partir do ambiente. Níveis mais elevados de SOD, CAT, e APX foram observados em variedades de samambaia mostrando sua participação ativa no mecanismo de desintoxicação do As. Maior atividade das enzimas antioxidantes em plantas de *P. vittata* tratadas com As resultaram em hiper acumulação de As sem sintomas de toxicidade (SRIVASTAVA et al., 2005).

1.2.8 Arsênio: toxicidade aos seres humanos

Mesmo a baixas concentrações, a exposição ao As pode dar origem a uma variedade de sintomas adversos. Os mais comuns são lesões no fígado, pulmão, pele, bexiga e rim (SMITH et al., 1992). Para tentar neutralizar seus efeitos negativos, o metabolismo humano tenta excretar o máximo possível deste elemento através da urina. Os passos básicos de desintoxicação incluem absorção de espécies inorgânicas no intestino, a metilação no fígado, principalmente DMA e excreção através da urina (SUZUKI et al., 2001). No entanto, um efeito colateral negativo do processo de desintoxicação é a produção dos intermediários metilados no fígado humano. Estes intermediários, ácidos metilados, são responsáveis por muitos efeitos negativos, sendo capazes de aumentar a toxicidade do produto inorgânico original ingerido, resultando em genotoxicidade e alteração enzimática (DOPP et al., 2010).

Adicionalmente inúmeros ensaios com citogenética vêm mostrando grande impacto do As no metabolismo humano (Tabela 1).

Tabela 1 - Ensaios de citogenética com exposição ao arsênio.

Modelo experimental	Forma química do arsênio	Dano observado	Referência
Allium cepa	DMA	CA	Nygren, 1949
Células fetais de rato, *in vivo*	Arsenio trioxido	CA	Nagymajtenyi et al., 1985
embriões de camundongos	Arsenito	MN	Muller et al., 1986
Ovelha	Arsênio inorgânico	SCE	Gebel et al., 1996
Linfócitos humanos, *in vitro*	Arsenato	CA	Petres et al., 1970
Fibroblastos da pele humanos	Arsenito	CA	Lee et al., 1986
Fibroblastos humanos	Arsenito	CA	Happle et al., 1973
Células da bexiga humanos, *in vivo*	Arsênio inorgânico	MN	Warner et al., 1994
Linfócitos humanos, *in vivo*	Arsênio inorgânico	SCE	Burgdorf et al., 1977

*Aberrações cromossômicas (CA); dano em cromátides (SCE); formação de micronúcleos (MN).

1.2.9 Estratégias de mitigação

Entre a nutrição e a toxicidade de micro nutrientes existe uma caminho muito estreito. Em se tratando de elementos benéficos ou como no caso do As, sem valor metabólico conhecido, a toxidez pode ocorrer já em doses muito baixas. Devido sua grande ocorrência no meio ambiente, tem sido dada atenção merecida no sentido de técnicas de mitigação para minimizar as concentrações de As principalmente em espécies que apresentam uma taxa de translocação significativa para o grão, como o arroz em grãos (MEHARG, 2004).

Neste sentido algumas estratégias têm sido bem sucedidas, entre elas:

- Manejo de irrigação. Sob inundação contínua a biodisponibilidade de As é aumentada para as plantas (XIE e HUANG, 1998; MASSCHELEYN et al., 1991). De acordo com Somanahalli, et al. (2011), a inundação intermitente pode reduzir até 30% do As em grãos de arroz. Spanu, et al. (2012) utilizaram um tipo de irrigação por aspersão reduzindo cerca 90% da translocação de As para grãos de 37 cultivares de arroz.

- Alteração do solo. O efeito da aplicação de P no solo influencia a absorção de As principalmente por adsorção competitiva entre AsV (condições aeróbicas) e, em menor medida, AsIII (QUAFOKU et al., 1999). Estudos demonstram um aumento na mobilidade de As com acréscimo de P sob condições de inundação (MEHARG, 2004; PERYEA , 1991). O fosfato desloca o As sorvido às partículas do solo, o que aumenta a solubilidade, fito-disponibilidade, e movimento para baixo do perfil do solo (ABEDIN et al., 2002). Muito embora bons resultados tenham sido descritos para arroz sequeiro com incremento de P, para arroz irrigado as informações ainda são

controvérsias, sendo assim inconclusivas (HUANG et al., 2010; ABEDIN et al., 2002). Assim, o impacto da aplicação de P em arroz irrigado não está completamente esclarecido.

- Uso de diferentes materiais genéticos. Trabalhos têm demonstrado uma grande diferença entre cultivares quanto a translocação e tolerância ao As (MEHARG, 2004, ABEDIN et al., 2002). Muito embora cultivares modernas apresentem um significativo estreitamento na variabilidade genética, ainda assim, é notável a diferença entre cultivares de um mesmo grupo.
- Outras estratégias. A atividade microbiana tem demonstrado impacto significativo sobre a biodisponibilidade de As (ZHANG et al., 2004; SOMENAHALLY et al., 2011) sendo um campo promissor a ser seguido. No ambiente do solo, esgotamento de oxigênio ocorre com a atividade microbiana e decomposição da matéria orgânica em condições alagadas (PILLAI et al., 2010). A concentração de espécies orgânicas tais como ácido dimetil arsênico (DMA) em solo foi positivamente correlacionada com a metilação microbiana de AsIII para DMA em condições anóxicas (SOMENAHALLY et al., 2011). Além disso, a presença de sorventes reduz fortemente a taxa de redução AsV por bactérias (HUANG et al., 2011).

1.3 PROPOSIÇÃO

Diante do exposto, este trabalho teve como objetivo avaliar a ecotoxicidade de As em diferentes sistemas experimentais, por meio de ensaios de toxidez com plantas de arroz e cebola, avaliando ainda a influência dos fatores manejo do solo, disponibilidade de P, manejo de irrigação, uso de diferentes cultivares e tipo de solo; visando subsidiar a revisão do impacto de As em grãos comerciais na América Latina e distinções fisiológicas entre cultivares.

1.4 MATERIAIS E MÉTODOS

A estratégia utilizada para a presente Tese foi primeiro avaliar inicialmente se as amostras comercializadas no Brasil e países vizinhos continham níveis elevados de As. A seguir, havendo As nas amostras comerciais, como seriam os níveis de As nos solos do RS destinados ao cultivo do arroz e se as concentrações de As poderiam variar entre os diferentes cultivares; havendo As, poderiam os diferentes nanejos do solo interferir com os níveis de As

presentes nas plantas? Diferentes cultivares teriam diferentes rotas metabólicas para a mitigação do As? Sabendo-se do potencial tóxico deste elemento para humanos, poderiam as alterações no DNA causados pelo As serem minimizadas com o uso de fitoterápicos?

Portanto, para os estudos em relação ao As, foram conduzidos análise de amostras comerciais de arroz; experimento a campo; estudo com citogenética para avaliação da toxicidade; cultivo em hidroponia e vaso de areia. Os resultados destes são discutidos na forma de sete manuscritos abaixo brevemente descritos.

1.4.1 Manuscrito 1

O manuscrito 1 refere-se à uma extensa coleta a campo. As amostras de solo e de arroz foram coletadas no Rio Grande do Sul, Brasil, durante a estação de crescimento 2012/13; além da análise de amostras comerciais de arroz da Argentina, Brasil, Paraguai e Uruguai.

Todas as áreas dos experimentos de campo tem um histórico de produção de arroz de longo prazo (pelo menos 15 anos). Três amostras de solo compostas (500 g) foram obtidas de 15 sub-amostras, coletados a partir da camada de solo 0 a 20 cm, e, em seguida, armazenados em sacos plásticos até a análise (Figura 5).

1.4.1.1.

Este estudo avaliou três diferentes cultivares: BR-IRGA 409, IRGA 424 e Puitá Inta CL, em 24, 24 e 25 áreas respectivamente, totalizando 73 áreas avaliadas.

Todas as lavouras foram marcadas utilizando GPS para análise da distribuição espacial. No geral, as áreas mostraram semelhanças quanto às pragas e gestão de fertilidade, no entanto com 4 estratégias distintas em relação ao período entre a colheita e a rotação de culturas: 1) a cultura do arroz exclusivo, sem cultivo durante a temporada de inverno; 2) uma safra com soja seguida por duas safras com o cultivo de arroz, sem cultivo durante a safra de inverno; 3) uma safra com o cultivo de arroz, seguido por duas ou três safras em pousio, com semeadura de azevém durante o inverno; e 4) a rotação das culturas de soja e arroz.

1.4.1.2 Análise química

Para especiação de As, lotes de até 48 amostras foram preparadas, incluindo 2 brancos e 2 controles de CRM (NIST 1568b farinha de arroz) que contém espécies As_i e DMA em

concentrações certificadas. As amostras foram digeridas em micro-ondas (CEM MARS 6). Para análise de multi elementos por ICP-MS, um procedimento de digestão mais agressivo era utilizado. As propriedades químicas das amostras de solo foram determinadas seguindo a metodologia proposta por Tedesco et al. (1995).

1.4.1.3 Mapas de distribuição espacial

Para confecção dos mapas de distribuição espacial, foi elaborada uma matriz geográfica utilizando GPS Track Maker, com um erro médio de 4 m, usando coordenadas UTM WGS84. A localização dos pontos de coleta foi definida a partir da conversão da localização geográfica em UTM. Geração de mapas de distribuição espacial foi realizada utilizando o módulo de análise espacial (Spatial Analyst) do s*oftwar*e ArcGIS Desktop 10.0 (ESRI), utilizando a interpolação de *Inverse Distance Square* (IQD) com expoente 2 conforme Reis et al (2005).

Para o cálculo do valor de ponto de interpolação foi utilizado o método DCI, que utiliza a seguinte equação:

$$Z(x) = \frac{\sum_{i=1}^{n} \omega_i Z(x_i)}{\sum_{i=1}^{n} \omega_i}$$

* Z (x) - é o valor do ponto que deseja interpolar; N - é o número de pontos utilizados no próximo ponto de interpolação x; Z (XI) - é o valor de xi; e ωi - é o valor do peso de XI / x. Para determinar ωi foi utilizada a seguinte equação:

$$\omega_i = \frac{1}{h(x, x_i)^p}$$

* h (x, x) - é a distância entre o ponto x e ponto xi; p - é o parâmetro de energia geralmente é igual a dois (MARCUZZO et al 2011).

A partir da matriz geográfica foram preparados grupos de mapas temáticos:.Concentração de arsênio (As), ferro (Fe), fósforo (P) e matéria orgânica (MO) no solo e total (AST) e arsênio inorgânico (Asi) em amostras de arroz interpolados por IQD

Figura 5 - Coleta de solo (A, B, C), lavoura de arroz durante o estágio vegetativo (D, E), separação de colmo principal e perfilhos marcados (F).

Fonte: autora

1.4.1.4 Amostras comerciais de arroz

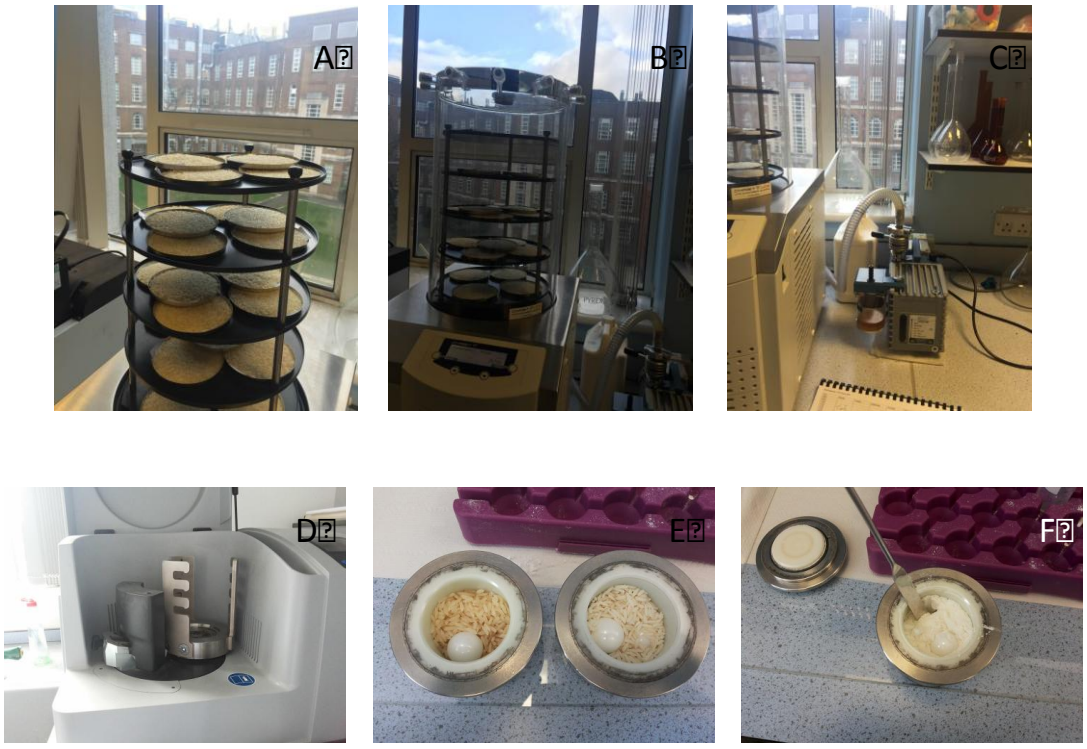
Amostras de arroz foram adquiridas de varejistas da Argentina, Brasil, Uruguai e Paraguai num total de 180 amostras (o percentual de 85% de arroz branco foi mantido para todos os países). Para o arroz brasileiro, as amostras foram obtidas das 5 regiões orizícolas (Norte, Nordeste, Centro-Oeste, Sudeste e Sul) e uma dessas regiões (Sul - Rio Grande do Sul) foi sub-amostrada em 4 regiões (Sudeste, Sul, Oeste e Central).

1.4.1.5 Especiação de As

Amostras de grãos de arroz foram desidratadas com auxílio de *freeze drier* (Figura 5) e posteriormente moídas em um moinho com esferas de zircônio (Figura 6). Para especiação de As, lotes de até 48 amostras foram preparados, incluindo 2 brancos e 2 amostras controle de CRM (NIST 1568b farinha de arroz) que contem espécies As_i e DMA em concentrações certificadas. As amostras foram digeridas com auxilio de micro-ondas (CEM MARS 6). Para

análise de multi elementos por ICP-MS, um procedimento de digestão mais agressivo foi utilizado.

Figura 6 - Amostras de arroz no freeze drier (A, B, C). Máquina para moagem de arroz com esferas de zircônio (D, E, F).



Fonte: autora

1.4.1.6 Estimativa da ingestão de espécies de arsênio diárias

Consumo diário estimado foi calculado como: EDI = Cce × Mrdc, / Bw onde EDI é a estimativa de ingestão diária do produto (mg / kg de peso corporal / dia), a CCE é a concentração média de As_i ou Al em arroz, ponderada pelo consumo de arroz brasileiro. Mrdc é a massa de arroz consumida diariamente no Brasil, com base em uma pesquisa nacional brasileira de consumo de arroz (IBGE, 2010). E BW é o peso corporal. Consumo diário estimado foi comparado com o *Provisional Tolerable Daily Intake* (PTDI) para As_i e Al conforme normativa da FAO/OMS

1.4.1.7 Análise estatística

As análises estatísticas foram realizadas utilizando o procedimento de Modelos SAS 8.2 Geral Linear. Teste de Kruskal-Wallace no Minitab v.16 . Sigma Plot (v9.0) e Minitab (v11) foram usados para ajustar as curvas e para a análise estatística descritiva de espécies de As, respectivamente.

1.4.2 Manuscrito 2

1.4.2.1 Cultivo da Allium Cepa e Rosmarinus Officinalis

Mudas de cebola (*Allium cepa* L.) foram obtidos pela Universidade Federal de Santa Maria (UFSM), RS, Brasil. As plântulas foram transplantadas para tabuleiros de plástico contendo substrato e cultivadas em uma estufa com sombreamento (50%). No final do ciclo, as plantas foram colhidas e utilizados para o teste de Allium cepa e análises bioquímicas. As plantas de alecrim foram cultivadas em casa de vegetação de 115 m^2, coberta com polietileno aditivado com anti-UV 200 mm de espessura, na UFSM, Rio Grande do Sul, Brasil. Vasos de polipropileno (2,8 dm^3) com uma planta por vaso foram enchidos com areia lavada (entre 1 mm e 3 mm de tamanho de grão). Durante o cultivo quatro irrigações diárias com solução nutritiva como descrito por Pardossi et al. (2011) foram realizadas para manter o nível de água nos vasos. As folhas de 10 plantas foram colhidas em 160 dias após o plantio. Estas folhas secaram à sombra durante 5 dias à temperatura ambiente, antes da preparação de extratos aquosos e a extração de óleo essencial.

1.4.2.2 Obtenção de extrato aquoso e óleo essencial de alecrim

As infusões foram obtidas com água destilada a 100 ° C, na concentração de 5 e 20 g L^{-1}, por 15 minutos (MARTINS et al., 2000). Estes extratos foram filtrados e analisados por cromatografia líquida de alta eficiência com detecção de arranjos de diodos (HPLC-DAD) para a identificação e quantificação dos compostos fenólicos.

Para a extração óleos essenciais, 30g de folhas das plantas, coletadas em quatro repetições, foram submetidas a hidrodestilação em um aparelho Clevenger, durante 3 horas, e

o óleo seco sobre sulfato de sódio anidro, armazenados a -4 ° C e analisados por cromatografia gasosa.

1.4.2.3 Análise citogenética (teste Allium cepa)

A análise citogenética de células meristemáticas obtidos a partir de radículas de A. cepa foram usadas para avaliar as modificações morfológicas e estruturais causadas pela exposição ao As e determinar o índice mitótico. Foram utilizados os seguintes tratamentos: T1 água destilada, como um controle negativo; T2 óleo de *R. officinalis* 0,8%; T3 óleo de *R. officinalis* 3%; T4 extrato de *R. officinalis* 5 g L^{-1}; T5 extrato de *R. officinalis* 20 g L^{-1}; T6 arsênio, para verificar o efeito individual de cada solução; T7 óleo de *R. officinalis* 0,8% (24 hs) + arsênio (24 hs); T8 óleo de *R. officinalis* 3% (24 hs) + arsênio (24 hs); T9 extrato de *R. officinalis* 5 g L^{-1} (24 hs) + arsênio (24 hs); T10 extrato de *R. officinalis* 20 g L^{-1} (24 hs) + arsênio (24 hs); T11 arsênio (24 hs) + óleo de *R. officinalis* 0,8% (24 hs), para verificar o efeito de *R. officinalis* antes da exposição ao arsênio; T12 arsênio (24 hs) + óleo de *R. officinalis* 3% (24 hs); T13 arsênio (24 hs) + extrato de *R. officinalis* 5 g L^{-1} (24 hs); T14 arsênio (24 hs) + extrato de *R. officinalis* 20 g L^{-1} (24 hs), para verificar o efeito de *R. officinalis* após exposição ao arsênio; T15 Arsênio com óleo de *R. officinalis* 0,8%; T16 arsênio com óleo de *R. officinalis* 3%; T17 arsênio com extrato *de R. officinalis* 5 g L^{-1}; T18 arsênio com extrato de *R. officinalis* 20 g L^{-1}, para verificar o efeito de *R. officinalis* usado concomitantemente com exposição ao arsênio; T19 etanol para verificar o efeito do álcool.

1.4.2.4 Análises bioquímicas e enzimas

A determinação de TBARS foi realizada conforme El-Moshaty et al (1993); a concentração de H_2O_2 foi determinada conforme Loreto e Velikova (2001); e atividade de POD de acordo com Chance e Maehly, 1955.

1.4.2.5 Análise estatística

Os experimentos foram realizados em delineamento inteiramente casualizado. As análises de variância foram calculadas sobre as diferenças estatisticamente significativas determinadas com base em F-teste. Os resultados são apresentados como a média ± SD de

pelo menos três replicatas independentes. As diferenças entre médias foram comparadas utilizando-Scott-Knott (P <0,05).

1.4.3 Manuscrito 3

1.4.3.1 Plantas e condições de crescimento

Mudas de arroz da variedade indica foram obtidas a partir da germinação de sementes fornecidas pelo IRGA (Instituto Rio Grandense do Arroz), RS, Brasil. As sementes de três cultivares de arroz usados no sul do Brasil, BR-IRGA 409, IRGA 423 e IRGA 424, foram utilizadas neste estudo. As sementes pré-germinadas foram transferidas para vasos de plástico revestidas com papel de filtro e colocadas em câmaras de crescimento parcialmente fechadas; estes vasos foram então irrigados com água destilada, durante dez dias. Depois de dez dias em água destilada, as mudas foram transferidas para vasos de plástico contendo 8 L de solução nutritiva.

O experimento em solução de cultivo foi finalizado em três fases:

Na fase I, todas as plântulas foram cultivadas em solução nutritiva completa (+ P) e sem As (-As) durante 5 dias.

Na fase II, metade das plantas foi enxaguadas três vezes com água destilada e transferidas para vasos contendo uma solução nutritiva sem fósforo (-P), e as demais plântulas foram cultivadas em solução de +P. Dessas, metade de cada grupo teve As adicionado na solução nutritiva como $Na_3AsO_4 \bullet 12\ H_2O$ (+ As) sob a concentração de 100 µM. Essa fase durou 5 dias.

Na sequencia, metade das plantas +P e -P com e sem exposição ao As foram coletadas (25 plantas por repetição, cada tratamento consistiu de 4 repetições) colhidas e separadas em parte aérea e raízes de forma aleatória.

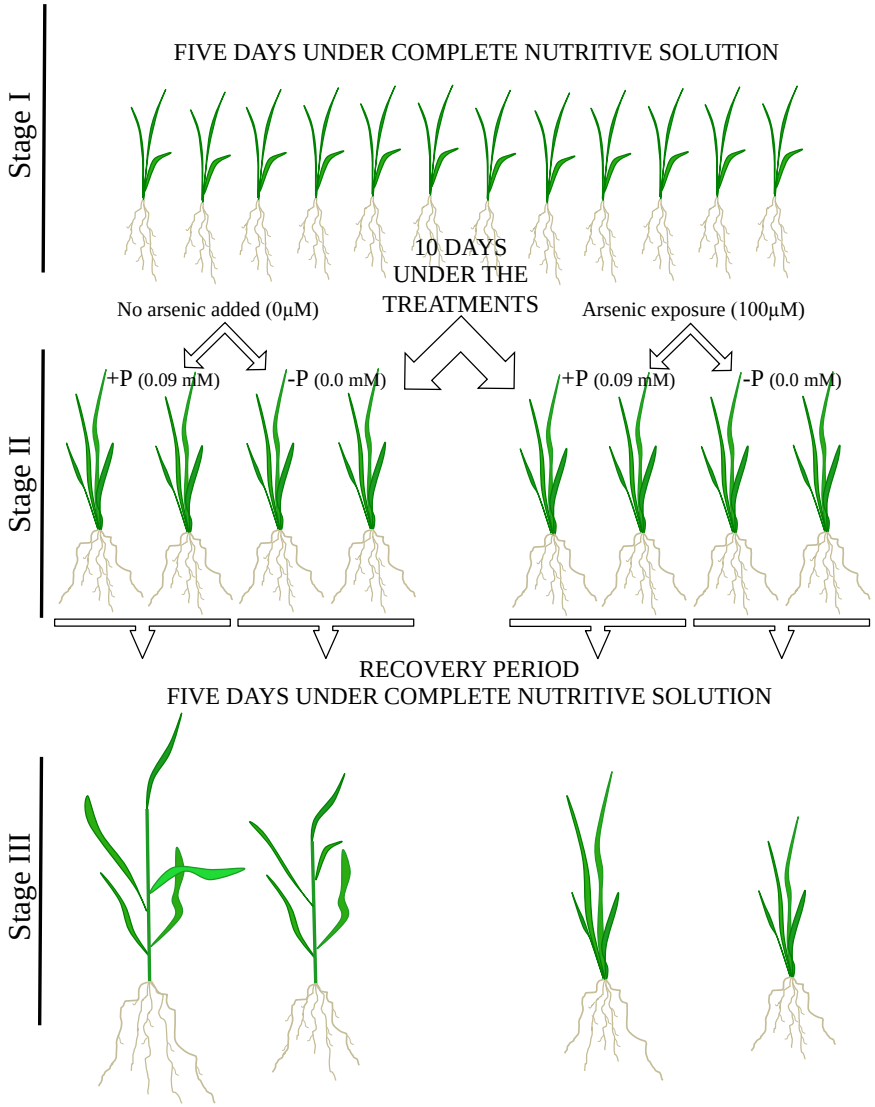
Fase III, plantas foram enxaguadas três vezes com água destilada e transferidas para vasos contendo uma solução nutritiva completa (+P) e sem As (-As). Esta fase durou 5 dias. Todas as plantas restantes foram coletadas (25 plantas por repetição, cada tratamento consistiu de 4 repetições) e separadas em parte aérea e raízes (Figura 9).

1.4.3.2 Análise dos elementos As e P

Para especiação As, lotes de até 48 amostras foram preparadas, incluindo 2 brancos e 2 controles de CRM (NIST 1568b farinha de arroz) que tem espécies As_i e DMA em concentrações certificadas. As amostras foram digeridas com micro-ondas, (CEM MARS 6). Para análise de multi elementos por ICP-MS, um procedimento de digestão mais agressivo era utilizado.

Para quantificação de P, as amostras secas em estufa foram trituradas e digeridas com 4 mL de HNO_3 concentrado. Decomposição de amostras foi realizada utilizando um bloco de aquecimento Velp Scientifica (Milão, Itália) em 130 °C durante 2 h. A concentração foi determinada por espectrometria de emissão óptica com plasma acoplado indutivamente (ICP-OES) usando um Perkin Elmer Optima 4300 DV (Shelton, EUA)

Figura 7 - Sistema experimental utilizado demonstrado em três estágios, climatização, exposição e recuperação.



Fonte: autora

1.4.3.3 Análise de dados

Concentrações dos elementos, em raízes e parte aérea, foram calculados com base no peso seco. Absorção de P total (TP), eficiência de absorção de fósforo (PUE), foram calculados como segue:

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

$T_{Root\text{-}P} = C_{Root\text{-}P} \square\square Roots_{\ biomass}$

$T_{Shoot\text{-}P} = C_{Shoot\text{-}P} \square\square Shoots_{\ biomass}$

$PUE = (T_{\ Shoot\text{-}P} / Shoot_{\ biomass})$ (WISSUWA et al., 2015).

1.4.4 Manuscrito 4

1.4.4.1 Plantas e condições de crescimento

Mudas de arroz da variedade indica foram obtidas do IRGA (Instituto Rio Grandense do Arroz), RS, Brasil. As sementes de cinco cultivares de arroz utilizadas no sul do Brasil, BR-IRGA 409, BR-IRGA 410, IRGA 420, IRGA 423 e IRGA 424, foram utilizados neste estudo. As sementes pré-germinadas foram transferidas para vasos de plástico revestidas com papel de filtro colocado em câmaras de crescimento parcialmente fechados; estes vasos foram então irrigados com água destilada durante cinco dias. O comprimento total do sistema radicular foi determinado de acordo com Tennant (1975), e o comprimento de raízes

1.4.4.2 Experimento hidropônico com e sem a presença de raiz seminal

Para avaliar o efeito da retirada da raiz seminal, após cinco dias em água destilada, as raízes seminais de metade das plantas de arroz semeadas foram removidas, e as plântulas foram transferidas para vasos de plástico contendo 180 mL de solução nutritiva Kimura B. Após sete dias de aclimatação, as plantas foram submetidas a três níveis de As (0, 20 e 50 μM) na solução nutritiva. Após 10 dias, 5 plantas por repetição (cada tratamento consistiu em 15 repetições) foram colhidas e separadas em parte aérea e raízes de forma aleatória (Figura 7).

1.4.4.3 Experimento hidropônico com um sistema radicular intacto

Após cinco dias em água destilada, as plântulas foram transferidas para vasos de plástico contendo 180 mL de um meio nutriente Kimura força solução B, como descrito anteriormente. O pH foi ajustado para 5,5, e a solução foi renovada a cada dois dias num

ambiente controlado. Após sete dias de aclimatação, as plantas foram submetidas a três níveis de As (0, 20 e 50 μM) na solução nutritiva. Depois de dez dias, 5 plantas por repetição (cada tratamento consistiu em 15 repetições) foram colhidas e separadas em parte aérea e raízes de forma aleatória (Figura 7).

1.4.4.4 Experimento hidropônico com um sistema raiz-dividida

Para avaliar o efeito local e sistêmico do As sobre a planta, um terceiro experimento foi realizado com raízes divididas. Após cinco dias em água destilada, as raízes seminais de todas as plantas de arroz semeadas foram removidas, e as plantas uniformes foram selecionadas e transferidas para um sistema raiz-dividida, em que as duas metades do sistema de raízes, cada uma em um vaso de 180 mL, foram expostas a meio nutriente Kimura. Após aproximadamente 2 semanas, estas plântulas foram cultivadas durante 10 dias com sete tratamentos de concentrações variáveis: Tratamento 1 [0/0 μM As, com ambas as metades de raiz sem As exposição]; tratamento 2 [0/20 μM As, com metade do sistema de raiz expostos a 0 uM As e a outra metade expostas a 20 μM As]; tratamento 3 [0/50 μM As, com metade do sistema de raiz expostos a 0 uM As e a outra metade do sistema radicular sendo expostas a 50 μM As]; tratamento 4 [10/10 μM As, com as duas metades expostas à mesma concentração de 10 μM As]; e tratamento de 5 [25/25 μM As, com ambas as metades expostas à mesma concentração de 25 μM As].

Depois de 10 dias, 5 plantas por repetição (cada tratamento consistiu em 70 repetições) foram colhidas e separadas em parte aérea, raízes de esquerda e direita aleatoriamente (Figura 7).

1.4.4.5 Biomass, e conteúdo nutriente mineral

A decomposição de amostras foi realizada utilizando um bloco de aquecimento Velp Scientifica (Milão, Itália) a 130 °C durante 2 h. O conteúdo de S e As foi determinada por espectrometria de emissão óptica com plasma indutivamente acoplado (ICP-OES), utilizando um aparelhoPerkinElmer Optima 4300 DV (Shelton, EUA)

1.4.4.6 Concentração de Grupos tióis não-protéicos (NPSH)

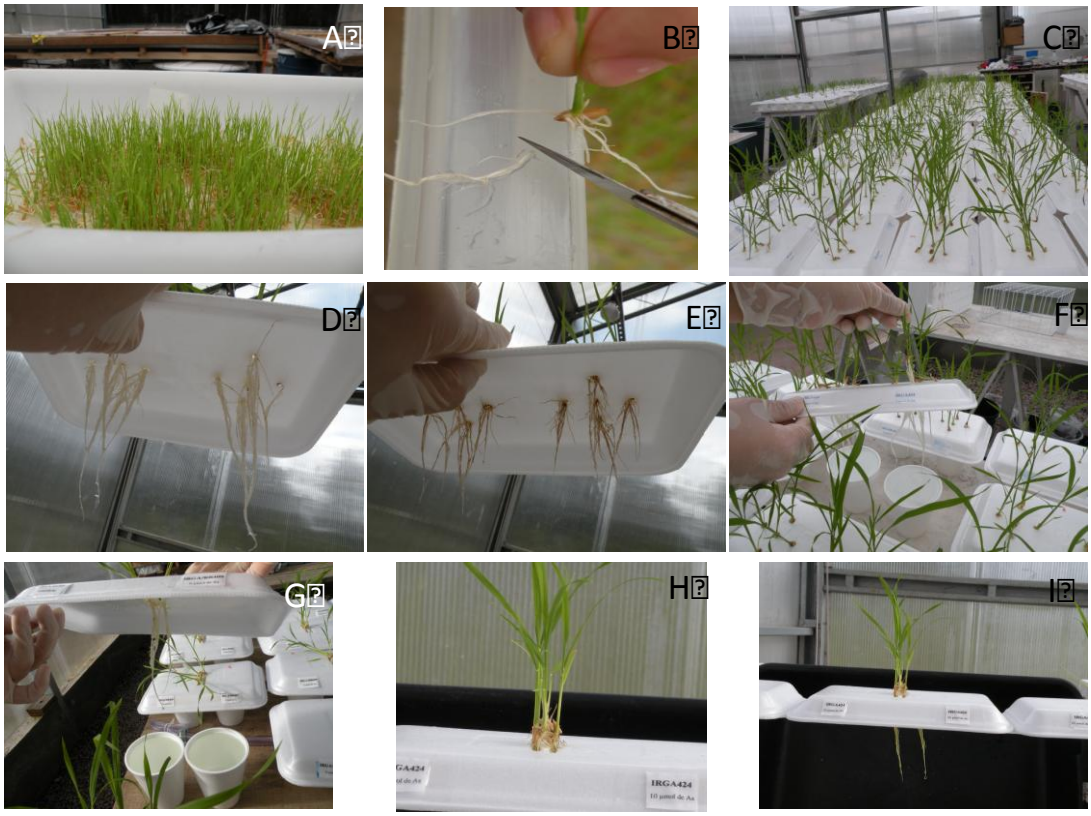
Amostras de raízes e folhas congeladas foram homogeneizadas em solução contendo 50 mM de Tris-HCl e 10% de Triton X-100 (pH 7,5) e centrifugadas a 6800 g durante 10 min. Ao sobrenadante, TCA a 10% foi adicionado numa proporção de 1:1 (v/v) seguido por centrifugação (6800 g durante 10 min) para remover as proteínas. O sobrenadante foi utilizado para determinar a concentração NPSH.

Uma alíquota do extratos de amostra (400 μL) foi adicionado num meio contendo 550μL 1 M de Tris-HCl (pH 7,4). Os produtos da reação foram quantificados à 412 nm após a adição de 10 mM de ácido 5,5'-ditiobis- (ácido 2-nitrobenzóico) (DTNB) (5 μL). Uma curva padrão utilizando cisteína foi usada para calcular o teor de grupos tiol das amostras.

1.4.4.7 Análise estatística

Os experimentos foram realizados com delineamento em blocos casualizados. As análises de variância foram computadas para diferenças estatisticamente significativas com base no F-teste. Os resultados são reportados como média± S.D. de pelo menos quatro repetições independentes. As diferenças entre as médias foram comparadas pelo teste de Tukey ($p < 0,05$).

Figura 8 - Plântulas de arroz cinco dias após a germinação (A). Retirada da raiz seminal (B). Sistema de cultivo com raiz intacta (C) sem As (D) e com As (E). Sistema com raízes divididas (F, G, H, I).



1.4.5 Manuscrito 5

1.4.5.1 Plantas e condições de crescimento

Sementes de arroz da variedades Indica foram obtidas do IRGA (Instituto Rio Grandense do Arroz), RS, Brasil. Sementes de cinco cultivares de arroz que são cultivadas no sul do Brasil, BR-IRGA 409, BR-IRGA 410, IRGA 420, IRGA 423 e IRGA 424, foram utilizadas no presente estudo. Sementes pré-germinadas foram cultivadas em papel de filtro com água destilada sob condições controladas durante sete dias.

1.4.5.2 Experimento hidropônico

Mudas de arroz foram transferidas para vasos de plástico contendo 12 litros de solução de nutritiva Kimura B (com metade da concentração original) e mantidas em estufa de

ambiente controlado, sendo adicionado As (0 (controle), 5, 20 ou 50μM) após 7 dias de climatização, sendo estas mantidas sob exposição durante 10 dias (Figura 8).

1.4.5.3 Experimento em vasos contendo areia

A unidade experimental constou de um vaso com quatro plantas. As plantas foram cultivadas em estufa parcialmente climatizada. Sete dias após o transplante, foi adicionado As à solução nutritiva a uma concentração final de 0 (controle), 2 ou 10 μM. Quando as plantas atingiram o estágio da quarta folha, a solução de nutrientes foi fornecida até atingir uma lâmina de 3 cm, que foi mantida até o final do ciclo de crescimento. Depois de 133-143 dias de exposição ao As, quatro plantas por repetição (cada tratamento constou de quatro repetições) foram colhidas e separadas em colmo principal e perfilhos. Os grãos foram processados e polidos e depois analisados por espectrometria de absorção atômica (ICP-MS) para determinar a concentração de um dos seguintes elementos: As, cálcio (Ca), potássio (K), magnésio (Mg), fósforo (P), cobre (Cu), ferro (Fe), manganês (Mn), e zinco (Zn) (Figura 8).

1.4.5.4 Análise de crescimento da Planta

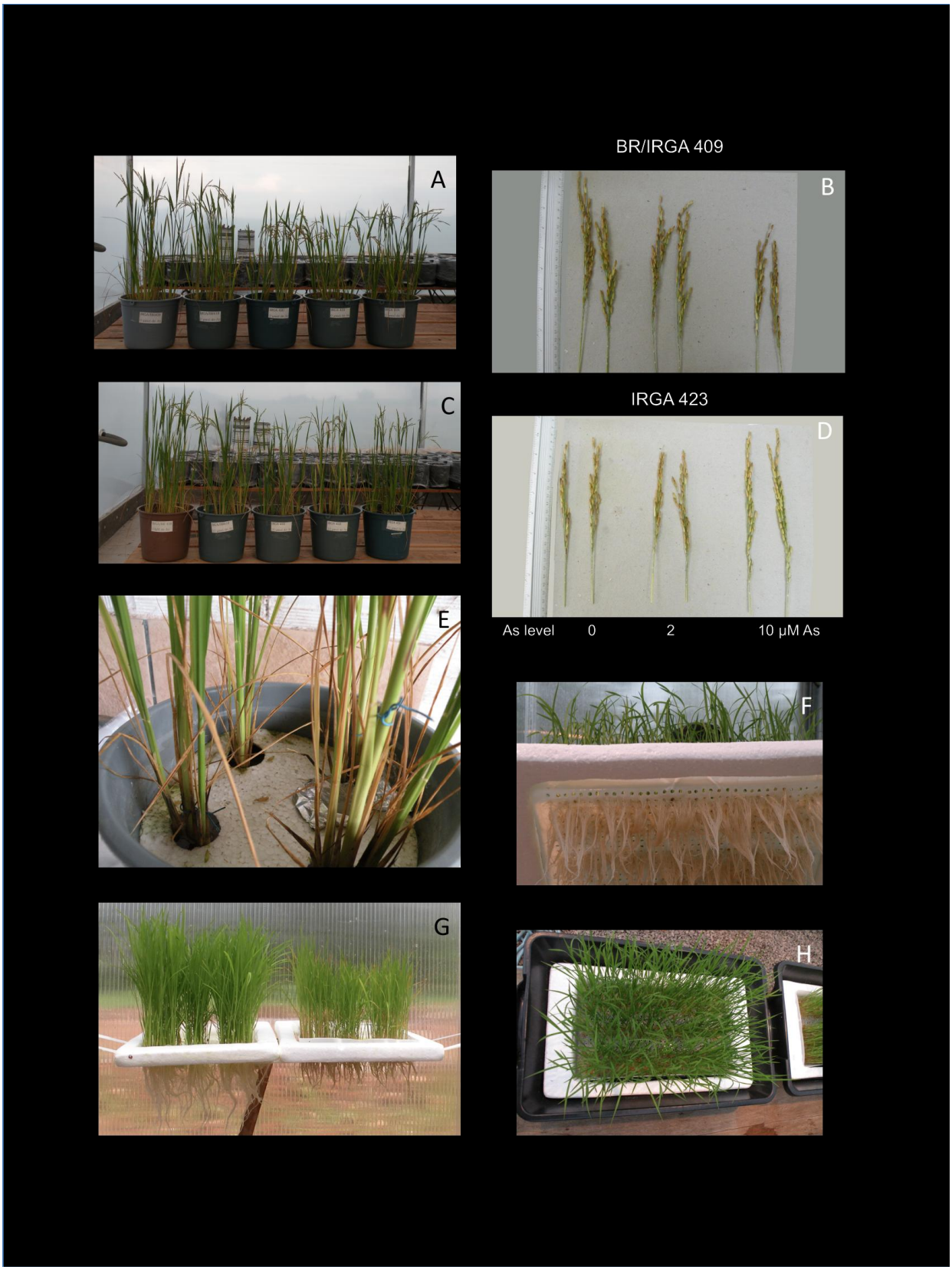
Na colheita, as plantas cultivadas em hidroponia foram divididos em raiz e parte aérea. A biomassa das plantas foi quantificada como peso seco. Comprimento radicular foi determinada de acordo com Tennant (1975), e comprimento da parte aérea foi medido com compassos de calibre de Vernier. Depois de 133-143 dias de exposição ao As, as plantas de arroz cultivadas em vasos com areia foram separados em colmo principal e perfilhos e medidos com compassos Vernier.

1.4.5.5 A análise multivariada

A análise de componentes principais (PCA) foi usada para avaliar as relações entre as variáveis e para identificar os padrões de distribuição dos dados obtidos a partir de diferentes tecidos durante o ciclo de cultivo de arroz.

Os dados coletados foram transformados pelo ranking em uma escala de 1-10. O valor médio para os parâmetros avaliados correspondeu a um valor de 5 na escala, sendo 1 o valor mais baixo e sendo 10 o valor mais elevado. Os dados médios foram analisados utilizando CANOCO "software estatístico (versão 4.5, Fa. Biometris, Ithaca, NY).

Figura 9 - Experimento com areia em vasos (A, B, C, D, E) e hidroponia *floating* (G, H).



Fonte : autor

1.4.6 Manuscrito 6

O experimento foi conduzido em quatro locais no Sul do Brasil, no Estado do Rio Grande do Sul. Dois sistemas diferentes foram testados: I) a gestão da água por meio de supressão de água em três áreas diferentes, com níveis de fósforo; II) a gestão da água através do uso de irrigação com a formação da lâmina de água ou sistema de pivô central.

1.4.6.1 Experimento I

O experimento foi realizado em três locais, Cachoeirinha, Camaquã e Restinga Seca (Figura 10), variando o teor de Fe no solo. Restinga Seca com alto conteúdo disponível Cachoeirinha e Camaquã, níveis intermediários.

Os seguintes tratamentos (Figura 10) foram utilizados: T1 - irrigação contínua com altura de lâmina 5 centímetros a partir das V3-V4 até o estágio de crescimento R6; T2 - irrigação com uma supressão de água entre as os estágios V6-V8, com a restauração da lâmina até o estágio R6; e T3 - irrigação com duas supressões de água, o primeiro entre os estágios V6-V8 restabelecendo a lâmina de água e realizando-se uma segunda supressão entre V8-V10, com a restauração da irrigação até R6 (Figura 10).

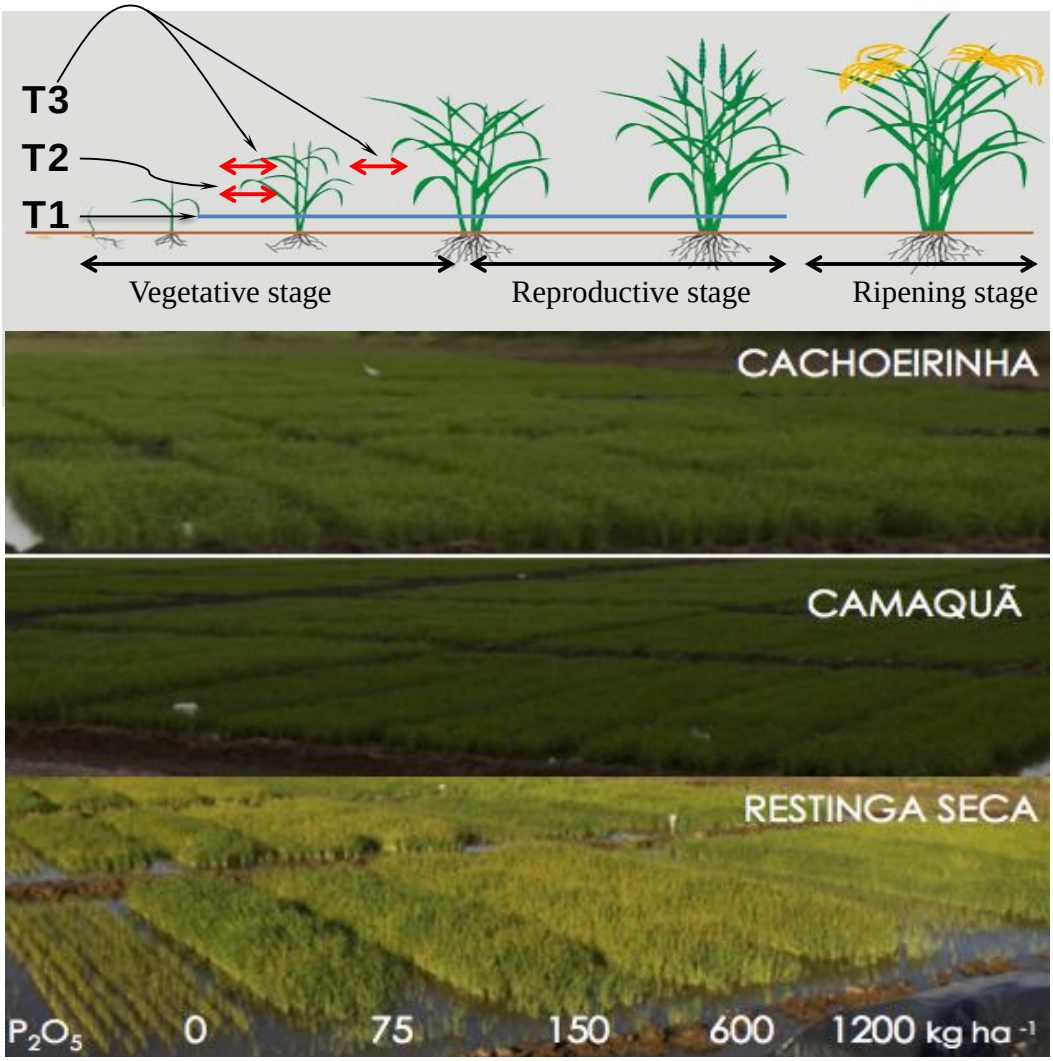
Foram utilizados dois cultivares contrastantes quanto à tolerância toxicidade Fe: IRGA 425 (tolerante) e BR-IRGA 409 (suscetível). A adubação fosfatada foi feita em doses crescentes de 0, 75, 150, 600, 1200 kg de P_2O_5 ha^{-1}.

1.4.6.2 Experimento II

Foram utilizados os seguintes tratamentos: T1 - irrigação contínua com lâmina de 5cm a partir de V3-V4 até o estágio de crescimento R6; T2 - irrigação contínua com a manutenção de 70-80% da capacidade de campo em sistema de pivô central durante todo o ciclo da cultura, ambos com 60 kg de P_2O_5 ha^{-1}. Foi utilizada a cultivar IRGA 409 (Figura 11).

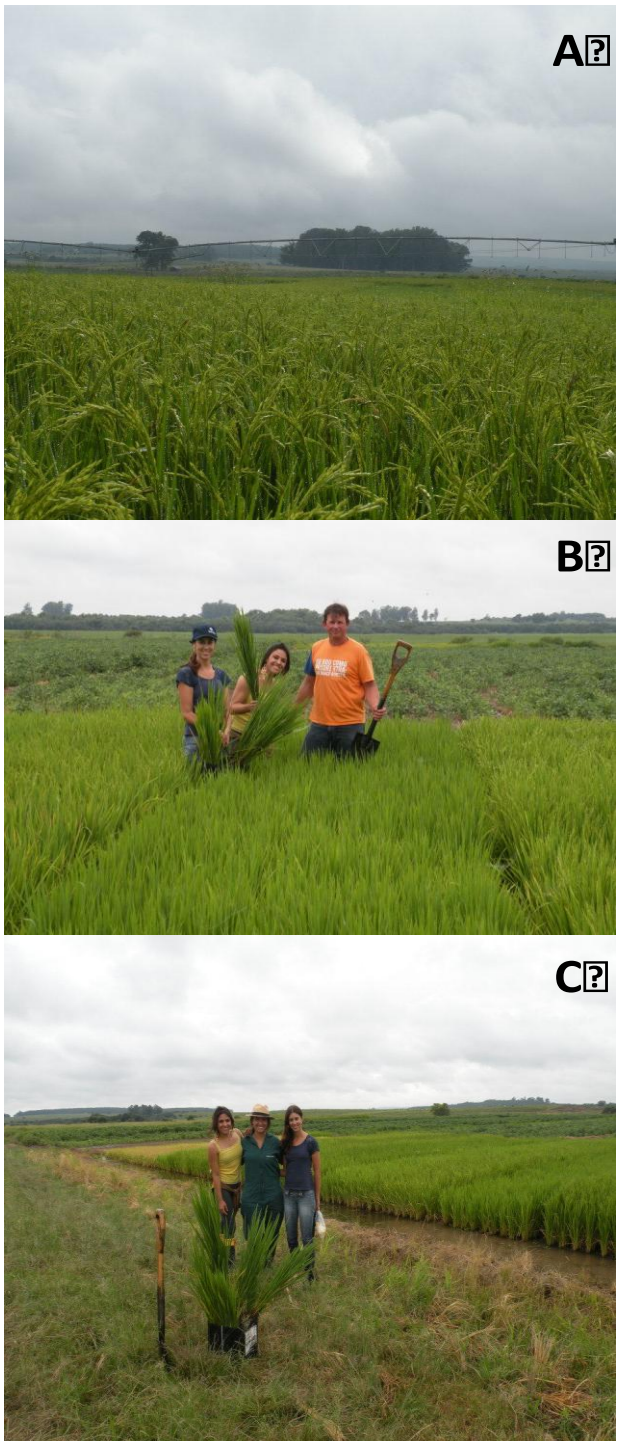
As análises referentes à especiação de As foram feitas conforme descritas anteriormente nos demais manuscritos e as análises de solo foram realizadas segundo Tedesco 1995.

Figura 10 - Esquema prático do manejo de água, sem supressão (T1), com uma supressão (T2) e com três supressões (T3), locais e níveis de fósforo.



Fonte: autora

Figura 11 - Lavoura de arroz irrigada com pivô central (A, B) e irrigada pelo método tradicional com lâmina de água (C).



Fonte: autora

2. MANUSCRITO 1 COMPREHENSIVE EVALUATION OF ARSENIC OCCURRENCE IN SOIL AND RICE GRAINS FROM SOUTH AMERICA

Manuscrito apresentado conforme as normas do jornal *Food Chemistry*

COMPREHENSIVE EVALUATION OF ARSENIC OCCURRENCE IN SOIL AND RICE GRAINS FROM SOUTH AMERICA

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ABSTRACT

The food chain is one of the major sources of human exposure to non-essential trace elements (TEs) present in soils. The global impact on public health due to trace elements in water and food is highlighted by an increasing number of countries worldwide reporting contaminations in drinking water and many food samples. In Latin America, the problem of arsenic (As) contamination in water is known in 14 out of 20 countries. To assess the quality of Southern Brazilian rice with respect to non-essential TEs, we evaluated arsenic (As), phosphorus (P), organic matter (OM), and iron (Fe) contents in soil of 73 crop fields as well as As speciation in rice grains from three cultivars. Additionally, commercial rice samples from Argentina, Brazil, Uruguay and Paraguay were analyzed. Significant differences were found in samples collected on field, for the rice grain As concentrations of the different cultivars and soil use managements. The levels of As found in this study were higher then in previous works made in Brazil, however in different regions; indicating that the concentrations of As in Brazilians crops needs attention in relation to exposure to human health. Our results showed high levels of As in commercial rice samples were region-dependent. These results provide information that may benefit countries where rice is imported, with a possible source of low- As_i rice for products where there is the greatest need; and also give the understanding base of rice response to soil biogeochemistry, field management and climatic conditions.

Keywords: arsenic, food contamination, South America, toxicity.

1 INTRODUCTION

Food security is achieved at the individual, household, national, regional, and global levels when people have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and preferences for an active and healthy life (Pinstrup-Andersen, 2009). It is well known that arsenic (As) pollution represents a serious threat to human health. Arsenic is widely distributed not only in the environment (mainly in soil and water) but also in some food (i.e. rice, vegetables and milk) (Meharg, Lombi, Williams, Scheckel, Feldmann, Raab et al, 2008; Meharg, Williams, Adomako, Lawgali, Deacon, Villada et al, 2009; Signes-Pastor, Mitra, Sarkhel, Hobbes, Burló, de Groot et al, 2008). Recently, studies highlighted the alarming issue of inorganic arsenic (As*i*) contamination in food as a non-threshold carcinogen (Stone, 2008; Carey, Jiujin, Farias, & Meharg, 2015; Signes-Pastor, Carey, & Meharg, 2016).

While non-essential trace elements (TEs) are present in many agricultural soils, their accumulation by plants and their translocation to edible and harvested parts depend on many factors, including climatic factors, soil attributes, input rates, and plant species and genotypes (McLaughlin, Parker, & Clarke, 1999). In this view, there is a growing concern over the As and its accumulation in rice grains, plant which has a high As absorption capacity and accumulation (Meharg, & Rahman, 2003).

For subsistence rice economies, agronomic practices such as cultivars selection and paddy management regimens (Meharg et al., 2003; Xu, McGrath, Meharg, & Zhao, 2008) and processing (milling and cooking) (Carey et al., 2015; Naito 2015) technologies that may lowering trace elements in ric e, ensuring a “premium product” to economies that import rice, thus commanding a higher price and allowing for expansion of rice production.

Several methods have been developed during the past few decades in this view. Selection and breeding of rice cultivars with low As accumulation have been practiced to reduce As in the rice grains (Zhang & Duan, 2008; Li, Wang, Qi, Huang, & Ye, 2012). Water management is another promising method that affects As bioavailability in soils and their subsequent uptake by rice (Arao, Kawasaki, Baba, Mori, & Matsumoto, 2009; Rahaman, Sinha, & Mukhopadhyay, 2011; Hu, Huang, Ouyang, Wu, Song, Wang et al, 2013), as well as cooking methods (Carey et al., 2015) and polishing treatments (Naito, Matsumoto, Shindoh, & Nishimura, 2015).

However, not much attention has being given to soil use management and its relationship with as accumulation in rice grains. Furthermore, there still lack of information regarding As concentration in Brazilian rice, considering the different soil use managements that occurs in the country. In Brazil the south region represents alone around 60% of the total country rice production (MAPA, 2011).

In view of the concern for food safety, this study assessed the contents of As, in the edible parts of major rice producer areas at southern Brazil, as well as the different soil management of each crop field; which is relevant not only for food security purposes, but also for commercial and

economics aspects. In this paper we also review the existing literature dealing with the occurrence of As worldwide, thus we evaluated a comprehensive number of commercial rice samples from Argentina, Brazil, Paraguay and Uruguay. Such critical synthesis of the As occurrence will assist us in evaluating pathways of human As exposure in Latin America that will also form a basis for risk assessment.

2 MATERIALS AND METHODS

2.1 Plant materials and rice fields

Soil and rice samples were obtained from crop fields conducted in Rio Grande do Sul state, Brazil, during the 2012/13 growing season. All areas in which the field experiments were located have a long-term rice production (at least 15 years). Three composite soil samples (500 g) were made up of 15 separate subsamples each, collected from the 0 to 20 cm soil layer and then stored in plastic bags until analysis.

This study evaluated three different cultivars: BR/IRGA 409, IRGA 424 and PUITA Inta CL, in 24, 24 and 25 fields respectively, being the total of fields evaluated 73.

All the crop fields were marked using GPS for spatial distribution analysis. Overall the areas showed very similar pest and fertility management; however they have 4 different managements in relation to the period between harvest and crop rotation. The managements were: 1) exclusive rice cultivation, with no cultivation during the winter season; 2) one crop season with soybean followed by two seasons with rice cultivation with no cultivation during the winter season; 3) a season with rice cultivation followed by two or three seasons on fallow, and ryegrass sown during the winter; and 4) Soybean and rice crop rotation.

To estimate grain yield, rice was harvested in an area of 8.0 m^2 when the grain moisture averaged was 20%. After weighing the grain, data were corrected to 13% moisture and converted into kg ha^{-1}. The 1000-grain weight (g) was determined from the average weight of four subsamples of 100 grains.

2.2 Spatial distribution maps

To create the geographical matrix, it was used GPS Track Maker field, with an average error of 4 m set to UTM WGS84 coordinates. The location of collection points was defined from the conversion of geographic location in UTM.

Generation of spatial distribution maps was performed using the spatial analysis module (Spatial Analyst) ArcGIS Desktop software 10.0 (ESRI) using the interpolation of Inverse Distance Square (IQD) with exponent 2 as Reis et. al. (2005).

For the calculation of the interpolation value point by the DCI method, it uses the following

equation:

$$Z(x) = \frac{\sum_{i=1}^{n} \omega_i Z(x_i)}{\sum_{i=1}^{n} \omega_i}$$

*Z (x) - is the value of the point you want to interpolate; n - is the number of points used in the next interpolation point x; Z (xi) - is the value of xi; and ωi - is the value of the weight of xi / x. To determine ωi it was used, the following equation:

$$\omega_i = \frac{1}{h(x, x_i)^p}$$

*h (x, x) - is the distance between point x and point xi; p - is the power parameter generally equals two (Marcuzzo, Andrade, & Melo, 2011).

From the geographical matrix it was prepared groups of thematic maps: Concentration of arsenic (As), iron (Fe), phosphorus (P) and organic matter (OM) in soil and total (As_t) and inorganic (As_i) arsenic in rice samples interpolated by IQD.

2.3 Rice sourcing

Market rice was purchased from retailers from Argentine, Brazil, Uruguay and Paraguay totaling 180 samples (the percentage of 85% of white rice was maintained for all countries). For Brazilian rice a detailed geographic breakdown of rice sourcing was known, with 5 Brazilians rice-growing regions sampled (North, Northeast, Midwest, Southeast and South) and one of these regions (South – Rio Grande do Sul state) was sub-sampled in 4 regions (Southeast, South, West and Central) (Figure 1).

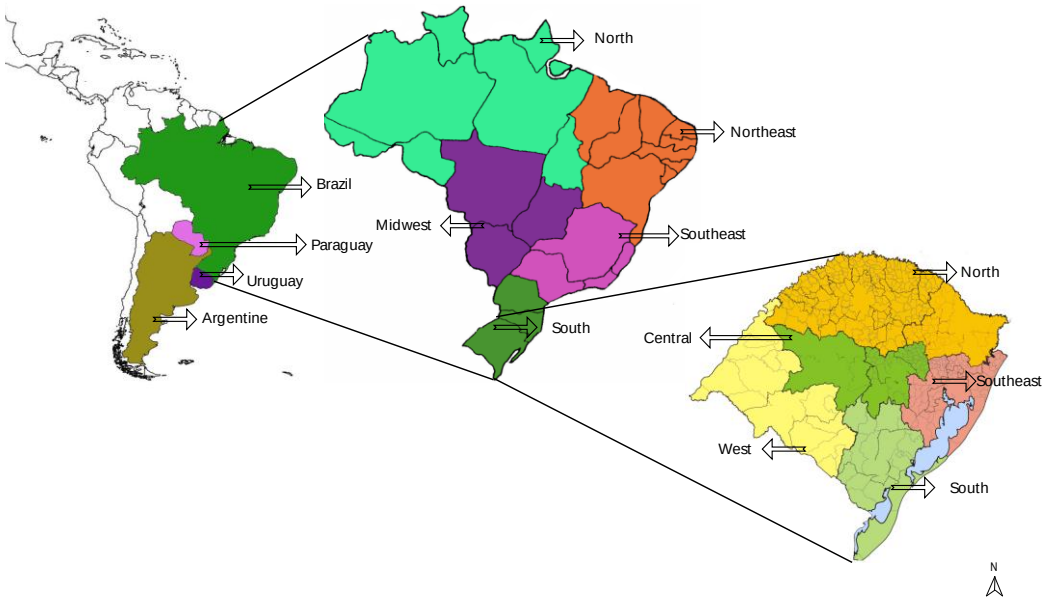

Figure 1. Schematic map of rice production sites. Left side evaluated Latin American countries (Argentina, Brazil, Uruguay and Paraguay); Center Brazilian regions (North, Northeast, Midwest, Southeast and South); right side regions of southern Brazil (Southeast, South, West and Central).

2.4 Chemical analysis

2.4.1 Arsenic speciation

For As speciation freeze-dried milled rice was weighed accurately to a weight of 0.1g into 50ml polypropylene centrifuge tubes to which 2ml 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 As species As_i and dimethylyarsonic acid (DMA) concentrations certified. Samples where then microwave digested in an CEM MARS 6 instrument for 30 min. at 95^{o}C using a 3 stage slow heating program: to 55^{o}C in 5 min. held for 10 min., to 75^{o}C in 5 min., held for 10 min. to 95^{o}C in 5 min., held for 30 minutes). The digestate, on cooling, was accurately diluted to 10ml with deionized distilled water and centrifuged at 3,500 rpm for 15 min. A 1 ml aliquot was transferred to a 2ml polypropylene vial and 10µl 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^{o}C in 5 min. hold for 10 min. to 135^{o}C then hold for 10 min., to 180^{o}C then hold for 30 min.) was employed, with 2ml of concentrated Aristar nitric acid and 2ml hydrogen peroxide added and left to soak overnight before microwaving. 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 analysed.

2.5 Statistics

Statistical analyses were done using the General Linear Models procedure of SAS 8.2 to test for As species differences. Kruskal-Wallace test in Minitab v.16 was used to analyze the global As data. Sigma Plot (v9.0) and Minitab (v11) were used to fit curves and for descriptive statistical analysis of As species, respectively.

3 RESULTS AND DISCUSSION

In the present study, a considerable range of soil chemistry characteristics, use management and arsenic (As) in grains was observed (Figure 2; Figure 3; Figure 4). Our results showed low natural phosphorus (P) content in these soils, which in Southern Brazilian is a common occurrence along with high content of aluminum (Al) and acid pH (Pallarés, Barreta, & Marasching, 2005).

Besides soil characteristics and soil use management there was another outstanding factor in this study, the average size of crop fields of different regions. The west area, known as Fronteira, had the average field size of 450 ha, in farms with average total size of 1250 ha. On the other hand, South and East areas had 80 and 250 ha respectively for crop field and total farm area, while the central area had 30 and 80 ha.

We believe that the size of crop field was a determinant factor to choose the soil use management. For example, the management of rice production followed by two or three years of follow (Figure 2) was only observed in bigger areas at the West part of the state. In these areas, after rice production, ryegrass is sown during the winter; and together with the spontaneous natural grasses that occur in these areas is used for cattle production. During this whole period the soil remains under aerobic conditions. Even though the producers stay two or three years without rice production they still have another economic source. However, in a small area this dynamics would be much less profitable.

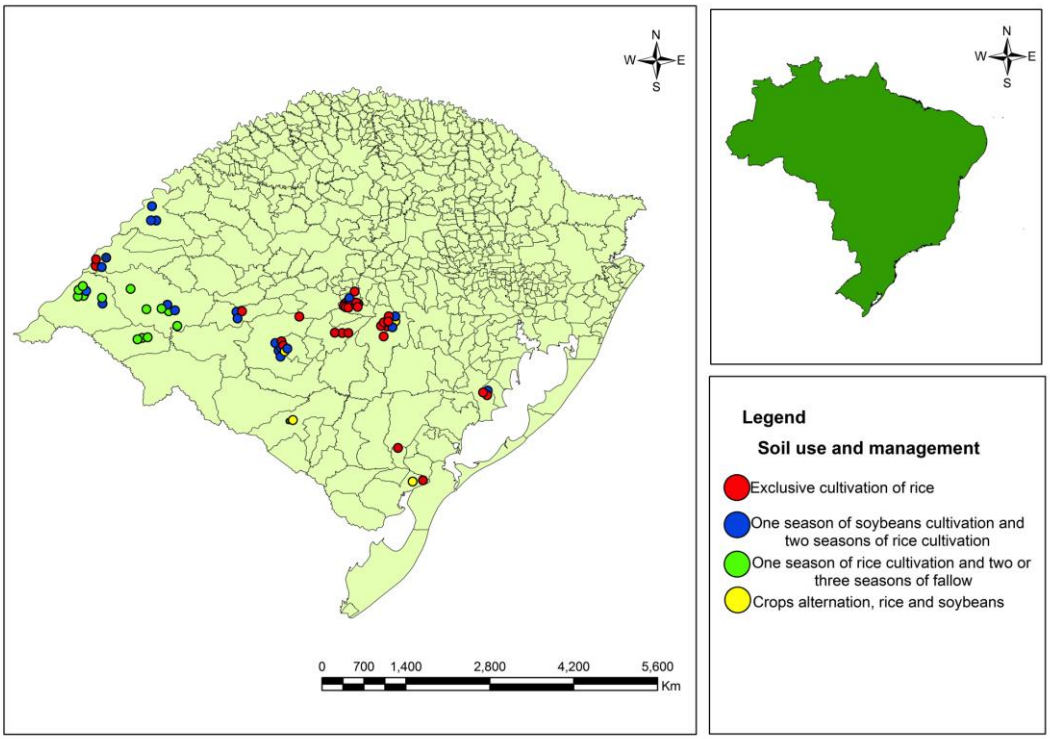

Figure 2. Soil use management in different areas of southern Brazil.

Another interesting fact is that the areas with the follow management showed the higher OM content and lowest contents of As in grains (Figure 2; Figure 3). A recent study indicated that the concentration of As in rice straw could be up to 92 mg kg^{-1} under As-contaminated conditions (Abedin, Cotter-Howells, & Meharg, 2002). However, in many countries the legal limit for straw fed to cattle is 0.2 mg kg^{-1} (Nicholson, Chambers, Williams, Unwin, 1999); which could be a negative factor for this type of soil use management since animals are introduced in this area and feed with the remain rice straw. However, studies showed that the As accumulated in animal tissues, can be partly transformed into organic As (As_o) through biomethylation processes (Centeno, Mullick, Martinez, Page, Gibb, Longfellow et al., 2002).

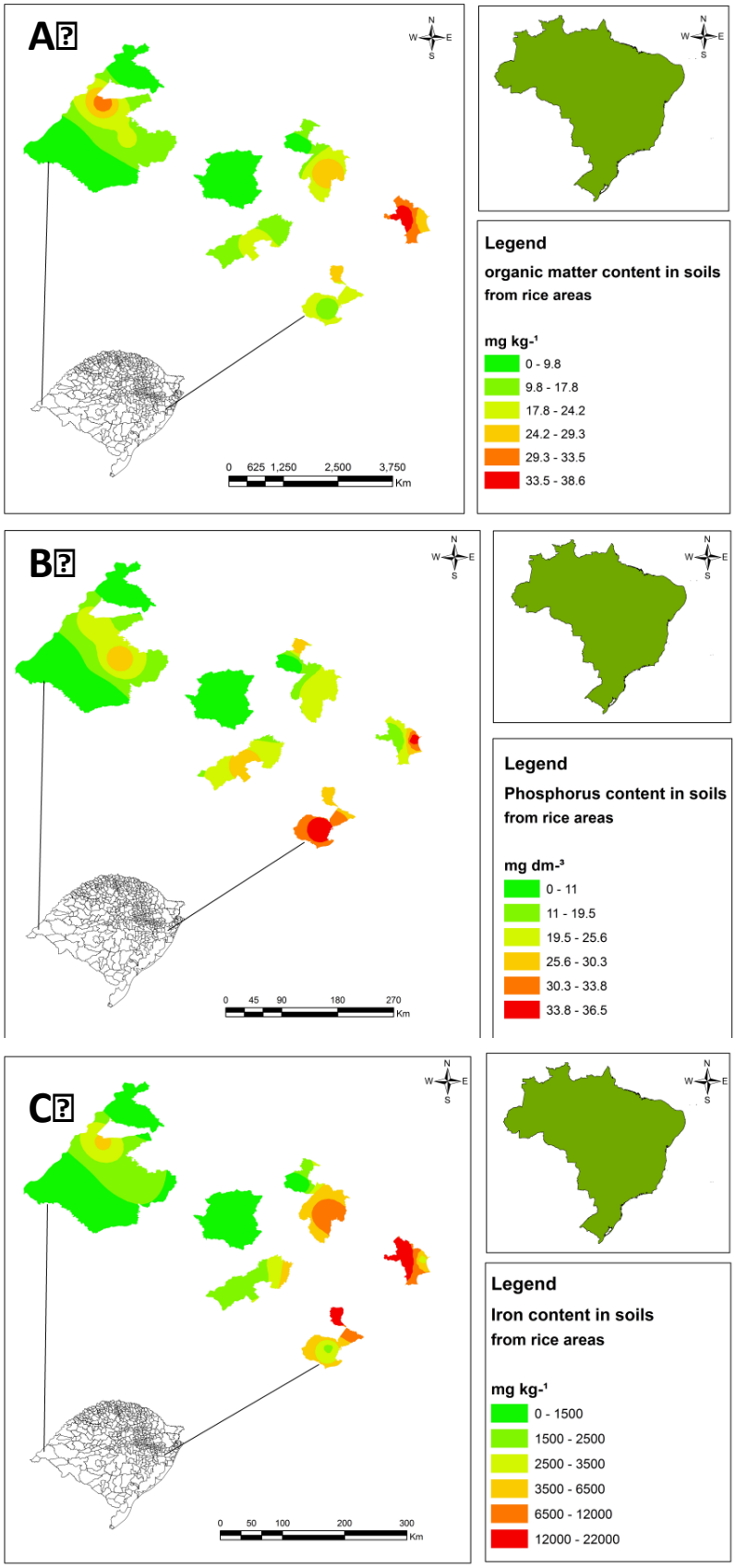

Figure 3. Geographical distribution of organic matter, phosphorus and iron in rice fields from southern Brazil.

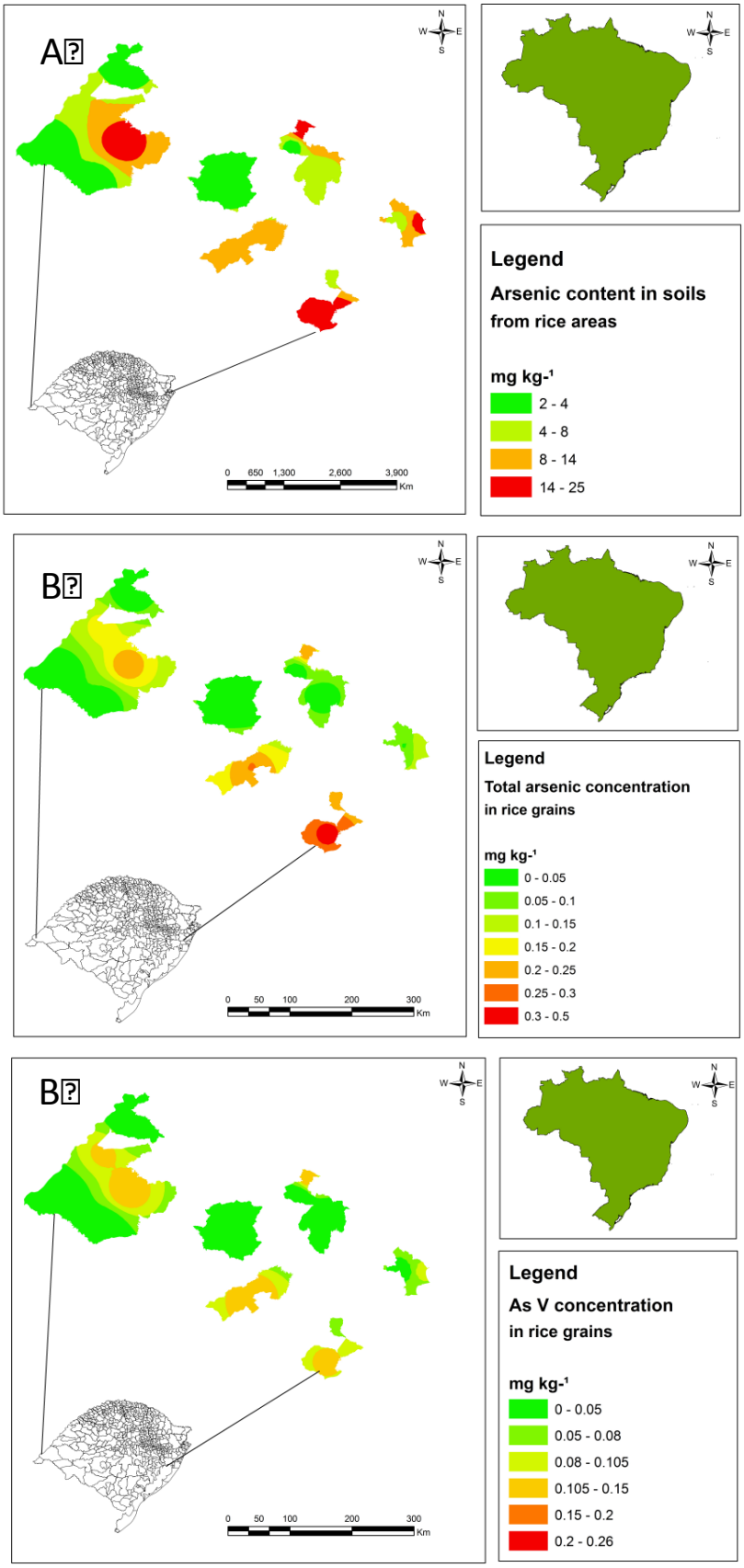

Figure 4. Geographical distribution of arsenic in soil (A), and grains (B, C) in rice fields from southern Brazil.

Crop rotation is a management practice used to avoid soil exhaustion while improving the physical, chemical, and biological properties of the soil and helping control pests and diseases (EMBRAPA, 2004). This management practice might also prevent the depletion or concentration of specific elements caused by single crops. Although other studies have shown significant decreases in Cd and Pb concentrations in plants cultivated under a crop rotation system (Wu, Norvell, Hopkins, & Welch, 2011), in the present study, no significant differences were detected in the As concentrations in the rice grains grown under crop rotation with soybean (Figure 2; Figure 4).

In our study a significant number of soil samples were above the range of maximum allowable concentrations of As in agricultural soils (15–20 mg/kg) (Kabata-Pendias & Mukherjee, 2007). Moreover, 48% of the rice samples had As_v concentrations above 0.1 mg/kg, which is now considered not suitable for infants and young children's food according to EU document 2015. On the other hand, samples from West region showed ultra-low As_i , with some samples under the limits of detection (LOD) (0.001-0.003 mg/kg) specially at the west area.

The identification of ultra-low As_i rice, such as with the grain from the these regions described here not only has the direct benefit of providing, for countries where rice is imported, a source of low-As_i rice for products where there is greatest need (foods for babies and young children and for those who suffer dietary restrictions/preferences that have diets strongly biased towards rice), but also, ultimately, an understanding of the rice germplasm, soil biogeochemistry, field management and climatic conditions.

In our study there was distinct pattern of As accumulation among the evaluated cultivars (Table 1) and grain yield (Table 1, Table 2). Cultivar BR-IRGA 409 accumulated more As in main culm tissue, showing low As translocation to tillers, which was not observed for the other cultivars. When compared de As concentration in main culm there was no difference among the cultivars, but considering the whole plant BR-IRGA 409 had an As concentration 50% lower as compared to the other cultivars (Table 1).

This cultivar also showed lower grain yield productive (Table 2), but still under the national average grain yield (MAPA, 2011). Besides the use of non-contaminated areas and rice polishing (Naito et al., 2015), cultivars can have a huge impact on As accumulation, thus resulting in a low cost solution for this problem.

Table 1. Arsenic concentration in main and tillers of three rice cultivars grown in southern Brazil.

Cultivar	Main culm	Tillers	Average per plant
BR/IRGA 409	0.174 Aa	0.054 Bb	0.087 Bb
IRGA 424	0.191 Aa	0.183 Aa	0.195 Aa
PUITÁ Inta CL	0.171 Aa	0.169 Aa	0.179 Aa

Means followed by lowercase letters indicate comparison among reproductive organ (ie. main, tillers) within the same cultivar, whereas capital letters indicate comparison between cultivars for the same reproductive organ, $\alpha = 0.05$.

The analysis of the As speciated CRM gave excellent recovery results, with 88.5±3.3% recovery for DMA and 94.4±4.4% recovery for As_i, the only two species found in that CRM that were also detectable in the rice analysed, giving a sum of species recovery of 94.6%. The CRM had a certified concentration of 0.182 and 0.092 mg/kg As for DMA and As_i respectively. The limits of detection (LOD) for both DMA and As_i (calculated from a DMA calibration) in rice was 0.0028 ±0.001 mg/kg DMA rice d.wt., n = 5.

Table 2. Rice grain yield of three cultivars grown in southern Brazil.

	1000-grains weight		
Cultivar	Main culm	Tillers	Average per plant
BR/IRGA 409	22,747	22,411	22,495
IRGA 424	26,438	25,492	25,729
PUITÁ Inta CL	25,643	25,858	25,804
	Grain yield		
BR/IRGA 409	8100 kg/ha		
IRGA 424	8900 kg/ha		
PUITÁ Inta CL	10080 kg/ha		

Means followed capital letters indicate comparison among rice cultivars.Teste Tukey, $\alpha = 0.05$.

When grain As_t was considered globally there wasn't considerable country specific variation in the median concentrations; ranging from 0.126 mg/kg for Argentina through to 0.254 mg/kg for the Paraguay (Figure 5). However, a considerable region specific variation occurred among Brazilians regions. Batista et al. (2008) also report differences among Brazilian areas, however with a very lower range of samples and evaluated areas. Also in this sense there is a quite difference regarding countries and regions of same country during the last years of As reports (Table 3).

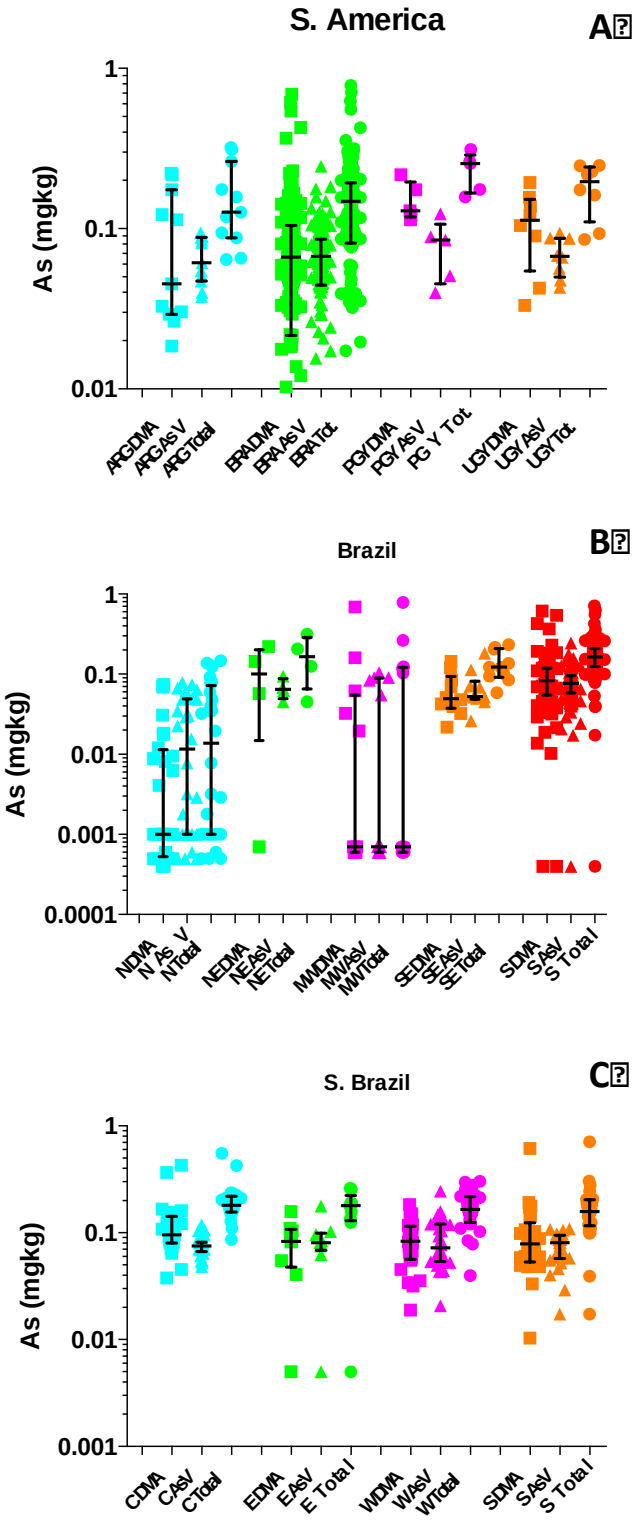

Figure 5. Distribution of DMA, As V and total arsenic concentrations reported for **A** –Argentina - Arg (11 samples), Brazil – Br (156 samples), Uruguay – UGY (8 samples) and Paraguay - PGY (5 samples) totaling 180 samples of rice.; **B** Brazilians regions, North – N, Northwest – NE, Midlewest – MW, Southeast – SE and South –S; **C** Southern Brazilians regions, Central (C), East – E, West- W and South –S. The whiskers (error bars) above and below the median (-) indicate the 95th and 5th percentiles.

Both North (N) and Midwest (MW) areas had grain As concentrations (As_t, As_V and DMA) below the LOD (Figure 5). These samples belong to a highland rice area, which is common in these regions of Brazil. On the other hand, lowland rice represents over 95% of the south production system, which also represent alone 70% of total rice production in Brazil (MAPA, 2011).

Table 3. Comparison of arsenic species composition in rice samples from different countries and studies.

Country of production	Rice type	Total As (mg/ Kg)	AsV (mg/ Kg)	DMA (mg/ Kg)	Reference
Argentina	Polished rice	0.134	0.0617	0.0712	This study
Argentina	Brown rice	0.300	0.0820	0.2160	This study
Bangladesh	Polished rice	0.610		0.1700	Meharg et al., 2008
Bangladesh	Polished rice	0.221			Ahmed et al.. 2011
Brazil	Polished rice	0.223	0.0340	0.0930	Batista et al., 2011
Brazil	Polished rice	0.136	0.0603	0.0751	This study
Brazil	Brown rice	0.348	0.0420	0.1270	Batista et al., 2011
Brazil	Brown rice	0.280	0.1150	0.1500	This study
Camboja	Polished rice	0.200			Seyfferth et al.. 2014
Canada	Polished rice	0.046		0.0660	Willians et al., 2005
China	Polished rice	0.230	0.0062	0.0400	Zhu et al., 2008
China	Polished rice	0.360		0.0900	Meharg et al., 2008
China	Polished rice	0.114		0.0260	Liang et al., 2010
India	Polished rice	0.131		0.0190	Willians et al., 2005
India	Polished rice	0.354		0.0050	Sanz et al., 2007
India	Polished rice	0.496	0.2430	0.2420	Signes-Pastor et al.. 2008
India	Polished rice	0.200	0.1800		Banerjee et al., 2013
India	Polished rice	0.283	0.0660	0.0140	Halder et al.. 2014
Italy	Polished rice	0.220		0.0850	Willians et al., 2005
Japan	Brown rice	0.259	0.2270	0.0390	Naito et al.. 2015
Paraguay	Polished rice	0.215	0.0597	0.1508	This study
Paraguay	Brown rice	0.292	0.0880	0.1620	This study
Spain	Polished rice	0.170		0.0500	Willians et al., 2005
Taiwan	Polished rice	0.383	0.0400	0.0370	Willians et al., 2005
Thailand	Polished rice	0.110		0.0300	Willians et al., 2005
Uruguay	Polished rice	0.163	0.0691	0.0915	This study
Uruguay	Brown rice	0.240	0.0977	0.1342	This study
USA	Polished rice	0.277		0.0770	Willians et al., 2005
USA	Polished rice	0.329	0.0420	0.1370	Zhu et al., 2008
USA	Polished rice	0.280	0.0170	0.1021	Meharg et al., 2008
USA	Polished rice	0.265		0.1550	Zavala et al., 2008
USA	Brown rice	0.440	0.0082	0.1400	Meharg et al., 2008
USA	Brown rice	0.331		0.1730	Zavala et al., 2008
Thailand	Polished rice		0.0683		Ruangwises et al.. 2012
Thailand	Brown rice		0.1240		Ruangwises et al.. 2012

The identification of ultra-low As_i rice, such as with the grain from these regions described here not only has the direct benefit of providing, for countries where rice is imported, a source of low-As_i rice for products where there is greatest need (foods for babies and young children and for those who suffer dietary restrictions/preferences that have diets strongly biased towards rice), but also, ultimately, an understanding of the rice response to soil biogeochemistry, field management and climatic conditions that produce low – As_i rice (Meharg & Zhao, 2012).

When compared the medians of different regions of the south there wasn't differences, however, the East (E) region of Rio Grande do Sul state, there were samples below the LOD of (As_t,

As_V and DMA) and also the highest level detected 0.784 As_t and 0.245 As_V (mg/kg) (Figure 2), which should enable a dissection of how to produce low inorganic arsenic rice. Southern Brazilian rice is dominated by low-land rainfed rice systems, *i.e.* paddy rice (GRiSP, 2013). The country's rice production is also characterized by high-input (fertilizers and pesticides) and elevated infrastructure investments, with an average production of 9 t/ ha (MAPA, 2011).

Irrigated rice is the most important system of production. In 2010, the irrigated system covered 50.1% of the total area under cultivation and accounted for 78.0% of the production; upland rice occupied 49.1% of the area and produced 21.4% of the total; while rainfed lowland rice occupied 0.8% of the area and produced 0.6% of the total (GRiSP, 2013).

Brazil is the ninth-largest rice-producing country and largest outside Asia, with 11.3 million t in 2010, 1.7% of the world's rice production. Because of several factors (e.g., Mercosul agreement, large country size, and market openings), Brazil is an importer as well as exporter of rice, being the largest South American rice importer at 590,000 t of milled rice and 82,146 t of paddy rice in 2009. The imported rice comes principally from Uruguay, Argentina, and Paraguay, which in 2009 sent 186,239, 131,926, and 58,440 t of milled rice and 29,913, 4,720, and 47,510 t of paddy rice, respectively (GRiSP, 2013).

Brazil became rice self-sufficient in 2002-03 and began exporting in 2004, with 36,717 t of milled rice, climbing to 511,919 t in 2008. Exports, which were less than 5% of rice production, were mainly for African countries such as Benin (108,138 t), Nigeria (80,998 t), South Africa (50,525 t), and Cameroon (16,547 t). Paddy rice was exported mainly to Venezuela (29,880 t) (MAPA 2011; GRiSP, 2013).

The fact that even large producers and exporters economies also import rice and the immense variation of grain quality among the producing areas of rice in a same country (Figure 5, Table 3) versus the variety of rice consumption by communities of different countries turns the awareness of the real rice production situational in different regions essential. Knowledge and appropriate description not only protect consumers but can also add value to samples with low concentrations of As. In this regard the imposition of limits as the one determined by the EU (COMMISSION REGULATION–EU- 2015) is crucial for decision-making of governments and producers.

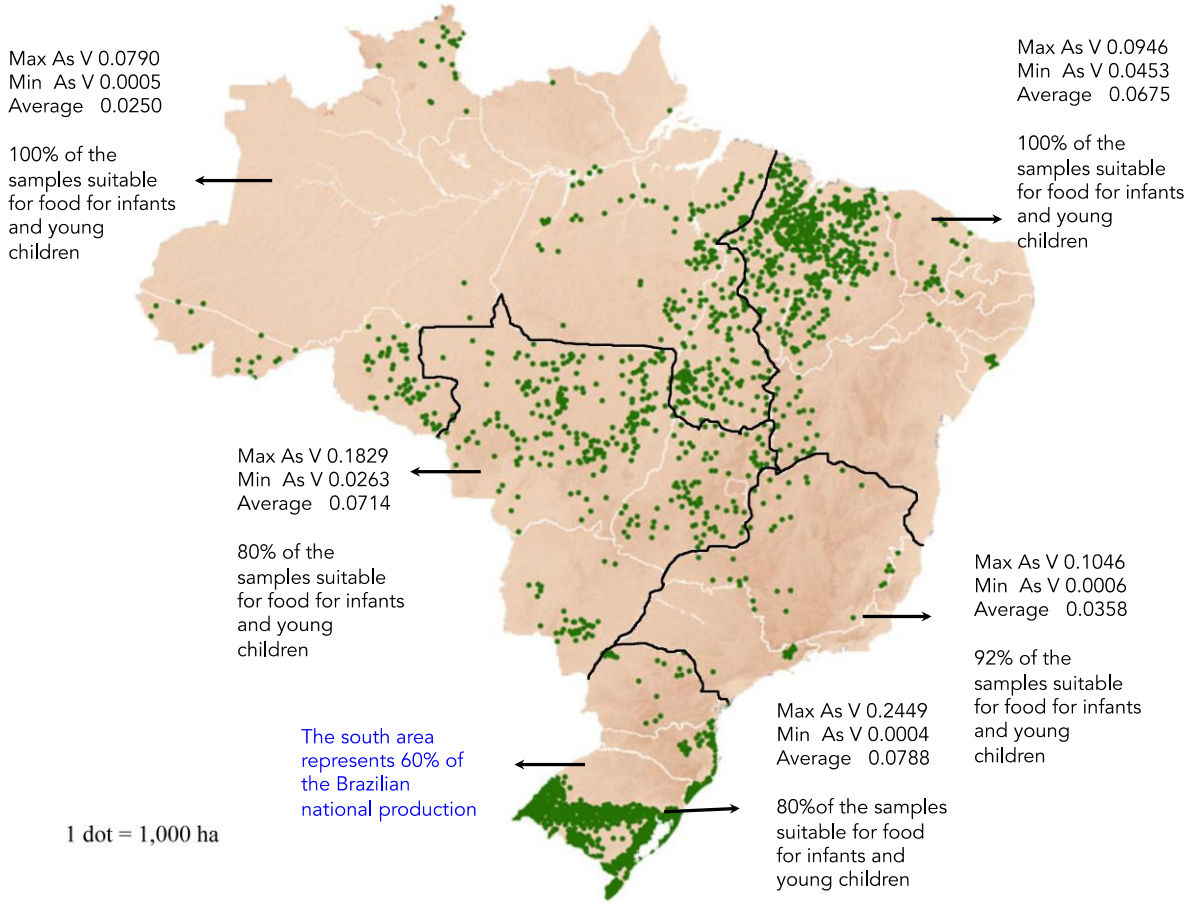

Figure 6. Rice production in Brazil (according to Global Rice Science Partnership -GriSP- 2013) and percentage of samples suitable for infants and young children's food according to Commission Regulation (EU) 2015 (0,10 mg As_i/ Kg).

For example, among the tested samples, Brazil was the only country with As_i concentrations over the EU limit for infants and young children's food (0,10 mg As_i/ kg). However, a considerable region specific variation occurred, with regions as north and northeast with 100% suitable samples and while the south region, which happens to be the main country producer, had 80% of samples suitable for infants and young children's food (Figure 6).

Arsenic toxicity depends on its chemical form. For inorganic arsenic, the lethal dose for 50% of rats varied from 15 to 293 mg As kg^{-1} body weight (Vallee, Ulmer & Wacker 1960). For humans, 70–80 mg of arsenic trioxide ingestion was reported as fatal (Aposhian & Aposhian, 2006). The oral route is the most common for inorganic arsenic exposure. However, in Brazil, a staple food such as rice is an important source of exposure. Average rice consumption in Brazil is 70 g day^{-1} $person^{-1}$ (FAO, 2008), of which 80% is polished rice (IBGE, 2010).

The basic staple foods in Brazil are rice, cassava, and beans; however, lately, beans and cassava have lost favor and wheat consumption has increased. At present, rice calorie intake is around 11% of total calorie intake and protein intake is almost 8%. Wheat and meat contribute more calories

and protein than rice, with values of more than 12% each (IRGA 2011; GRiSP, 2013).

Estimated daily intake of As_i through rice consumption was under (Provisional Tolerable Weekly Intake) PTDI value for low (0.0004 mg/kg), average (0.136 mg/kg) and high (0.244 mg/kg) concentrations of As_i founded in this study (Figure 7). These values were estimated based on the mean daily intake of 60-140 g of rice and body weight of 50-100 kg. However, it can be pointed out that a consumption of more than 350 g of rice per day is very common by Brazilian males. Then, the intake of inorganic arsenic from rice could easily reach the PTDI for arsenic. It is also important to mention that part of the Brazilian rice production is exported and can be consumed by communities with higher rice intake.

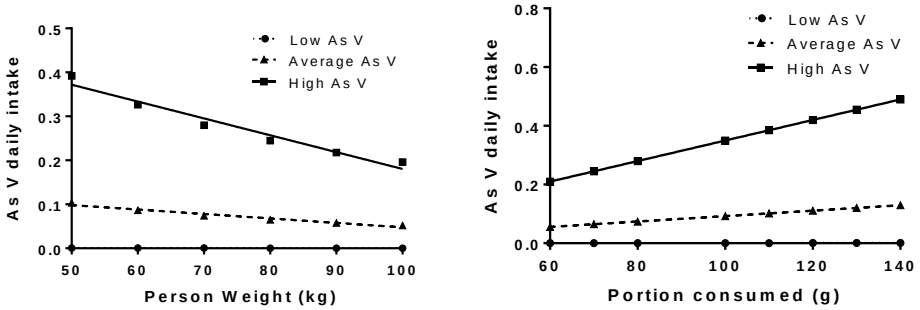

Figure 7. Estimated daily intake of As*i* considering the potential health risk for Brazilians of different regions, based on the POF/IBGE.

In this view, Banerjee, Banerjee, Bhattacharjee, Mondal, Lythgoe, Martínez et al. (2013) reported that the average of 200 mg/kg total arsenic in rice was equivalent to approximately 180 mg/kg inorganic arsenic and to a mean daily intake of inorganic arsenic of 2.0 mg/kg-bw/day. This intake value, resulted in genotoxic effects (ie. micronuclei occurrence), is marginally lower than the PTWI of 2.1 mg/kg-bw/day previously recommended by the WHO. Their study brought further highlights the inconsistency of current national and international regulation and guidelines for arsenic in drinking water, and rice as well as contributing to the increasing evidence that, irrespective of the exposure route, exposures to arsenic much lower than the equivalent of 2.1 mg/kg-bw/day.

According to Zavala, Gerads, Gürleyük & Duxbury, (2008) rice may be divided into two types, depending on the form of arsenic in the grain: inorganic arsenic-type and DMA-type. They reported that as arsenic levels rise, rice contains more methylated arsenic (DMA) but Meharg et al. (2009) noted that the amount of DMA is dependent on the rice cultivar.

As shown in Figure 8, Brazilian, Argentine, Uruguay and Paraguay rice samples did not tend toward the inorganic or DMA type since there was a high correlation between DMA and As V and the sum of arsenic species (Figure 5). However, it seems that rice samples from Brazil contained more fractions of DMA as compared to the others countries (Figure 5).

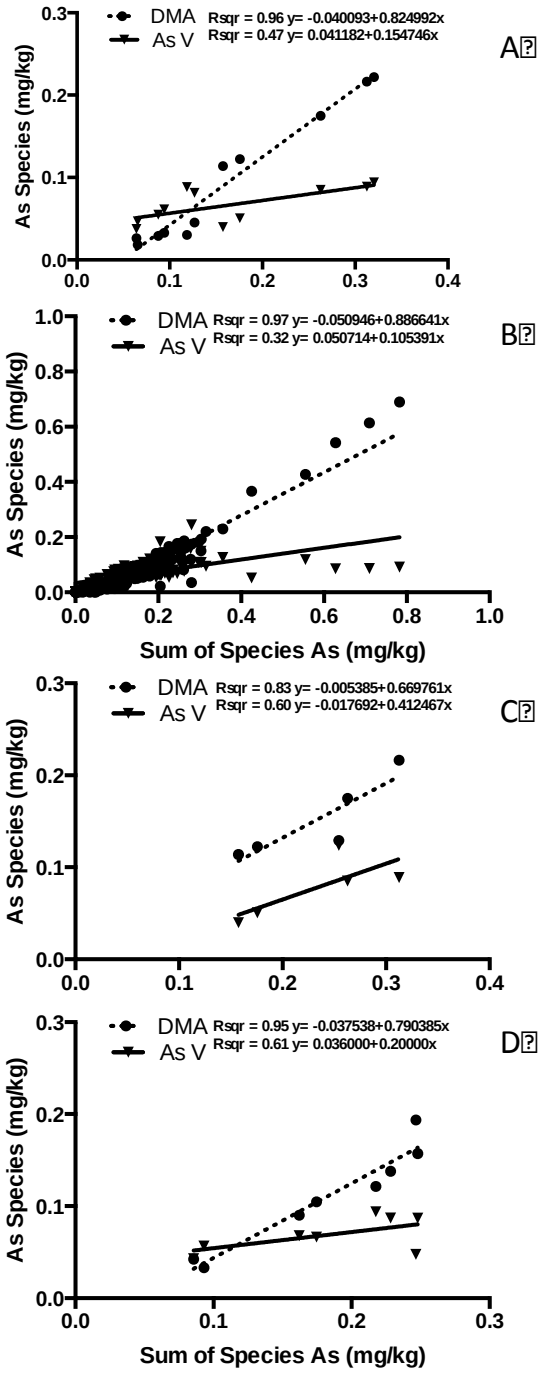

Figure 8. Arsenic species relationship to sum of species of total As for Argentina (A), Brazil (B), Paraguay (C) and Uruguay (D) rice grain samples.

CONCLUSION

This study showed the importance of As_i quantification in rice. We have comprehensively compiled, compared and assessed As in rice grains from South America. Total As content and As speciation were affected by soil use management, rice cultivar and geographic location. Moreover our results highlight the importance of increasing rice production under regions with low As since a

considerable area showed values of rice grain As concentration above the suitable As concentration for infants. Since Brazil is the major producer, thus exporting part of the produced rice, the high variance among different producers regions highlight the importance of increasing rice production under managements and regions with low As as well as to limit the use of grains with high content to babies and children's products. It is clear the need for rice certification in Brazilian rice regarding this matter.

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3. MANUSCRITO 2 CHEMICAL PROPERTIES AND THE PROTECTIVE EFFECT OF *ROSMARINUS OFFICINALIS*: MITIGATION OF LIPID PEROXIDATION AND DNA-DAMAGE FROM ARSENIC EXPOSURE

O manuscrito apresentado conforme as normas do jornal *Revista Brasileira de Plantas Medicinais - RBPM*

Chemical properties and the protective effect of *rosmarinus officinalis*: mitigation of lipid peroxidation and dna-damage from arsenic exposure

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ABSTRACT: Recent studies have implicated dietary factors in the cause and prevention of important diseases, with strong evidence that plant's compounds can protect against these diseases. Moreover, food security and environmental contamination are topics in focus at the moment. In this view, contamination by arsenic (As) has received much attention as well as some spices with medicinal properties. Among these plants, the use of *Rosmarinus officinalis* L. has demonstrated antioxidant properties besides being used for circulatory disorders, as antiseptic and for cicatrization. Therefore, we measured the mitotic index of *Allium cepa L.* and characterized the antioxidant effects to determine the capacity of *R. officinalis* to ameliorate arsenic-induced DNA damage. *R. officinalis* extract showed no mutagenic effects and exhibited antimutagenic potential, reducing the DNA damage, anaphase-telophase and micronuclei chromosome aberrations that result from treatment with the arsenic. Additionally, reduction in arsenic- induced lipid peroxidation was also observed.

Key words: arsenate, cytogenetics, heavy metal, medicinal plant, rosemary.

INTRODUCTION

Rosmarinus officinalis L. is a species of the Lamiaceae family native from the Mediterranean Region, popularly known as rosemary (Ferrari et al., 2011). According to Anvisa (2010), the extracts of rosemary leaves can be used for combating circulatory disorders, and is recommended as an antiseptic and for cicatrization.

Among the chemical constituents of rosemary, the flavonoids and phenolic acids are the two major groups of phenolic compounds (Cunha et al., 2012). These substances are noted for their antioxidant, anti-inflammatory, antitumor and estrogenic properties, suggesting the role of certain phenolic compounds in the prevention of coronary heart diseases and cancer (Tomás- Barberan, 2000; Espín, 2001).

Thus, studies to improve the potential of plant production quality to drug use are of great importance, especially when reporting on the chemical composition of the extracts of these plants growing under hydroponic condictions, which is a way to optimize the production.

Chronic arsenic (As) toxicity from ingestion of contaminated drinking water has been reported in many countries and is an environmental problem of colossal proportions with a wide range of deleterious health impacts, including hyperpigmentation, keratosis, skin and internal cancers, and vascular diseases (NRC, 2001; Argos et al., 2010). Additionally, over three billion people across the world consume rice as a staple food, which has also been identified as a major exposure route, as evidenced by observations of a strong association between rice consumption and urinary arsenic (Meharg; Zhao, 2012; Bae et al., 2002; Misbahuddin, 2003; Kile et al., 2007; Mondal; Polya, 2008).

The present study aimed to evaluate the concentration of phenolic compounds of *R. officinalis* extracts grown off the ground and its effects on arsenic-induced DNA damage and oxidative stress through *Allium cepa* test and biochemical analysis.

MATERIALS AND METHODS

Plant cultivation

Onion cultivation

Onion plantlets (*Allium cepa* L.) were obtained from Universidade Federal de Santa Maria (UFSM), RS, Brazil. The plantlets were transplanted to plastic trays containing substrate and cultivated in a greenhouse with shading (50%), at a spacing of 10 cm and a density of 100 per m of surface seedlings. These plants were administered with three daily irrigations (15 min each) with complete nutrient solution containing (mg L^{-1}): 155.90 N; 46.40 P; 5271.00 S; 123.00 Ca; 30.00 Mg; 253.60 K; (mmol L^{-1}) 2622.00 B; 133.00 Na; 277.00 Mo; 2274.00 Zn; 636.00 Cu; 6501.00 Mn and (mg L^{-1}) 1.20 Fe.

At the end of the cycle, the plants were collected and the produced bulbs were subsequently used for the *Allium cepa* test and biochemical analyses.

Rosemary cultivation

The rosemary plants were grown in a 115 m^2 polyethylene shelter, covered with polyethylene additive anti-UV 200 mm thick, at UFSM, Rio Grande do Sul, Brazil.

Polypropylene vessels (2.8 dm^3) with one plant per pot were used for this experiment. The vessels were filled with washed sand (between 1 mm and 3 mm grain size). During the cultivation four daily irrigations with nutrient solution were performed to keep the water level in the vessels.

The nutrient solution used had the following ionic composition in mmol L^{-1}: 11.02 NO_3^-; 2.32 NH_4^+; 0.8 $H_2PO_4^-$; 6.0 K^+; 1.75 Ca^{2+}; 1.25 Mg^{2+} and 1.25 SO_4^{2-}. Micronutrients were supplied in concentrations in mg L^{-1}, 0.03 Mo; 0.26 B; 0.06 Cu; 0.50 Mn; 0.22 Zn; through a stock solution Fe was separately provided at a concentration of 1.0 mg L^{-1} in the chelate form. The pH was maintained between 5.5 and 6.0.

The leaves of 10 plants were harvested at 160 days after planting (DAP). These leaves were dried under the shade during 5 days at room temperature, to reduce leaf

humidity (Khordishi et al., 2009), before the preparation of extracts and essential oil extraction.

Preparation of aqueous extracts

The extracts were prepared in the Laboratory of Cytogenetic and Plant Genotoxicity of UFSM, by infusion of the leaves at 5 and 20 g L^{-1}, using distilled water as liquid extractor.

The infusions were obtained by boiling distilled water at 100 °C and pouring water on whether the chopped plant material to facilitate the action of water. After mixing, the container remained capped for 15 minutes.

These extracts were filtered and, after reaching room temperature, were analyzed by high performance liquid chromatography with diode arrangements Detection (HPLC-DAD) for identification and quantification of the phenolic compounds, and used for the *A. cepa* test.

Extraction of volatile oil of rosemary

For the extraction of essential oil composite samples of 30 g were prepared from plants collected in four replications. The extraction of the essential oil of the leaves was carried out by hydrodistillation method in a Clevenger apparatus for 3 hours, and stored at -4° C until the chromatographic analysis and evaluation of the effect on the cell cycle through *A. Cepa* test.

High Performance Liquid Chromatography

The analysis by High Performance Liquid Chromatography (HPLC-DAD) was performed on HPLC system (Shimadzu, Kyoto, Japan) with auto injector (sIL 20A) equipped with reciprocating pumps (Shimadzu LC-20AT) connected to the degasser (DGU 20A5) with integrator (CBM 20A) detector UV / VIS diode array (SPD-M20A) and LC software solution 1.22 SP1. The analysis were performed in reversed phase under gradient conditions using C18 column (4.6 mm x 150 mm) packed with 5µm diameter particles.

The mobile phase was composed of: water containing acetic acid 2% (A) and methanol (B), and composition gradient was: 5% B for 2 min, 25% B to 10 min, 40, 50, 60, 70 and 80% (B) every 10 min, following the method described by Coelho et al. (2013), with slight modifications. The mobile phase extracts were filtered through a 0.45 µM membrane filter (Millipore) and then degassed in a sonication bath. Before use, *R. officinalis* samples were analyzed at a concentration of 5 and 20 mg ml^{-1}. The flow rate was 0.9 ml min^{-1}, injection volume of 50 L and wavelengths were 285 nm for carnosic acid, 325 nm for caffeic, chlorogenic and rosmarinic acid, and 365 nm for quercetin, rutin and kaempferol.

The reference standard solutions were prepared in HPLC mobile phase in concentrations of from 0.031 to 0.250 mg ml^{-1} to kaempferol, quercetin and rutin, and 0.100 to 0.250 mg ml^{-1} to rosmarinic acid, carnosic acid, chlorogenic acid and caffeic. The chromatographic peaks were confirmed by comparison of retention time and DAD spectra (200 to 600nm) with reference standards. Calibration curve for chlorogenic acid: Y = 12573x + 1206.5 (r = 0.9997), caffeic acid: Y = 11872x + 1570.3 (r = 0.9996), rosmarinic acid: Y = 13569x + 1344. 9 (r = 0.9995); carnosic acid: Y = 12278x + 1305.4 (r = 0.9999); rutin: Y = 15983x - 1321.5 (r = 0.9998), quercetin: Y = 16134x - 1422.6 (r = 0.9996) and kaempferol: Y = 16423x - 1853.2 (r = 0, 9998). All chromatographic operations were performed at room temperature in triplicate.

Chromatographic analysis of the volatile oil of Rosmarinus officinalis

Gas chromatography (GC-FID)

The gas chromatography (GC) analyses were carried out using an Agilent Technologies 6890N GC-FID system, equipped with DB-5 capillary column (30 m x 0.25 mm; film thickness 0.25 mm) and connected to an FID detector. The injector and detector temperatures were set to 280 °C. The carrier gas was helium, at a flow rate of 1.3 ml min^{-1}. The thermal programmer was 50-300 °C at a rate of 5°C/min. Two replicates of samples were processed in the same way. Component relative concentrations were calculated based

on GC peak areas without using correction factors. The injection volume of the oil was 1 µL (Tzakou; Loukis, 2009; Verma et al., 2010).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent Technologies AutoSystem XL GC-MS operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). The transfer line temperature was 280 °C. Helium was used as carrier gas (1.3 ml min^{-1}) and the capillary columns used were an HP 5MS (30 m x 0.25 mm; film thickness 0.25 mm) and an HP Innowax (30 m x 0.32 mm i.d., film thickness 0.50 mm). The temperature program was the same as that used for the GC analyses. The injected volume was 1 µL of the volatile oil.

Identification of the components

Identification of the constituents was performed on the basis of retention index (RI), determined with reference of the homologous series of *n*-alkanes, C_7-C_{30}, under identical experimental conditions, comparing with the mass spectra library search (Nist and Wiley), and with the mass spectra literature date Adams (1995). The relative amounts of individual components were calculated based on the CG peak area (FID response).

Cytogenetic analysis

Cytogenetic analysis of meristematic cells obtained from *A. cepa* radicles were used to evaluate morphological and structural cell modifications and determine the mitotic index.

Nineteen groups of five bulbs were placed in distilled water, constituting 19 treatments with 5 replicates each. The following treatments were used: to check the individual effect of each solution; T1: distilled water, as a negative control; T2: *R. officinalis*

oil 0.8%, T3: *R. officinalis* oil 3%, T4: *R. officinalis* extract 5 g L^{-1}, T5: *R. officinalis* extract 20 g L^{-1}, T6: arsenic (100 µM), to check the effect of *R. officinalis* prior to arsenic exposure; T7: *R. officinalis* oil 0.8% (24 hs) + arsenic (24 hs), T8: *R. officinalis* oil 3% (24 hs) + arsenic (24 hs), T9: *R. officinalis* extract 5 g L^{-1} (24 hs) + arsenic (24 hs), T10: *R. officinalis* extract 20 g L^{-1} (24 hs) + arsenic (24 hs), T11: arsenic (24 hs) + *R. officinalis* oil 0.8% (24 hs), to check the effect of *R. officinalis* after arsenic exposure; T12: arsenic (24 hs) + *R. officinalis* oil 3% (24 hs), T13: arsenic (24 hs) + *R. officinalis* extract 5 g L^{-1} (24 hs), T14: arsenic (24 hs) + *R. officinalis* extract 20 g L^{-1} (24 hs), to check the effect of *R. officinalis* use concomitantly with arsenic exposure;T15: arsenic with *R. officinalis* oil 0.8%, T16: arsenic with *R. officinalis* oil 3%, T17: arsenic with *R. officinalis* extract 5 g L^{-1}, T18: arsenic with *R. officinalis* extract 20 g L^{-1}, T19: ethanol to check alcohol effect.

For the analysis of cell division, approximately 2 cm of onion radicles were collected and hydrolyzed in 1 N HCl for 5 min, followed by staining with 2% acetic orcein in accordance with Guerra and Souza (2002). The meristematic region was crushed using a small glass rod, and the material was placed onto a coverslip. The slides were observed under the microscope at 40 X magnification. Subsequently, the total cell count (interphase and cell division) was obtained, and the mitotic index (MI) was calculated based on the percentage of dividing cells. A total of 1000 cells were counted per bulb, totaling 4000 cells per group for each treatment and each species studied.

Biochemical parameters

For the biochemical assays, the root samples were collected, immediately placed in liquid nitrogen and pulverized to a fine powder using a porcelain mortar. The level of lipid peroxidation was estimated using a TBARS Assay (thiobarbituric acid reactive substances assay) according to the method of El-Moshaty et al. (1993), where the concentration of malondialdehyde (MDA) was measured as an end product of lipid peroxidation through the reaction with thiobarbituric acid (TBA). The frozen root samples were homogenized in 0.2 M

citrate phosphate (pH 6.5) containing 0.5% Triton X-100 at a ratio of 1:10 (w/v). The homogenate was centrifuged for 15 min at 20,000 g. One milliliter of the supernatant fraction was added to an equal volume of 20% (w/v) TCA containing 0.5% (w/v) TBA.

The mixture was heated at 95 ^{o}C for 40 min and quickly cooled in an ice bath for 15 min. After centrifugation at 3,600 rpm for 15 min, the absorbance of the supernatant was measured at 532 nm. The absorbance value obtained at 600 nm was subtracted to correct for the non-specific turbidity.

The H_2O_2 concentration in the roots was determined according to Loreto and Velikova (2001). Approximately, 0.05 g of the frozen sample was homogenized in 2 mL of 0.1% (w/v) TCA. The homogenate was mixed with 0.5 mL of a 10 mM potassium phosphate buffer (pH 7.0) and 1.0 mL of 1 M KI, and the mixture was centrifuged at 12,000 g for 15 min at 4^{o}C. The H_2O_2 concentration of the supernatant was evaluated through a comparison of its absorbance at 390 nm with the standard calibration curve.

Non specif peroxidase activity

The activity of peroxidase (POD, EC 1.11.1.7) nonspecific present in the extract was determined according Zeraik et al. (2008), using guaiacol as substrate. The reaction mixture contained 1.0 mL of 100 mM potassium phosphate buffer (pH 6.5), 1.0 mL of 15 mM guaiacol and 1.0 mL of 3 mM H2O2. After homogenization of this solution was added 50 uL extract. The oxidation of guaiacol (a tetraguaiacol) was measured by the increase in absorbance at 470 nm (Chance; Maehly, 1955).

Statistical analysis

The experiments were performed using a randomized design. The analyses of variance were computed on statistically significant differences determined based on appropriate F-tests. The results are presented as the means ± SD of at least three independent replicates. The mean differences were compared using Scott-Knott ($P<0.05$).

RESULTS AND DISCUSSION

Chromatography and cytogenetic analysis

The HPLC-DAD revealed that quercetin, rutin and kaempferol (flavonoids) and chlorogenic acid, caffeic acid, rosmarinic and carnosic acid (phenolic acids) are the main components found in aqueous extracts by infusion of *R. officinalis* (Table 2). Similar results have being reported by Yesil- Celiktas et al., (2007) and Frescura et al., (2013). Yesil-Celiktas et al., (2007) demonstrated that there are significant variations in rosmarinic acid content and carnosic acid according to the harvest season and place of rosemary cultivation. In present study, using the hydroponic cultivation, we obtained 5.99 mg ml^{-1} rosmarinic acid values, indicating that this culture allows to obtain plants with good yield, minimizing the effects of the harvest season

The compound found in higher amounts in aqueous extracts of *R. officinalis* was rosmarinic acid (Table 2), which is widely spread in the Lamiaceae family (Simões et al, 2001). The presence of rosmarinic acid gives the extract antioxidant activity, reducing numerous events deleterious to the organism, such as formation of reactive oxygen species, lipid peroxidation and DNA fragmentation (Izzo; Capasso, 2007; Ji; Zhang, 2008). The rosmarinic acid, together with chlorogenic acid and caffeic acid derivatives have also beening studied as potential taxonomic markers in family Lamiaceae (Simões et al., 2001).

The carnosic acid is described as the active ingredient responsible for antimicrobial activity exhibited by the extract from the leaves of *R. officinalis* (Bernardes et al., 2009) and has clinical application for diseases affecting the outer retine, including age-related degeneration, in which oxidative stress is probably one factor that contributes to disease progression (Rezaie et al., 2012).

The rutin has among other activities, the improvement in symptoms of impaired lymphatic and venous vessels associated with some bleeding disorders or high blood pressure, capillary fragility symptoms are also improved, including, loss of visual acuity and

visual field changes (Pathak et al., 1991). The quercetin offers many benefits of health promotion, including improved cardiovascular health, eye diseases, allergic diseases, arthritis, reducing the risk of cancer and many others (LakhanpaL; Rai, 2007). Still, kaempferol and quercetin have protective action against pancreatic hypertrophy and hyperplasia (Rawel et al., 2002).

The major compounds of the volatile oil were the monoterpenes - camphor (21.33%), 1.8 cineole (16.78%), α-pinene (11.15%), β-myrcene (8.18%,) and verbenone (7.20%) corresponding to 64.64% of 91.37%. Besides the major compounds, other ones were identified, which are described in Table 4. In study carried by Ribeiro et al. (2012), the dominant constituents found were α-pinene (19.8%), β-myrcene (24.2%), 1.8 cineole (22.2%) and verbenona (9.3%), and Rahman et al. (2007) found as major compounds the camphor compounds (26.40%), 1.8 cineole (23.48%), α-pinene (9.94%) and verenone (3.32%).

TABLE 1 - Concentration of phenolic compounds, mg g^{-1}, in extracts of *R. officinalis*, prepared with two concentrations (5g of leaves L^{-1} and 20 g of leaves L^{-1}).

phenolic compounds	*R. officinalis* extract	
	5 g L^{-1}	20 g L^{-1}
rosmarinic acid (mg ml^{-1})	5.99	26.3
chlorogenic acid (mg ml^{-1})	2.30	8.45
carnosic acid (mg ml^{-1})	1.83	5.09
caffeic acid (mg ml^{-1})	1.33	4.87
canpferol (mg ml^{-1})	0.61	2.40
quercetin (mg ml^{-1})	1.45	5.21
rutin (mg ml^{-1})	0.73	5.71

The *Allium cepa* test has previously been used to evaluate DNA damage in conjunction with animal tests, yielding similar results (Vicentini et al., 2001; Teixeira et al., 2003) with proven efficacies. In addition, the International Program on Chemical Safety (IPCS, WHO) and the United Nations Environment Program (UNEP) have validated the method of chromosomal aberration in *A. cepa* roots as an effective test for the *in situ* analysis and monitoring of the environmental genotoxicity of substances (Cabrera; Rodriguez, 1999).

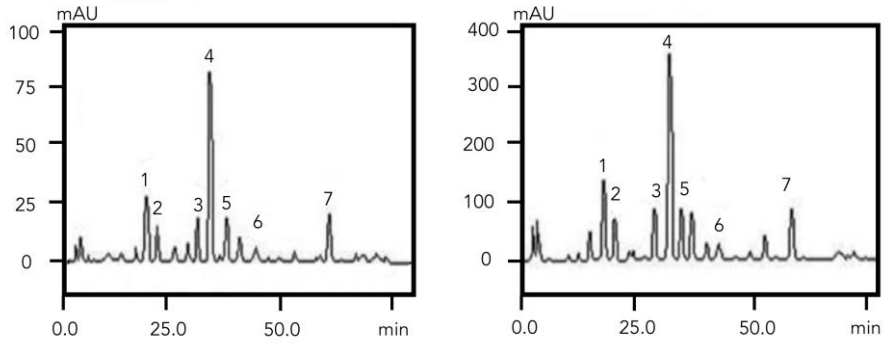

FIGURE 1 **-** Liquid Chromatography Profile High Efficiency *R. officinalis* extract under the concentration of 5 g L^{-1} (right side) and 20 g L^{-1}(right side). The peaks refer to chlorogenic acid (peak 1), caffeic acid (peak 2), rutin (peak 3), rosmarinic acid (peak 4), quercetin (peak 5), canpferol (peak 6), carnosic acid (peak 7).

The results demonstrate that both volatile oil, in concentrations of 0.8 and 3%, and aqueous extracts of *R. officinalis* in concentrations of 5 and 20 g L^{-1}, have no mutagenic effect as compared to arsenic treatments (Table 3), and slight different from control treatment, consistent with the results of other studies using different techniques (Amin; Hamza, 2005) which is beneficial in terms of the safety of volatile oil and aqueous extracts of *R. officinalis* as a phytomedicine.

Genotoxicity of arsenic compounds has been observed in a variety of cultured human cells (Yih; Lee, 1999; Yih et al., 2005), animals (Gebel, 2001; Patlolla; Tchounwou, 2005) and arsenic-exposed human populations (Mahata et al., 2003). In the present study, *R. officinalis* (both volatile oil and aqueous extract) reduced arsenic-mediated chromosome aberrations, especially the occurrence of micronucleus (Table 3; Figure 2). When added after 24h exposure to As, the aqueous extract was able to completely reverse the damage caused by exposure to As. When used concomitant, there was no occurrence of chromosomal aberrations (Table 1; Figure 2). Some studies have reported the antimutagenic effects of *R. officinalis* (Wang et al., 2012; Horvathova et al., 2014**),** but to our knowledge, there were no studies evaluating the effect of *R officinalis* in to repair the DNA damage caused by exposure to As.

The most common abnormality caused by the arsenic exposure in this study was the occurrence of micronuclei, which was already reported by other studies (Yi et al., 2007; Banerjee et al., 2013). Banerjee et al. (2013) reported the association between micronuclei frequency in urothelial cells from men, women, smoker group and non-smokers group, and arsenic content in cooked rice. They indicated a strong positive correlation of mean urinary arsenic with mean cooked rice arsenic content among the tested groups.

The mitotic index and DNA replication are used as indicators of adequate cell proliferation (Gadano et al., 2002) and can be measured using the *Allium cepa* test (Fachinetto; Tedesco, 2009), being a good indicator to access the impact of As exposure, i.e. trough contaminated rice consumption.

According to the Mitra (2004) populations that may suffer from folate, anemia, animal protein, and vegetable fibre deficiency, have increased risk of toxic effects arising out of chronic As exposure. Global anemia prevalence improved slightly between 1995 and 2011, decreased from 33% (29–37) to 29% (24–35) in non-pregnant women, from 43% (39–47) to 38% (34–43) in pregnant women, and from 47% (43–51) to 43% (38–47) in children. This prevalence translated to 496 million (409–595 million) non-pregnant women, 32 million (28–36 million) pregnant women, and 273 million (242–304 million) children with anaemia in 2011 (Stevens et al., 2013). In Brazil, at the age of 12-16 months, the overall prevalence of anemia, iron deficiency and iron deficiency anemia is 63.7, 90.3 and 58.8%, respectively, thus a large part of this population have low income being rice and pasta the main sources of energetic food (Bortolini; Vitolo, 2010).

TABLE 2 **-** Chemical composition of *Rosmarinus officinalis* volatile oil.

	Compounds	RI[a]	RI[b]	Concentration %
M	*α*-pinene	936	939	11.15
	α-camphene	954	953	4.52
	β-pinene	980	980	3.29
	β-myrcene	991	991	8.18
	α-phellandrene	1003	1005	0.25
	p-cymene	1026	1026	0.44
	1.8 cineol	1037	1033	16.78
	Camphor	1143	1143	21.33
	δ –terpinene	1065	1062	1.28
	Linalool	1098	1098	2.54
	Isoboneol	1156	1156	2.74
	Borneol	1165	1166	1.82
	Menthol	1173	1173	0.94
	Terpin-4-ol	1178	1177	0.51
	Pinocarvone	1160	1162	1.11
	Naphthalene	1178	1179	0.34
	α-terpineol	1189	1189	1.70
	piperitol <cis->	1195	1193	0.93
	Verbenone	1205	1204	7.20
	Pulegone	1237	1237	0.15
	Eugenol	1356	1356	0.73
S	α-copaene	1376	1376	0.01
	Caryophyllene	1417	1418	1.34
	Aromadendrene	1440	1439	0.48
	α-muurolene	1498	1499	0.07
	α-bisabolene	1504	1504	0.46
	α-cadidene	1538	1538	0.25
	Caryophyllene oxide	1580	1581	0.83
	Total identified (%)			91.37

Relative proportions of the essential oil constituents were expressed as percentages. [a]Retention indices experimental (based on homologous series of *n*-alkane C_7-C_{30}). [b]Retention indices from literature (Adams, 1995); M= monoterpenes; S= sesquiterpenes.

TABLE 3 **-** Effect of *Rosmarinus officinalis* oil and extract on mitotic index and chromosomal aberrations in root tip cells of *A. cepa* exposed to arsenic.

TREATMENTS	Mitotic index (%)	Total abnormalitie (%)	Micronuclei	Chromosomal breakage and lost chromosomes	Anaphasic telophasic bridges
T1 distilled water *	5.37 a	0.25 c	0	0	1
T2 *R. officinalis* oil 0.8%	5.00 b	0.50 c	1	0	1
T3 *R. officinalis* oil 3%	3.15 d	0.75 c	3	0	0
T4 *R. officinalis* extract 5 g L^{-1}	1.37 f	0.50 c	1	0	1
T5 *R. officinalis* extract 20 g L^{-1}	0.80 g	0.75 c	2	0	1
T6 arsenic	4.27 c	3.50 a	10	0	4
T7 *R. officinalis* oil 0.8% (24 hs) + arsenic (24 hs)	1.15 f	2.00 b	7	0	1
T8 *R. officinalis* oil 3% (24 hs) + arsenic (24 hs)	0.20 h	1.50 b	5	0	1
T9 *R. officinalis* extract 5 g L^{-1} (24 hs) + arsenic (24 hs)	0.20 h	1.00 c	3	0	1
T10 *R. officinalis* extract 20 g L^{-1} (24 hs) + arsenic (24 hs)	0.10 h	0.75 c	1	0	2
T11 arsenic (24 hs) + R. officinalis oil 0.8% (24 hs)	3.95 c	2.00 b	6	1	1
T12 arsenic (24 hs) + *R. officinalis* oil 3% (24 hs)	1.00 g	0.25 c	1	0	0
T13 arsenic (24 hs) + *R. officinalis* extract 5 g L^{-1} (24 hs)	1.00 g	0.50 c	1	0	1
T14 arsenic (24 hs) + *R. officinalis* extract 20 g L^{-1} (24 hs)	0.95 g	0.00 c	0	0	0
T15 arsenic with R. officinalis oil 0.8%	4.10 c	2.00 b	5	1	2
T16 arsenic with *R. officinalis* oil 3%	2.35 e	0.25 c	1	0	0
T17 arsenic with *R. officinalis* extract 5 g L^{-1}	0.75 g	0.50 c	2	0	0
T18 arsenic with *R. officinalis* extract 20 g L^{-1}	0.20 h	0.00 c	0	0	0
T19 ethanol	4.25 c	0.50 c	2	0	0

Different low letters show significant differences among the treatments. *negative control

Interestingly, developed countries may have specific sub-populations with high As_i consumption rates from rice such as babies and young children, gluten intolerant and lactose intolerant individuals. Moreover, many communities with low income purchasing have high rice consumption levels due to its low price and ease of preparation (Meharg et al., 2003).

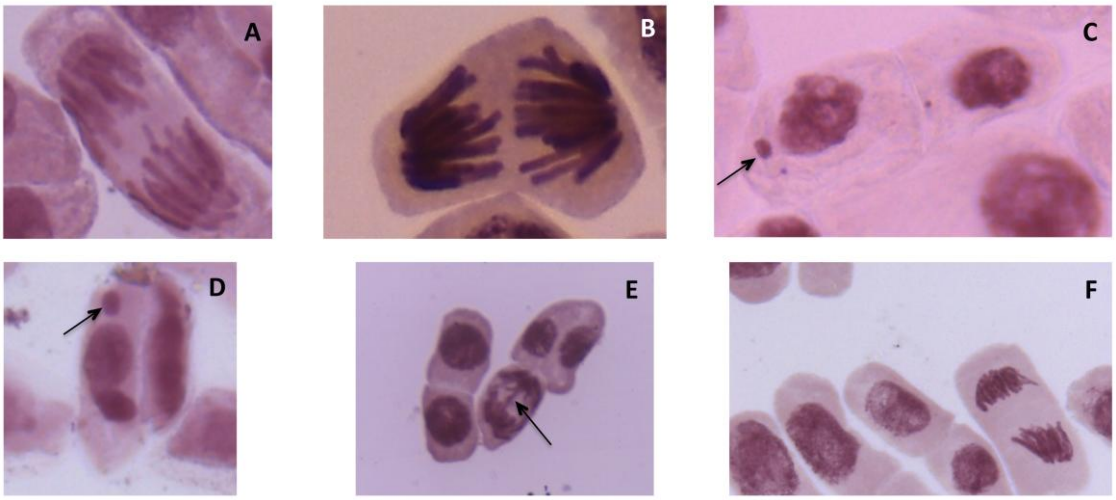

FIGURE 2 **- A B:** Allium cepa cells during normal anaphase (T1 distilled water A and T5 Rosmarinus officinalis extract B); **C D** Allium cepa cells during interphase exhibiting micronuclei (T6 treated with Arsenic (24hs); and T7 R. officinalis oil 0.8% (24 hs) + arsenic (24 hs)); **E:** Allium cepa cells exhibiting anaphase chromosome bridges (T6 treated with arsenic); **F:** Allium cepa cells during normal anaphase (T18 arsenic with R. officinalis extract 20 g L^{-1}).

Oxidative stress

R. officinalis, as others Lamiaceae species present in its chemically flavonoids and phenolic acids (Cunha et al., 2012; Coelho et al, 2013). Phenolic compounds can slow down oxidative reactions in biological systems. The study of phenolic compounds is also associated with important antioxidant activity of these compounds, suggesting that diseases caused by oxidative reactions in biological systems can be delayed by the intake of natural antioxidants found in plants such as *R. officinalis* (Simões et al., 2001). In the present study, the high content of antioxidant compounds enables us to confirm the beneficial effect of the aqueous extract, in reducing the deleterious effects of exposure to As.

The exposure to As causes oxidative stress, as can clearly be evidenced by TBARS formation, increased content of H_2O_2 and POD activity. In the present study, the application of *R. officinalis* extract reduced the TBARS concentration in cells exposed to arsenic, as well as the H_2O_2 concentration regardless the moment of

exposure (Table 3). On the other hand, the volatile oil did not alter TBARS or H_2O_2 concentration under the 0.8% dose when used only 24hs before arsenic exposure (Table 3).

Essential oils present in their chemical constitution terpenic hydrocarbons, alcohols, aldehydes, ketones and esters among others. These compounds exhibit high antibacterial and anticancer activity, however with low antioxidant effect as compared to polyphenolic compounds found in the aqueous extract (Simões et al., 2001) which may explain our results.

Wang et al. (2012) assessed the comparative antibacterial and anticancer activities of *R. officinalis* essential oil as well as its compounds, being the three main components 1,8-cineole, α-pinene and β-pinene. Even though *R. officinalis* essential oil exhibited the strongest antibacterial and cytotoxic activities towards SK-OV-3, HO-8910 and Bel-7402 human tumor cell lines. It is important to point that even though in this study the *R. officinalis* essential oil didn't show high antioxidant activity, it is well known its beneficial proprieties to human heath (Posadas et al., 2009).

TABLE 4. Levels of H_2O_2, TBARS and POD in cells obtained from *Allium cepa* radicles exposed to arsenic and *Rosmarinus officinalis* oil and extract.

TREATMENTS	TBARS (MDA nM g^{-1} FW)	H_20_2(uM g^{-1} FW)	POD (umol min^{-1} mg FW)
T1 distilled water *	0.567^{d}	1.602^{c}	22.217^{d}
T2 *R. officinalis* oil 0.8%	0.587^{d}	2.203^{c}	26.728^{d}
T3 *R. officinalis* oil 3%	0.564^{d}	1.456^{c}	28.984^{c}
T4 *R. officinalis* extract 5 g L^{-1}	0.498^{d}	1.467^{c}	26.728^{d}
T5 *R. officinalis* extract 20 g L^{-1}	0.476^{d}	2.202^{c}	19.961^{d}
T6 arsenic	1.876^{a}	10.267^{a}	55.173^{a}
T7 R. officinalis oil 0.8% (24 hs) + arsenic (24 hs)	1.654^{a}	8.467^{a}	36.428^{c}
T8 *R. officinalis* oil 3% (24 hs) + arsenic (24 hs)	1.132^{c}	7.233^{a}	29.661^{c}
T9 *R. officinalis* extract 5 g L^{-1} (24 hs) + arsenic (24 hs)	1.109^{c}	5.533^{b}	31.917^{c}
T10 *R. officinalis* extract 20 g L^{-1} (24 hs) + arsenic (24 hs)	1.187^{c}	4.822^{b}	36.428^{c}
T11 arsenic (24 hs) + R. officinalis oil 0.8% (24 hs)	1.543^{b}	8.467^{a}	38.684^{b}
T12 arsenic (24 hs) + *R. officinalis* oil 3% (24 hs)	0.765^{d}	6.875^{a}	31.917^{c}
T13 arsenic (24 hs) + *R. officinalis* extract 5 g L^{-1} (24 hs)	0.708^{d}	4.076^{b}	29.661^{c}
T14 arsenic (24 hs) + *R. officinalis* extract 20 g L^{-1} (24 hs)	0.698^{d}	4.897^{b}	29.554^{c}
T15 arsenic with R. officinalis oil 0.8%	1.106^{c}	8.467^{a}	34.172^{c}
T16 arsenic with *R. officinalis* oil 3%	0.706^{d}	5.876^{b}	43.195^{b}
T17 arsenic with *R. officinalis* extract 5 g L^{-1}	0.654^{d}	4.035^{b}	43.194^{b}
T18 arsenic with *R. officinalis* extract 20 g L^{-1}	0.698^{d}	4.876^{b}	40.706^{b}

Different low letters show significant differences among the treatments. *negative control

The POD activity was enhanced by As exposure as compared to the others treatments (Table 3). Among the treatments with *R. officinalis*, the concomitant exposure to As and *R. officinalis* resulted in a higher activity as compared to *R. officinalis* prior or after As exposure (Table 3).

However, in general terms this activity of the *R. officinalis* extracts wasn't enough to prevent DNA damage in a higher level as compared to treatments with *R. officinalis* extracts being used after As exposure (Table 3).

CONCLUSION

This study demonstrated that the *Rosmarinus officinalis* extract exerted no mutagenic effects and showed antimutagenic potential, reducing the DNA damage and lipid peroxidation resulting from treatment with As exposure.

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4. MANUSCRITO 3 ARSENIC UPTAKE AND METABOLISM IN RICE CULTIVARS DIFFERING IN USE EFFICIENCY AND RESPONSE TO PHOSPHORUS

O manuscrito apresentado conforme as normas do jornal *Anais da Academia Brasileira de Ciências*

Arsenic uptake and metabolism in rice cultivars differing in use efficiency and response to phosphorus

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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 three rice cultivars, BR-IRGA 409, IRGA 423 and IRGA 424. The experiment occurred in three stages: I 5 days of acclimatization under complete nutritive solution; II 10 days under P (0.0 and 0.09 mM) and As (0.0 and 100 μM) treatments and III five days under recovery, with complete nutritive solution without As. The As exposure had significant effects on dry weights of shoots or roots, thus 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. On the other hand 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. Thus 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.

Key words: mineral nutrition**,** *Oryza sativa*, phosphate, 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. Arsenic exists in the environment in inorganic and organic forms, and both arsenite and arsenate are often found in anaerobic and aerobic soil environments (Meharg and Macnair, 1992; Meharg et al. 2003).

Arsenate is an analogue of phosphate, competing for the same sorption sites in the root apoplast and for the same uptake carriers in the root plasmalemma (Meharg and Macnair, 1992; Meharg and Hartley-Whitaker, 2002). Phosphate can decrease or increase the uptake of As by plants, depending on the speciation of As, plant species and plant growth medium (Tsutsumi, 1980; Otte et al. 1990).

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 of free metalloid ions via thiol-mediated complexation (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 evaluated its relationship with As tolerance.

MATERIAL AND METHODS

Plant materials and growth conditions

Rice seeds of the *indica* variety were obtained from ***IRGA* (*Instituto Rio Grandense do Arroz*)**, RS, 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 $(NH_4)_2SO_4$, 0.27 mM $MgSO_4.7H_2O$, 0.09 mM KNO_3, 0.18 mM $Ca(NO_3)_2.4H_2O$, and 0.09 mM P_2O_5 and the micronutrients 20 μM NaEDTA-$Fe.3H_2O$, 6.7 μM $MnCl_2.4H_2O$, 9.4 μM H_3BO_3, 0.015 μM $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.15 μM $ZnSO_4.7H_2O$, and 0.16 μM $CuSO_4.5H_2O$. 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. Than, half of each group seedlings had As added in the nutrient solution as $Na_3AsO_4 \cdot 12\ H_2O$ (+As) under the concentration of 100 μM. 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 remaining 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.

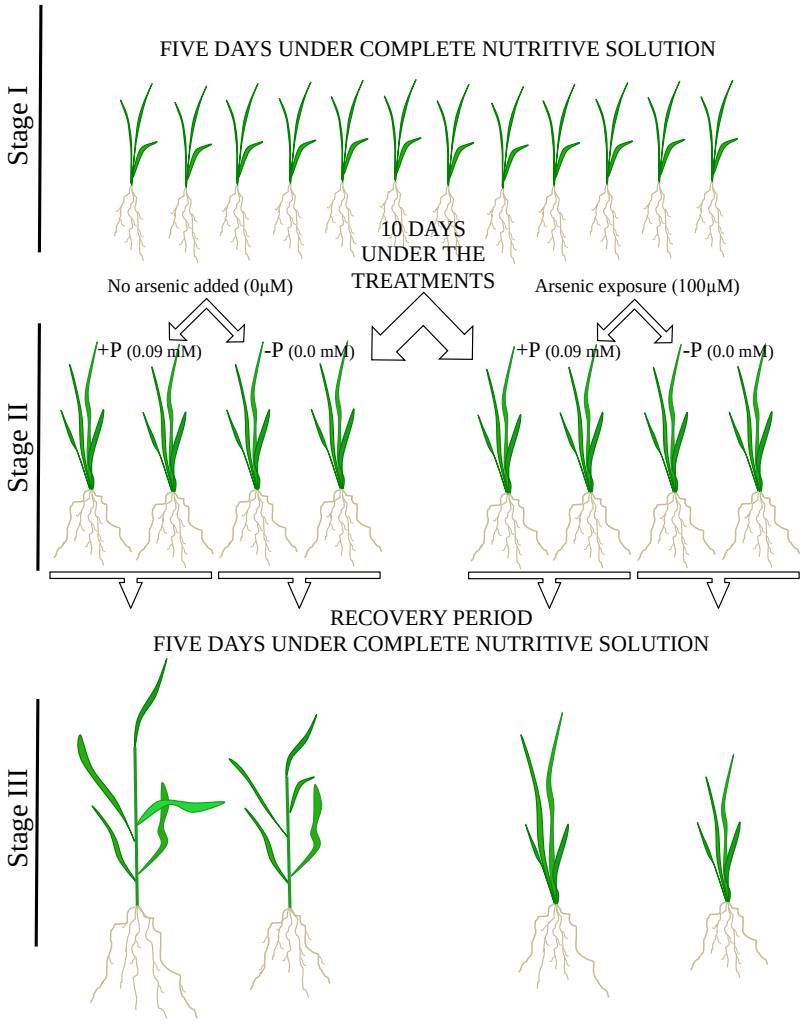

Figure 1. Practical scheme of the experimental design used in this study.

Tissue elements analysis

Arsenic speciation - The roots and shoots of the seedlings were oven-dried at 65°C to a constant mass for the determination of biomass and than 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 dimethylyarsonic acid (DMA) concentrations certified. Samples where then microwave digested in an CEM MARS 6 instrument for 30 min. at 95 ^{o}C using a 3 stage slow heating program: to 55 ^{o}C in 5 min. held for 10 min., to 75^{o}C in 5 min., held for 10 min. to 95 ^{o}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 ^{o}C in 5 min. hold for 10 min. to 135^{o}C then hold for 10 min., to 180^{o}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 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 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 the 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 the 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:

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

$T_{Root\text{-}P} = C_{Root\text{-}P} \times Roots_{biomass}$

$T_{Shoot\text{-}P} = C_{Shoot\text{-}P} \times Shoots_{biomass}$

$PUE = (T_{Shoot\text{-}P} / Shoot_{biomass})$ (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 of 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, ie. 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).

Even though 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 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).

CULTIVAR	NO ARSENIC ADDED		ARSENIC EXPOSURE (100 μM)	
	0 mM P_2O_5	0.09 mM P_2O_5	0 mM P_2O_5	0.09 mM P_2O_5
	Root Dry weight (mg plant^{-1})			
BR-IRGA 409	10.507 ± 0.28 Ba	20.09 ± 0.26 Ba*	8.55 ± 0.23 Cb	11.51 ± 0.21 Bb
IRGA 423	10.820 ± 0.63 Ba	23.36 ± 0.52 ABa	13.64 ± 0.33 Bb	15.05 ± 0.30 Ab
IRGA 424	30.143 ± 0.30 Aa	32.25 ± 0.77 Aa	16.55 ± 0.15 Aa	22.95 ± 0.46 Aa*
	Shoot Dry weight (mg plant^{-1})			
BR-IRGA 409	58.08 ± 0.92 Aa	70.18 ± 0.97 Ba	28.68 ± 0.65 Bb	38.58 ± 0.67 Cb
IRGA 423	66.88 ± 0.77 Aa	81.32 ± 0.88 Ba	39.01 ± 0.43 Ab	50.47 ± 0.83 Bb
IRGA 424	61.17 ± 1.01 Aa	125.44 ± 1.23 Aa*	47.64 ± 0.75 Ab	71.06 ± 1.20 Ab*
	Total root length (cm plant^{-1})			
BR-IRGA 409	83.50 ± 5.76 Ba	100.35 ± 7.30 Ba*	46.88 ± 4.03 Bb	61.02 ± 7.33 Bb
IRGA 423	99.38 ± 12.03 Ba	101.23 ± 10.53 Ba	55.00 ± 5.08 Bb	72.80 ± 5.20 Bb*
IRGA 424	123.37 ± 11.44 Aa	133.24 ± 10.05 Aa	87.00 ± 6.22 Ab	112.30 ± 12.33 Aa*
	Number of root tips (plant^{-1})			
BR-IRGA 409	168.00 ± 33.50 Ba	186.50 ± 22.33 Bb	125.75 ± 25.33 Aa	204.25 ± 16.33 Ba*
IRGA 423	217.25 ± 44.33 Aa	168.50 ± 33.50 Ba	106.50 ± 23.33 Ab	167.00 ± 15.50 Ca*
IRGA 424	244.25 ± 32.33 Aa	230.50 ± 21.33 Aa	155.50 ± 32.50 Ab	247.75 ± 20.33 Aa*

Figure 2. Biomass and root system parameters of rice plants of three cultivars exposed to 100 μM arsenic (+As) and without arsenic (–As), with phosphorus 0.09 mM (+P) and without phosphorus (–P) 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, $\alpha = 0.05$.

It seems that 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 beneficial effect of adding mineral elements to plant substrate in order to improve plant growth has been known in agriculture for over 2000 years (Marschner, 1995). The increment on mineral nutrition may also mitigate toxic effects of other elements, through 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 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. Thus, BR/IRGA 409 cultivar which had a high susceptibility to As and is well known for it susceptibility to excess levels of Fe (Stein et al., 2009), had the smallest 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 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). Interestingly, in the present study, the As present in shoot tissue was mainly inorganic As, with no significant changes in other As species such as DMA with As increment in shoot tissue (Figure 5).

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 Whitaker, 2002).

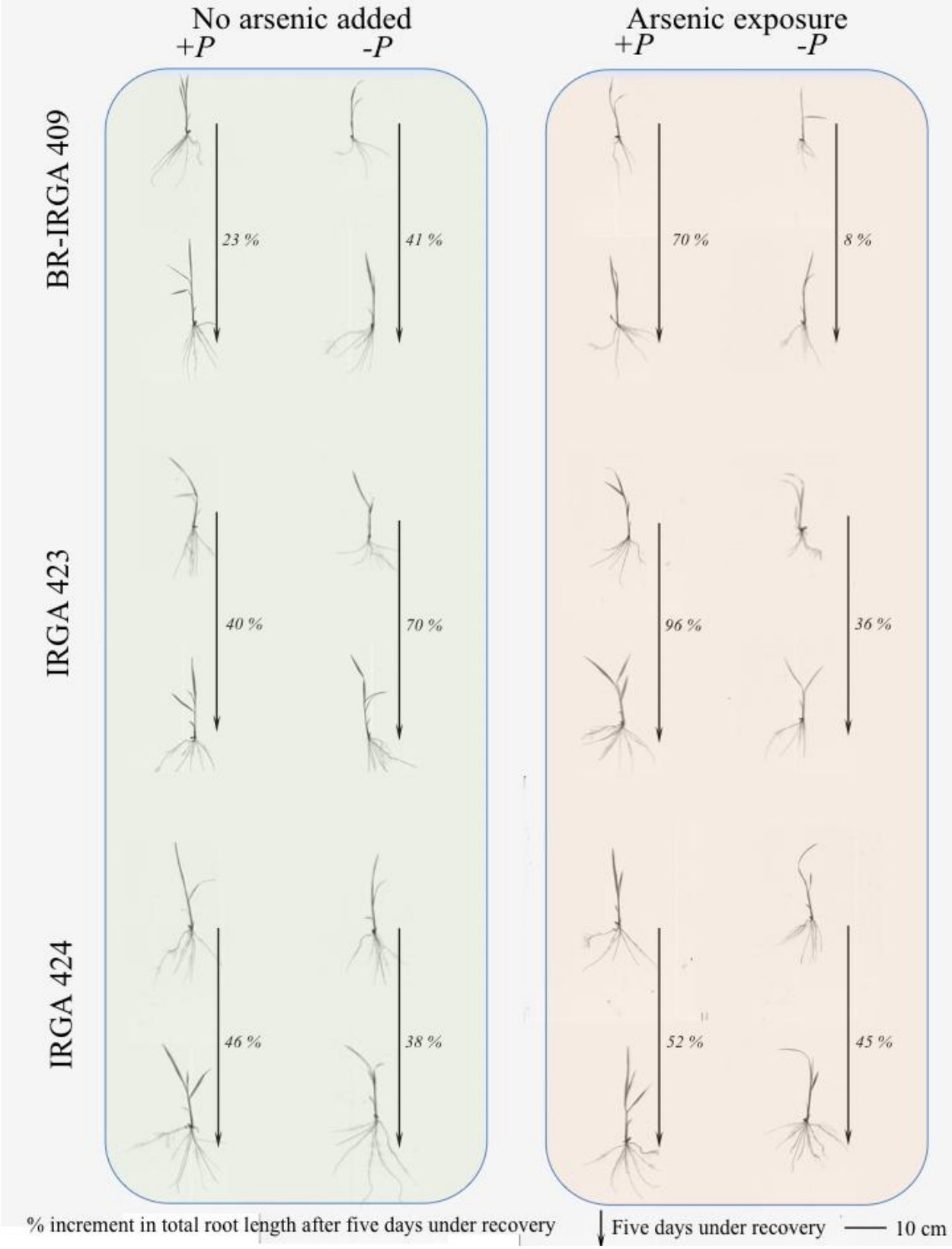

Figure 3. Effect of phosphorus levels with or without arsenic exposure in rice plants on total root length increment (percentage inside the box) of plants after five days in control solution, recovery stage.

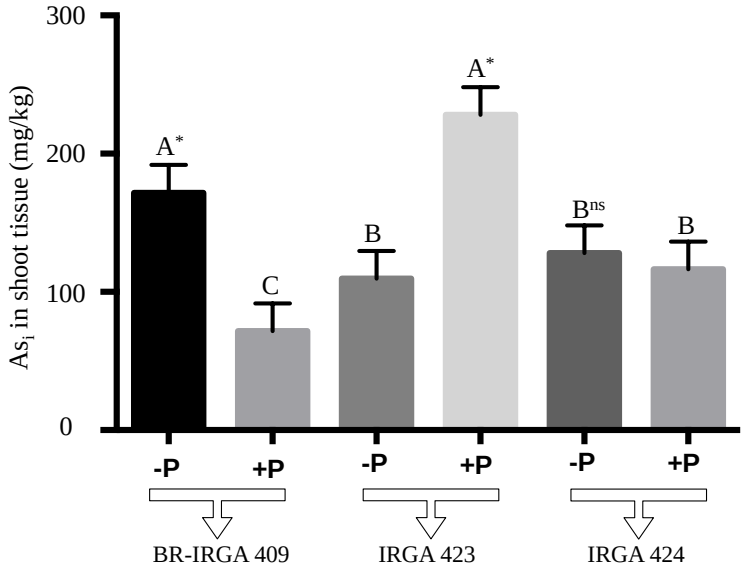

Figure 4. Inorganic arsenic (As_i) concentration in shoot tissues of BR-IRGA 409, IRGA 423 and IRGA 24 cultivars under phosphorus starvation (-P) and normal supply (+P). Means followed by capital letters indicate comparison between cultivars within the same phosphorus and arsenic levels, whereas asterisk indicate comparison between phosphorus levels for the same cultivar and arsenic exposure. Tukey test, $\alpha = 0.05$.

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 6). The results of the present study indicated that rice genotypes had different nutrient partitioning requirements (Figure 6). 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).

Phosphorus (P) is an essential nutrient for plant growth and development. Due to the diverse functional and structural roles of P in plants, P-use efficiency (PUE) is a complex trait to dissect. Phosphorus-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 6). 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).

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 7).

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).

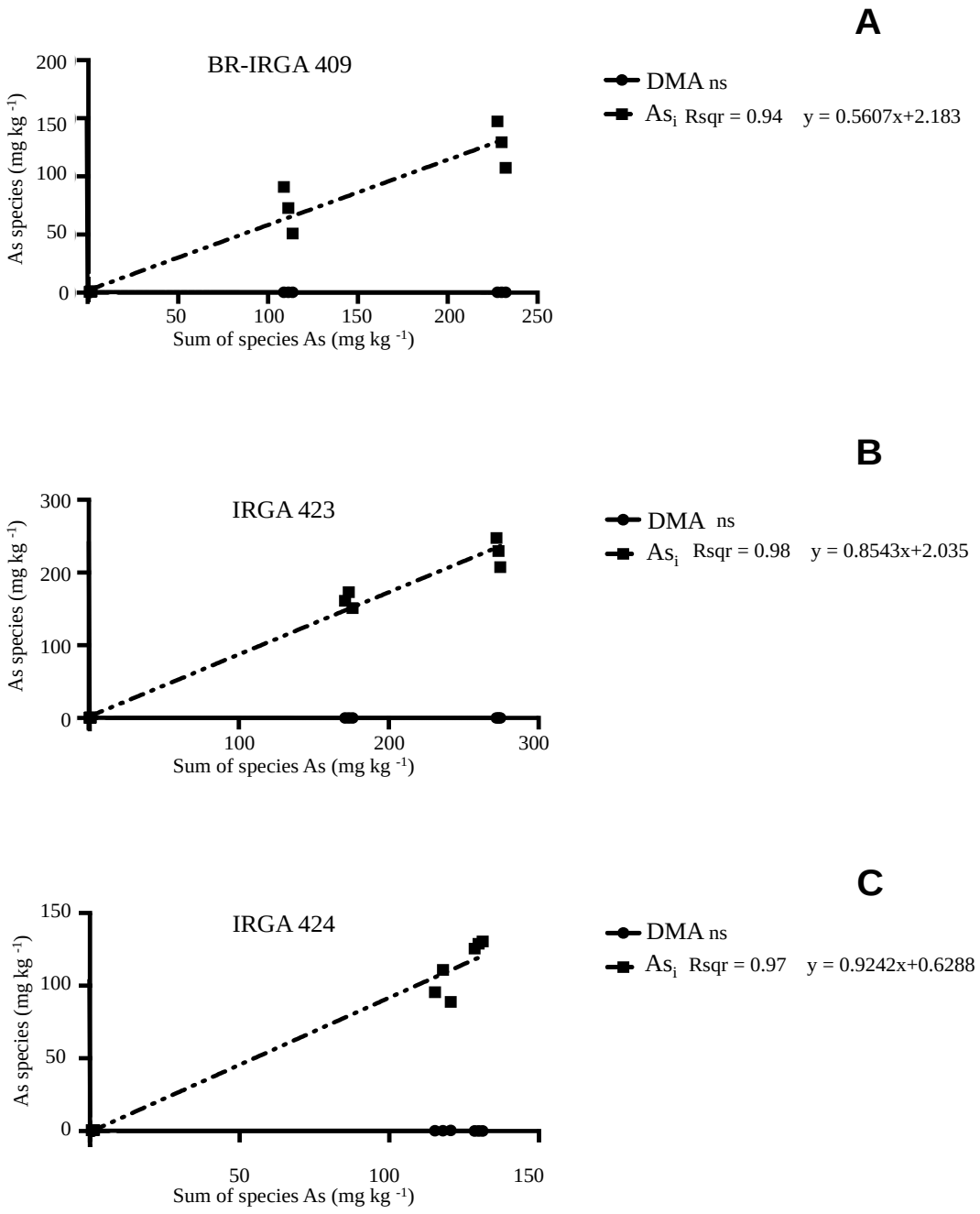

Figure 5. Arsenic species relationship to sum of species of total As for BR-IRGA 409, IRGA 423 and IRGA 424 cultivars in shoot tissue samples.

Under P starvation and P starvation combined with As exposure, IRGA 424 had higher PUE as compared to the other cultivars (Figure 7). 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). And this is not an important adaptive mechanism because in low P 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 reach the root (Aerts and Chapin, 2000).

CULTIVAR	NO ARSENIC ADDED		ARSENIC EXPOSURE (100 μM)	
	0 mM P_2O_5	0.09 mM P_2O_5	0 mM P_2O_5	0.09 mM P_2O_5
	Phosphorus concentration in root tissue (mg kg^{-1})			
BR-IRGA 409	243.84 ± 34.60 Aa	1780.00 ± 165.40 Aa*	219.45 ± 25.07 Aa	1602.33 ± 98.70 Aba*
IRGA 423	214.25 ± 27.60 Aa	1564.00 ± 155.33 Aba*	228.08 ± 29.27 Aa	1665.50 ± 90.04 Aa*
IRGA 424	205.07 ± 20.43 Aa	1497.33 ± 144.50 Ba*	202.19 ± 25.94 Aa	1476.00 ± 80.33 Ba*
	Phosphorus concentration in shoot tissue (mg kg^{-1})			
BR-IRGA 409	905.91 ± 70.45 Aa	5565.00 ± 324.50 Ba*	510.00 ± 80.45 Ab	5210.00 ± 272.50 Ba*
IRGA 423	926.27 ± 88.20 Aa	6889.00 ± 220.70 Aa*	581.91 ± 77.12 Ab	6401.00 ± 334.33 Aa*
IRGA 424	1054.45 ± 97.33 Aa	7199.00 ± 354.20 Aa*	618.73 ± 92.33 Ab	6806.33 ± 257.00 Aa*

Figure 6. Biomass and root system parameters of rice plants of three cultivars exposed to 100 μM arsenic (+As) and without arsenic (–As), with phosphorus 0.09 mM (+P) and without phosphorus (–P) 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, $\alpha = 0.05$.

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 necessarily relate to As uptake and accumulation (Figure 8).

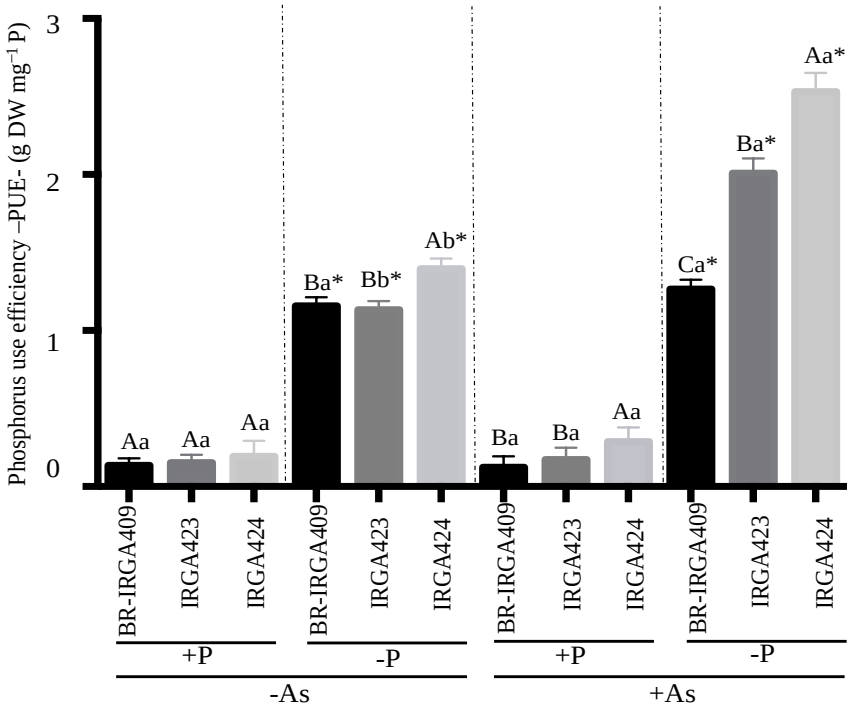

Figure 7. Phosphorus use efficiency of rice plants of three cultivsars exposed to 100µM arsenic(+As) and without arsenic (-As). Means followed by capital letters indicate comparison between cultivars within the same phosphorus and arsenic levels, whereas lowercase letters indicate comparison of the arsenic exposure for the same cultivars and phosphorus level and asterisk indicate comparison between phosphorus levels for the same cultivar and arsenic exposure. Tukey test, $\alpha = 0.05$.

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). While Siddiqi and Glass (1981) use total plant biomass, Wissuwa (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 interesting potential for further studies. Thus, for this cultivar PUE was related with total root length and shoot dry weight (Figure 8), 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, 8).

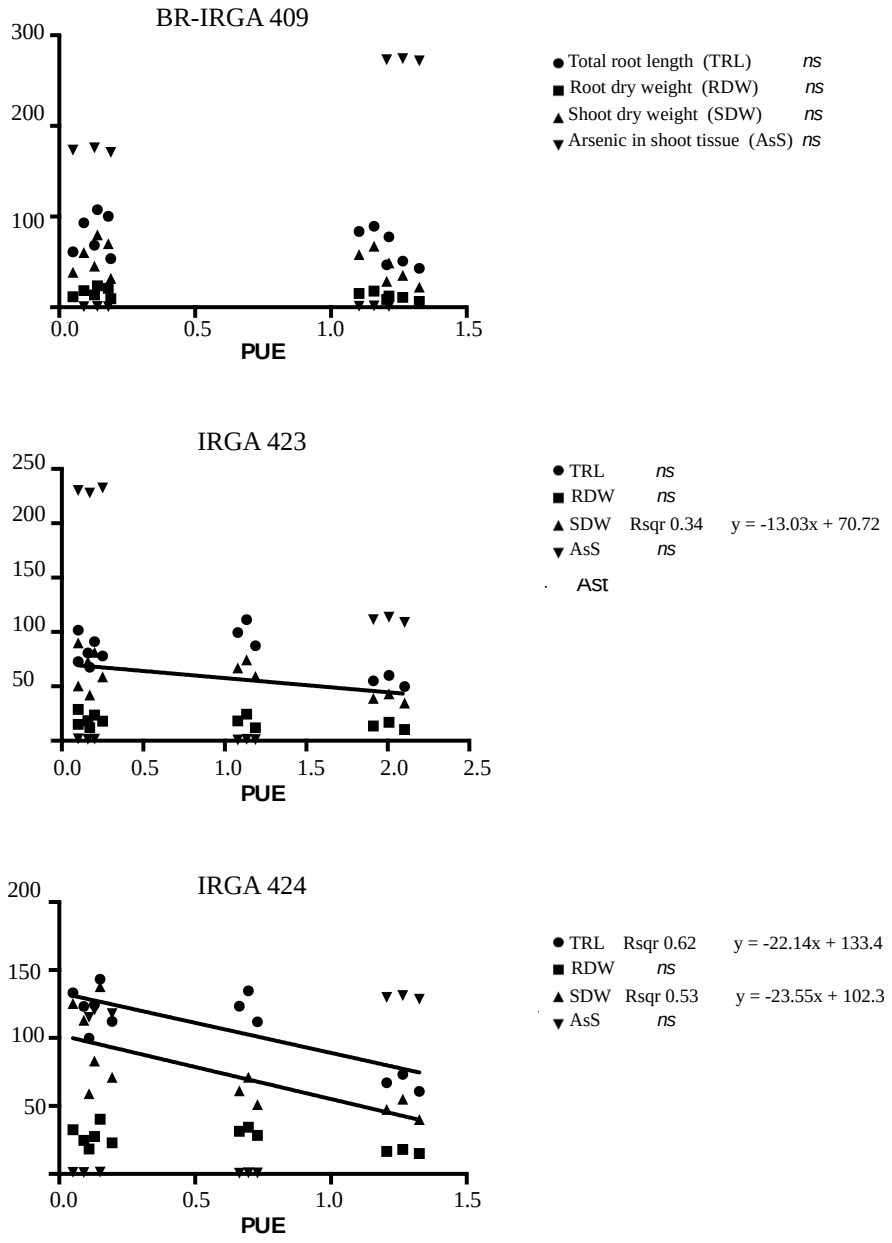

Figure 8. Phosphorus use efficiency (PUE) relationship to sum of species of total As, shoot dry weight, root dry weight and total root length for BR-IRGA 409, IRGA 423 and IRGA 424 cultivars in shoot tissue samples.

CONCLUSION

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.

RESUMO

Um experimento hidropônico foi realizado para investigar o efeito do fósforo (P) sobre a absorção e translocação de arsênio (As) em três cultivares de arroz, BR-IRGA 409, IRGA 423 e IRGA 424. O experimento ocorreu em três etapas : I 5 dias de aclimatização sob solução nutritiva completa; II 10 dias sob os tratamentos de P (0,0 e 0,09 mM) e As (0,0 e 100 mm) e III cinco dias em recuperação, com solução nutritiva completa sem As. A exposição ao As teve efeitos significativos sobre a material seca de raízes e parte aérea, resultando também em concentrações elevadas de As nos tecidos. A cultivar BR-IRGA 409 apresentou a maior susceptibilidade ao As em parâmetros de produção de biomassa e do sistema radicular, independentemente do nível P. Por outro lado, o efeito do P foi marcante para a recuperação de plantas de todas as cultivares sob expostas preveamente ao As. A separação mais clara de cultivares para a eficiência do uso de fósforo (PUE) ocorreu conjuntamente com os menores teores de P no tecido de parte áerea, ou seja, em níveis mais elevados de estresse por deficiência de P. Assim, nossos resultados ajudam na compreensão do papel da nutrição de P e efeitos do As em tecidos da parte aérea de plantas de arroz, podendo a continuação de trabalhos como este formar a base das práticas de gestão para reduzir a acumulação de As à níveis tóxicos.

Palavras-chave: Nutrição Mineral, *Oryza sativa*, fosfato, absorção.

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5 MANUSCRITO 4 - MORPHOLOGICAL, MINERAL AND BIOCHEMICAL ADAPTATIONS TO ARSENIC-INDUCED STRESS IN INDICA RICE CULTIVARS

Abstract Crop plants may show physiological and biochemical responses to oxidative stress caused by heavy metals and metalloids. In this context, we evaluated the effect of arsenic (As) toxicity on root morphology and concentrations of sulfur (S), As and non-protein thiol groups (NPSH) of five indica rice cultivars (BR/IRGA 409, BR/IRGA 410, IRGA 420, IRGA 423 and IRGA 424). The cultivars were evaluated at the seedling stage grown with intact roots or with split root system under increasing levels of As (0, 10, 20, 25 and 50 μM). The rice root system showed great plasticity in response to As exposure. A common response among the cultivars was the reduction of the total root system length, an increase in the root S concentration and a decrease in the shoot S concentration. Under split root system, tem cultivars with lowest As concentration in shoot tissues showed a higher As remobilization to roots not exposed to As. Also in this sense, the cultivar with lowest As concentration in shoot (IRGA 420) also had the lowest S concentration in these tissues. In addition, non-protein thiol groups concentration was much higher in the roots than in shoots of seedlings grown under As stress. IRGA 424 showed a greater increase in the NPSH concentration compared with those of the other cultivars.

Keywords: As toxicity, glutathione, heavy metal, root system, sulfur.

5.1 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 (MEHARG and RAHMAN, 2003; 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 (BLEEKER et al., 2006). 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 (TRIPATHI et al., 2007; MISHRA et al., 2008; 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 (DWIVEDI et al., 2010; TRIPATHI et al., 2012). 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 deep attention.

Plant roots are characterized by very high adaptability. Their growth and development involve complex interactions with both the soil environment and the shoot part (MARSCHNER, 1995). Plant roots are able to respond to the heterogeneous soil environment by improving root growth in more favorable pockets (KERLEY et al., 2000), which is described as a plastic response of the root system (FELDMAN, 1984) and could be related to toxic element tolerance.

Another important fact to be considered is the adaptive capacity of each genotype, including mineral nutrition and As transport and remobilization. In this context, the presents 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 cultivars grown with their roots under two different system, i.e., normal and split root, under increasing As levels.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials and growth conditions

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. The total

root system length was determined according to Tennant (1975), and the adventitious root length was measured with Vernier calipers; both lengths are expressed in cm.

5.2.1.1 Hydroponic experiment with and without the presence of seminal root

To evaluate the effect of seminal root removal, an experiment was conducted with and without the presence of seminal roots. After five days in distilled water, the seminal roots of half of the rice seedlings were removed, and the seedlings were transferred to plastic pots containing 180 mL of one-half strength Kimura B nutrient solution. The nutrient solution contained the macronutrients 0.18 mM $(NH_4)_2SO_4$, 0.27 mM $MgSO_4.7H_2O$, 0.09 mM KNO_3, 0.18 mM $Ca(NO_3)_2.4H_2O$, and 0.09 mM KH_2PO_4 and the micronutrients 20 μM NaEDTA-$Fe.3H_2O$, 6.7 μM $MnCl_2.4H_2O$, 9.4 μM H_3BO_3, 0.015 μM $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.15 μM $ZnSO_4.7H_2O$, and 0.16 μM $CuSO_4.5H_2O$. The pH was adjusted to 5.5, and the solution was renewed every two days in a controlled environment. After ten days of acclimation, seedlings were submitted to two As levels (0 and 10 μM). After ten days, 5 plants per replicate (each treatment consisted of ten replicates) were randomLy harvested and separated into shoots and roots.

5.2.1.2 Hydroponic experiment with an intact root system

After five days in distilled water, the seminal roots of half of the rice seedlings were removed, and the seedlings were transferred to plastic pots containing 180 mL of one-half strength Kimura B nutrient solution, as previously described. The pH was adjusted to 5.5, and the solution was renewed every two days in a controlled environment. After seven days of acclimation, plants 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.

5.2.1.3 Hydroponic experiment with a split-root system

To evaluate the effect of local and systemic As levels on the plant, a third experiment was conducted with split roots. After five 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, as previously described (supplemental data figure 1). After approximately 2 weeks, these seedlings with split roots were cultivated for 10 days with seven treatments of varying concentrations and locations of As as follows: treatment 1 [0/0 µM As, with both root halves without As exposure]; treatment 2 [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]; treatment 3 [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]; treatment 4 [10/10 µM, with both halves being exposed to the same concentration of 10 µM As]; and treatment 5 [25/25 µM, with both halves being exposed to the same concentration of 25 µM As].

After ten days, 5 plants per replicate (each treatment consisted of 70 replicates) were randomLy harvested and separated into shoots, left roots and right roots.

5.2.2 Biomass, As and mineral nutrient content determination

The roots and shoots of the rice seedlings were oven-dried at 65 °C to a constant mass for the determination of the 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.

5.2.2.1 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.

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.

5.2.3 Statistical analysis

The experiments were performed in a randomized block design. The analyses of variance were computed for statistically significant differences based on the appropriate F-tests. The results are reported as the means and S.D. of at least four independent replicates. The mean differences were compared using the Tukey test ($p < 0.05$).

5.3 RESULTS AND DISCUSSION

5.3.1 Morpho-physiological adaptive capacity of roots

In this study, the growth of rice cultivars was also characterized before the experiments installation, i.e., prior to the period of acclimation (seeds soaked in distilled water for five days). There was a large discrepancy in the total root length among the tested cultivars. Three of the five cultivars tested had a total root length between 114 and 124 cm. Cultivar IRGA 424 showed a slightly smaller root length (102 cm) compared to the other cultivars. However, cultivar BR/IRGA 409 showed a drastic lower root length (only 54 cm) compared to the other cultivars (Supplemental data Table 1), which demonstrate a clear physiological separation among the tested cultivars.

The contribution of the seminal root to total root length during the initial stage of growth was also evaluated. Overall, in the present study the removal of the seminal root resulted in increased production of biomass regardless of the As concentration tested, although a subtle effect has been observed for most of the parameters. However, with 10 µM of As, a greater reduction in the root biomass of cultivar BR/IRGA 409 was observed in plants without seminal roots as compared to plants with intact root systems (Suplemental data Table 2).

Interestingly, BR/IRGA 409 (which showed the lower root length, approximately 50% than the other cultivars, prior to As exposure) had an increase in the number of roots per plant upon both the removal of the seminal root and exposure to As occurred (Suplemental data table 1; table 2). This data suggests a positive adaptive response to As exposure.

The rice root system consists of seminal, adventitious, and lateral roots. The seminal root develops during embryogenesis, whereas the adventitious and lateral roots develop post-embryonically. The seminal roots persist for only a short time after germination, and they are soon replaced by the secondary system of adventitious roots, which originates from the lower nodes of the stem (MATSUO and HOSHIKAWA, 1993).

Besides the intrinsic difference among cultivars, the main factors influencing the root growth and root hairs are the plant physiological age, the oxygen supply, moisture content, temperature, nutrient availability, osmotic pressure of the soil solution, presence of toxic elements, the presence of pathogens in the soil, the soil texture and the method of cultivation (YOSHIDA, 1981).

Recently, a large number of studies have investigated the impact of hormones on root growth and development. There is also a line of research focused on the impact of these hormones on the elongation of seminal roots (ZHANG et al., 2001; PAREDES et al., 2009; TAN et al,. 2012; LIU et al., 2013). However, there is little information in the literature concerning the impact of the seminal roots on the growth and development of rice plants. Park and Back (2012) developed a study with transgenic rice genotypes overexpressing serotonin N-acetyltransferase (NAT) that produced more melatonin compared to the wild-type plants. They observed the promotion of seminal root growth by melatonin without the alteration of either the adventitious root number or shoot growth. These data suggest that the removal of seminal root, as in the present study, may have a small impact on initial growth of rice seedlings.

In the experiment with plants containing intact root system, As exposure significantly reduced the length of the root system, but this response was cultivar- and dose-dependent. For plants not exposed to As, the IRGA 424 genotype had the lowest contribution to this parameter. However, with exposure at 50 μM As, cultivar BR/IRGA 409 showed the smallest root system, while IRGA 424 had the largest (Table 1).

Similar to the response of the total root system length, the total adventitious root length and average length of adventitious roots were in general negatively affected by increasing As levels in the nutrient solution (Figure 1). Interestingly, we observed root damage as necrosis in adventitious roots with and without lateral roots for the cultivars BR/IRGA 409, BR/IRGA 410, IRGA 420 and IRGA 423; while for IRGA 424 no visual damage was found in roots with lateral roots (Figure 2).

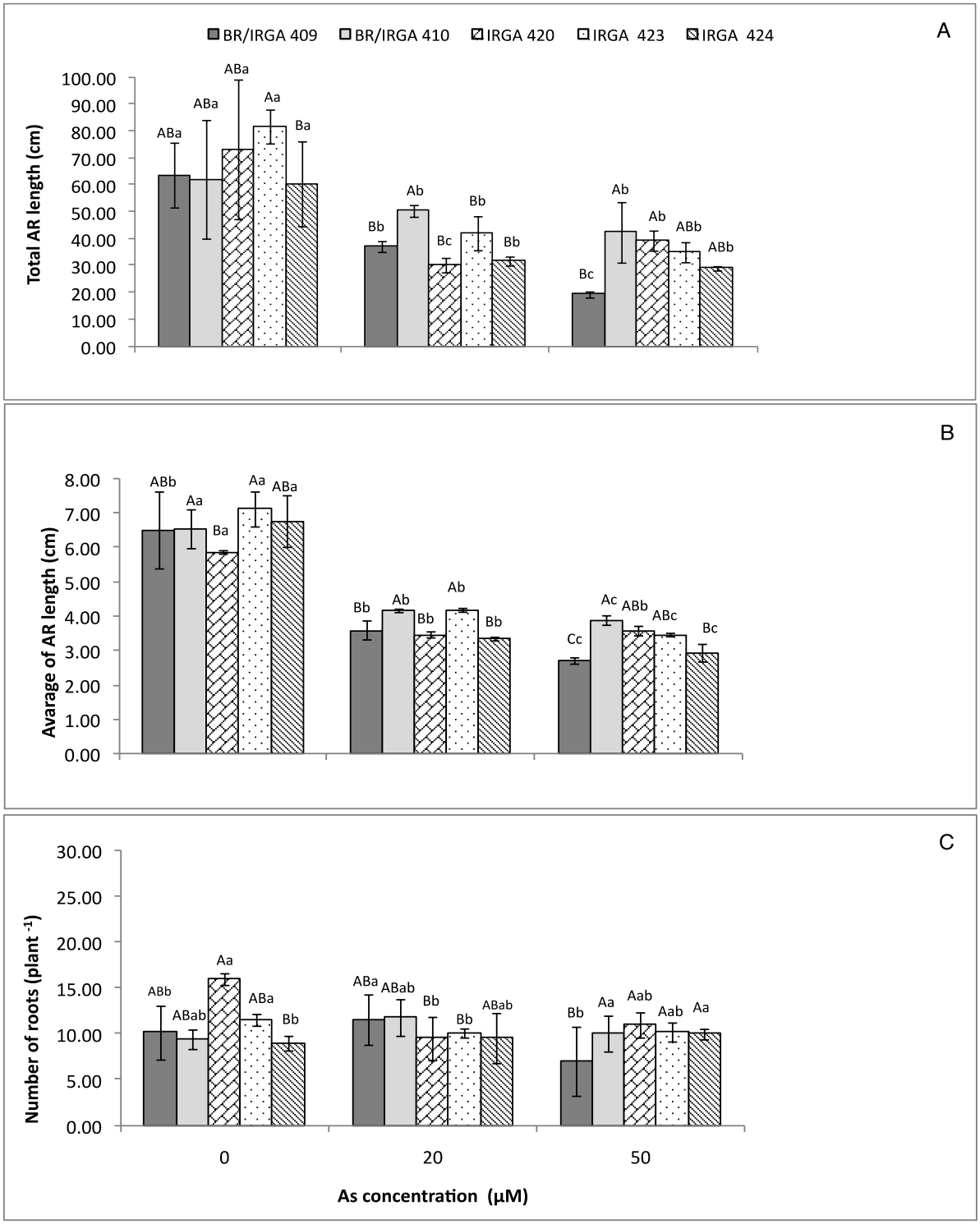
Table 1 - Effect of Arsenic exposure on root length and root dry weight.

Cultivar	As concentration µM	Root system length (cm plant $^{-1}$)	Root system dry weight (mg plant $^{-1}$)
BR/IRGA 409	0	734.50 Ba	38.00 Ba
	20	384.70 Ab	19.33 Bb
	50	213.22 Bc	10.00 Bbc
BR/IRGA 410	0	892.33 Aa	43.00 Aba
	20	372.35 Ab	25.50 Abb
	50	198.25 Bc	15.30 Ab
IRGA 420	0	902.00 Aa	47.10 Aa
	20	407.53 Ab	23.00 Bb
	50	234.55 ABc	12.21 Bc
IRGA 423	0	937.25 Aa	53.40 Aa
	20	453.33 Ab	29.00 Aab
	50	285.98 Ac	16.22 Ab
IRGA 424	0	787.67 Aba	49.31 Aa
	20	401.68 Ab	28.00 Ab
	50	248.56 Abc	13.10 Abbc

Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$).
Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$).

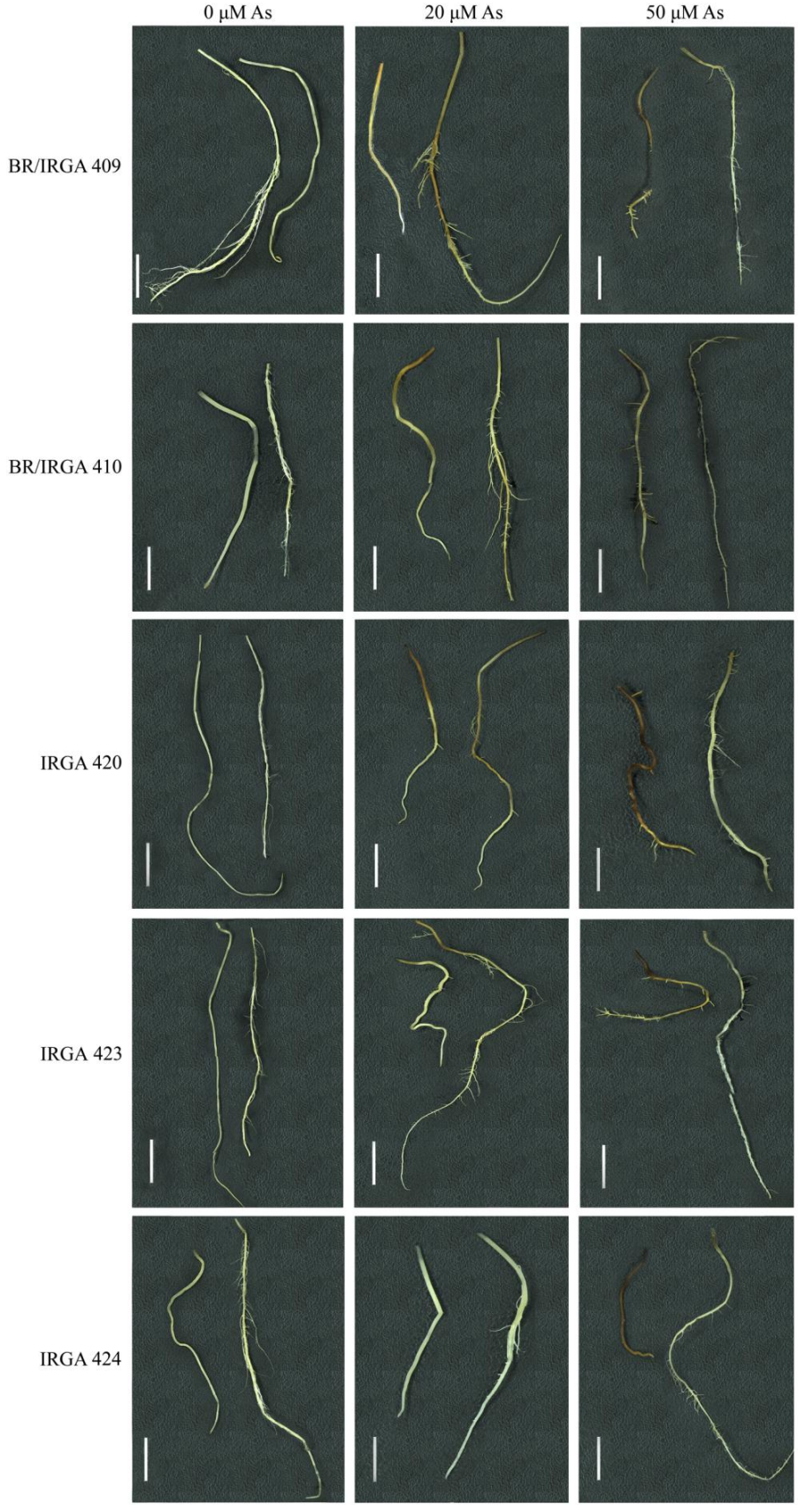
Recent published data showed that the endodermis, pericycle (especially in the mature root zone), and xylem parenchyma cells are the main locations of As storage in rice roots, while the outer cells in the rice roots store little As. A concentration effect may be expected as a result of the radial transport of solutes from the outer cells to the stele (MOORE et al., 2011). Additionally, it is possible that mature roots (with lateral roots) demonstrate more pronounced As uptake, thus resulting in root damage as compared to young roots (without lateral roots).

Figure 1 - Effect of As exposure on the total length of adventitious roots (AR) per seedling (A), average length of AR per seedling (B) and number of adventitious roots per seedling (C).



Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$).

Figure 2 - Rice roots exposed to different As levels during 10 days under hydroponic system. Were collected both mature (with lateral roots) and young roots in order to characterize the morphological As effects



The choice of parameters to characterize the physiological tolerance/susceptibility to a particular stressor is extremely important for proper evaluation and screening of genotypes. However, the chosen parameters are often unable to efficiently differentiate between the genotypes. A clear example of this pattern is the root system evaluation performed in this study, which raised many questions as (i) at what level should a cultivar be considered susceptible? (ii) Will the reduction in biomass partition or even the reduction in its production always be considered related to susceptibility? (iii) Could this biomass reduction be a strategy of tolerance or adaptation aimed at leaving descendants? These questions have received increasing attention in the scientific community. Therefore, the present study sought to examine different parameters to answer these questions. Of these parameters, we assessed the number of adventitious roots per plant, which unlike the abovementioned parameters, showed distinct patterns among cultivars (Figure 1).

For this parameter (Figure 1), we observed three patterns: (I) Reduction in the number of adventitious roots per plant (cultivars BR/IRGA 409 and IRGA 420) with an increasing As levels; (II) No change in the number of adventitious roots of cultivars BR/IRGA 410 and IRGA 423 regardless As exposure; and (III) Increase in the number of roots of cultivar IRGA 424 with increasing As levels in the solution.

In the experiment performed with the split-root system, more consistent data became available for the discussion of adaptive mechanisms of rice seedlings. In this experiment, plants where both root halves were exposed to the same As concentration (treatment 6 [10/10 μM] and treatment 7 [25/25 μM]) showed no significant differences for the parameters tested between the root halves. Thus, for a better understanding, to both of these treatments only an average value for all parameters in the figures and tables were presented.

Both BR/IRGA 409 and 410 cultivars showed higher root biomass production in the control treatment (treatment 1 [0/0 μM As]) as compared to the other cultivars tested (Figure 3). For cultivars IRGA 420 and IRGA 424 at 0/20 μM As treatment, the root halves not exposed to As did not differ from the control plant roots. In contrast, in the 0/50 μM As treatment, the root halves that were not exposed to As showed a reduction in root biomass as compared to the control, but were not different from the other half, which was exposed to 50 μM As (Figure 3).

For IRGA 423, the halves of roots not exposed to As in the 0/20 μM As treatment showed greater root biomass production compared to that of the control plants. This cultivar also maintained the biomass production similar to control in the halves not exposed to As in

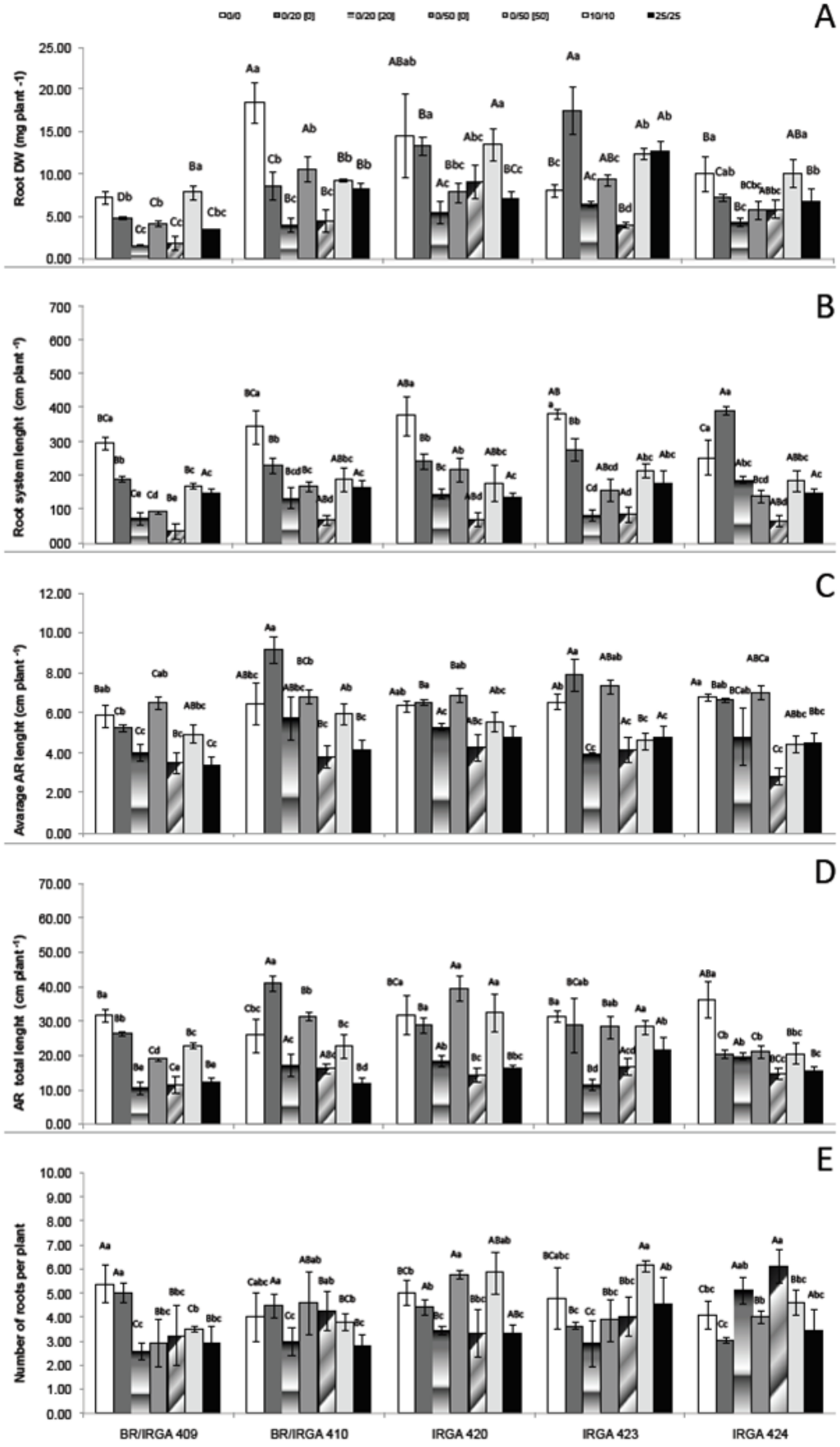
the 0/50 μM treatment. However, the halves that were exposed to 50 μM As showed reduced root biomass as compared to both the control halves and the halves without exposure (Figure 3).

Apparently, the total length of root system, which includes adventitious roots, lateral roots and root hairs visible to the naked eye, is the most sensitive parameter with respect to As exposure. With the exception of the halves of IRGA 424 not exposed to As in the 0/20 μM As treatment, all of the roots exposed directly or indirectly to As showed lower total root length compared to that of the control treatment (Figure 3).

However, the average length of the adventitious roots, the total length of adventitious root and the number of adventitious roots per plant had a distinct pattern of response compared to the total root system length (Figure 3). Through these data, it is clear that the deleterious effect of As on the lateral roots as well as to root hairs is much more extensive as compared to the damage in the primary roots. Root hairs are very important in the uptake of nutrients and water from the soil by increasing the absorptive surface area (PETERSON and FARQUHAR, 1996). Ma et al. (2001) compared two rice (*Oryza sativa* L. cv Oochikara) root mutants, RH2 and RM109, which are defective in the formation of root hairs and lateral roots, respectively, to a wild type (WT) regarding Si absorption and transportation. Uptake experiments over the short term (up to 12 h) and relatively long term (26 days) revealed similar uptake of Si in WT and RH2; in contrast, RM109 showed a lower Si concentration in both the shoot and root tissues compared to the others.

Interestingly, under absence of As in the present study, the IRGA 424 cultivar showed a root system with a lowest contribution of lateral roots and root hairs (217 cm per plant), while IRGA 423 had the highest contribution (351 cm per plant). However, under stress conditions in the 0/20 μM As treatment, both root halves of IRGA 424 showed the highest contribution of lateral roots and root hairs compared to the other cultivars (374 and 167 cm per plant for the halves exposed to 0 and 20 μM As, respectively), while BR/IRGA 9 was the cultivar with the lowest contribution (164 and 64 cm per plant for halves exposed to 0 and 20 μM As, respectively).

Figure 3 - Effect of As levels on rice plants grown under split root system



[0/0 mM As]; [0/20 mM As] with half of the root system being exposed to 0 mM As; [0/20 mM As] half root system exposed to 20 mM As; [0/50 mM As] half root system exposed to 0 mM As; [0/50 mM As] half root system exposed to 50 mM As; [10/10 uM] both halves exposed to the same concentration; and [25/25 uM] both halves exposed to the same concentration. Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$). * AR- adventitious root.

Conversely, when both root halves were exposed to As (treatments of 10 and 25 μM As), IRGA 424 no longer was the cultivar with the most lateral root extensions; again IRGA 423 showed the highest contribution of lateral roots and root hairs.

As we did not separately evaluate the lateral roots and root hairs, we cannot determine which of these structures were more affected. Regarding the average length of adventitious roots, the BR/IRGA 409 cultivar showed similar behavior in the 0/20, 0/50 and control treatments when comparing the halves of the root system without direct exposure to As. In contrast, the BR/IRGA 410 and IRGA 423 cultivars showed an increase in the average size of the primary root in the 0/20 treatment compared to the control (Figure 3).

For the total length of adventitious roots, the BR/IRGA 409 cultivar showed the following order with the various treatments: 0/0 > 0/20 [0] > 10/10 > 0/50 [0] > 0/20 [20] = 0/50 [50] = 25/25 (Figure 3). We observed an interesting pattern in response to As for the IRGA 424 cultivar. This cultivar showed the highest sensitivity to As in the total length of adventitious roots with direct or indirect exposure to As (Figure 3). Interestingly, this cultivar maintained the average length of adventitious roots in halves without direct exposure to As; however, a decrease in the number of roots was observed (Figure 3).

In the root halves where there was a reduction in the average length and direct exposure to As (treatments 0/20 and 0/50), an increase in the number of adventitious roots was noticed (Figure 3). This result suggests a different strategy in relation to the use of assimilates under As exposure. From examining the raw data of dry weight, a default behavior exists between the cultivars exposed to As. However, in further evaluating the root system it seems that there are a number of distinctions among the tested cultivars.

5.2.2 Biochemical and mineral response to As contamination

In the experiment with intact roots, all of the cultivars showed an increase in sulfur (S) concentration in the root tissue with increasing As levels in the nutrient solution. This increase was followed by an increase in the root tissue As. However, in the shoot tissue, there

was an increase in the As concentration followed by a decrease in the S tissue concentration (Table 2, 3).

Excess metals/metalloids are harmful to plants because they alter the metabolism. These elements stimulate the formation of reactive oxygen species (ROS). The increased production of ROS during stress can be a threat to the cells, but ROS production may signal the activation of the defense processes and stress response (SCANDALIOS, 1997). Whether the effect of ROS is beneficial is determined by the delicate balance between ROS production and scavenging at the local and temporal levels through the action of the antioxidant defense system. This metabolic imbalance and the consequent formation of ROS are directly related to the ability of the plant to reduce/prevent the absorption and translocation of elements to toxic levels.

Table 2 - Effect of Arsenic exposure on Arsenic concentration in root and shoot tissues.

Cultivar	**As concentration µM**	**Arsenic concentration µg g $^{-1}$**	
		root	**shoot**
BR/IRGA 409	0	<8.00	<8.00
	20	384.33 ± 53.87 Ab	55.33 ± 1.23 Bb
	50	415.90 ± 32.87 Bc	67.25 ± 343 Bbc
BR/IRGA 410	0	<8.00	<8.00
	20	382.25 ± 72.87 Ab	72.00 ± 2.76 Abb
	50	483.00 ± 24.76 Bc	80.15 ± 1.76 Ab
IRGA 420	0	<8.00	<8.00
	20	480.50 ± 22.54 Ab	55.76 ± 2.76 Bb
	50	493.00 ± 8987 ABc	82.48 ± 3.40 Bc
IRGA 423	0	<8.00	<8.00
	20	355.25 ± 15.87 Ab	53.08 ± 2.87 Aab
	50	506.26 ± 54.87 Ac	72.53 ± 1.22 Ab
IRGA 424	0	<8.00	<8.00
	20	673.33 ± 43.58 Ab	62.27 ± 4.65 Ab
	50	680.50 ± 65.77 Abc	70.55 ± 3.75 Abbc

Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$).

After the arsenic being absorbed and translocate into the shoots, an adaptive strategy against As toxicity is the As remobilization, as in rice plants that show 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. In the 0/20 As treatment, BR/IRGA 409 exhibited major remobilization in the root tissue and lower translocation in the shoot for the 0/20 treatment

compared to the other cultivars. Notably, the IRGA 423 cultivar showed the highest remobilization in the root system for the 0/50 treatment and greater translocation for the 0/20 treatment in the shoot (Table 4). This cultivar was the only oner where the tissue As concentration at 0/20 treatment did not differ that at 10/10 As treatment (Table 4).

Table 3 - Effect of Arsenic exposure on Sulfur concentration in root and shoot tissues.

Cultivar	As concentration µM	Sulfur concentration µg g^{-1}	
		root	shoot
BR/IRGA 409	0	1495.00 ± 46.54 Ab	2778.54 ± 46.54 Ba
	20	2032.50 ± 53.89 ABa	2269.06 ± 53.89 Cb
	50	2188.79 ± 71.03 ABa	1839.15 ± 7103 Bc
BR/IRGA 410	0	1216.73 ± 71.85 Bb	3409.68 ± 71.85 Aa
	20	2254.15 ± 46.54 Aa	2587.38 ± 46.54 Cb
	50	2142.40 ± 62.05 Aba	2009.64 ± 62.05 Bc
IRGA 420	0	1411.26 ± 72.67 Ab	2888.30 ± 72.67 Bab
	20	2240.65 ± 76.75 Aa	3213.18 ± 76.75 Aa
	50	2316.73 ± 84.09 ABc	2460.71 ± 84.09 Ab
IRGA 423	0	1348.32 ± 106.14 ABb	2964.23 ± 106.14 Ba
	20	2152.39 ± 61.64 Aa	3216.92 ± 61.64 Aa
	50	2293.49 ± 69.89 Aa	1966.78 ± 69.89 Bb
IRGA 424	0	1172.62 ± 47.14 Bc	3044.83 ± 47.14 ABa
	20	1759.15 ± 70.71 Bb	2918.99 ± 7071 Ba
	50	2076.13 ± 82.06 Ba	1998.27 ± 82.06 Bb

Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$).
Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$).

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). 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 phloem transport of As, 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 DMA was transported to the immature grain approximately 30 times more efficiently than arsenite. A 2011 study (CAREY et al., 2011) agreed 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 4 - Effect of Arsenic exposure on Arsenic concentration in root and shoot tissues under split root system.

Cultivar	Treatment	As concentration for the evaluated roots	Arsenic concentration	
			root	shoot
3R/IRGA 409	0*0	0 µM	<3.1	<3.1
	0*20	0 µM	20.00 ± 3.10 Ce	3.50 ± 0.70 Cd
	0*20	20 µM	397.00 ± 31.00 Aa	
	10*10	10 µM	310.00 ± 5.00 Ab	11.30 ± 1.20 Cc
	0*50	0 µM	68.60 ± 7.40 Ad	20.70 ± 1.20 Cb
	0*50	50 µM	226.00 ± 5.00 Cc	
	25*25	25 µM	407.00 ± 20.00 Ba	43.80 ± 1.60 Ba
3R/IRGA 410	0*0	0 µM	<3.1	<3.1
	0*20	0 µM	30.10 ± 3.00 Bc	6.90 ± 0.50Bc
	0*20	20 µM	286.00 ± 15.00 Cb	
	10*10	10 µM	326.00 ± 5.00 Aa	15.10 ± 0.60 Bb
	0*50	0 µM	35.40 ± 3.80 Cc	50.70 ± 3.90 Aa
	0*50	50 µM	312.00 ± 11.00 Bab	
	25*25	25 µM	343.00 ± 6.00 Ca	49.30 ± 2.50 Ba
RGA 420	0*0	0 µM	<3.1	<3.1
	0*20	0 µM	28.90 ± 1.70 Bd	5.50 ± 0.50 Bd
	0*20	20 µM	254.00 ± 16.00 Cb	
	10*10	10 µM	295.00 ± 8.00 Ab	26.90 ± 1.20 Ab
	0*50	0 µM	44.30 ± 4.10 Bc	12.50 ± 2.90 Cc
	0*50	50 µM	245.00 ± 8.00 Cb	
	25*25	25 µM	351.00 ± 5.00 Ca	60.90 ± 2.30 Aa
RGA 423	0*0	0 µM	<3.1	<3.1
	0*20	0 µM	68.80 ± 13.40 Ad	12.10 ± 0.60 Ac
	0*20	20 µM	356.00 ± 14.00 Bb	
	10*10	10 µM	294.00 ± 9.00 Ac	11.20 ± 0.70 Cc
	0*50	0 µM	47.50 ± 10.10 Bd	40.90 ± 0.80 Ba
	0*50	50 µM	369.00 ± 16.90 Ab	
	25*25	25 µM	439.00 ± 6.00 Aa	19.90 ± 0.80 Cb
RGA 424	0*0	0 µM	<3.1	<3.1
	0*20	0 µM	35.30 ± 10.80 Abd	11.90 ± 1.40 Ad
	0*20	20 µM	324.30 ± 18.00 Bb	
	10*10	10 µM	255.10 ± 11.9 Bc	30.00 ± 3.20 Ab
	0*50	0 µM	43.40 ± 4.10 Bd	18.80 ± 3.20Cc
	0*50	50 µM	320.10 ± 20.40 Bb	
	25*25	25 µM	401.90 ± 2.60 Ba	41.00 ± 5.40 Ba

Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$). Treatment 1 [0/0 mM As]; treatment 2 [0/20 mM As] with half of the root system being exposed to 0 mM As; treatment 3 [0/20 mM As] with half of the root system being exposed to 20 mM As; treatment 4 [0/50 mM As] with half of the root system being exposed to 0 mM As; treatment 5 [0/50 mM As] with half of the root system being exposed to 50 mM As; treatment 6 [10/10 uM] with both halves being exposed to the same concentration; and treatment 7 [25/25 uM] with both halves being exposed to the same concentration.

Table 5 - Effect of Arsenic exposure on Sulfur concentration in root and shoot tissues under split root system.

Cultivar	Treatment	As concentration for the evaluated roots	Sulfur concentration			
			root		shoot	
BR/IRGA 409	0*0	0 µM	3541.00 ± 122.00	BCb	3609.00 ± 26.00	Bb
	0*20	0 µM	2315.00 ± 20.00	Cd	3026.00 ± 23.00 Cd	
	0*20	20 µM	3564.00 ± 97.00	Cb		
	10*10	10 µM	4450.00 ± 153.00	Ba	6168.00 ± 186.00	Aa
	0*50	0 µM	2527.00 ± 30.00	Ad	3463.00 ± 16.00 Ac	
	0*50	50 µM	3050.00 ± 21.00	Cc		
	25*25	25 µM	4132.00 ± 141.00	Aa	3729.00 ± 28.00	Bb
BR/IRGA 410	0*0	0 µM	3371.00 ± 105.00	Cb	4066.00 ± 70.00	Ab
	0*20	0 µM	2337.00 ± 86.00	Cc	3301.00 ± 34.00 Bd	
	0*20	20 µM	3644.00 ± 111.00	Cab		
	10*10	10 µM	3836.00 ± 46.00	Ca	4475.00 ± 82.00	Ca
	0*50	0 µM	2357.00 ± 58.00	Cc	3699.00 ± 142.00 Ac	
	0*50	50 µM	3633.00 ± 61.00	Aa		
	25*25	25 µM	3484.00 ± 80.00	Bb	3589.00 ± 83.00	Cc
IRGA 420	0*0	0 µM	3786.00 ± 90.00	Bb	2584.00 ± 79.00	Cd
	0*20	0 µM	2545.00 ± 57.00	Bd	2927.00 ± 89.00 Cc	
	0*20	20 µM	3636.00 ±126.00	Cb		
	10*10	10 µM	3953.00 ± 58.00	Cab	4892.00 ± 50.00	Ba
	0*50	0 µM	2434.00 ± 12.00	Bd	2901.00 ± 40.00 Cc	
	0*50	50 µM	3039.00 ± 129.00	Cc		
	25*25	25 µM	4089.00 ± 46.00	Aa	3897.00 ± 96.00	Ab
IRGA 423	0*0	0 µM	4025.00 ± 112.00	Aa	4189.00 ± 17.00	Aa
	0*20	0 µM	2627.00 ± 42.00	Ad	3344.00 ± 32.00 Bc	
	0*20	20 µM	4141.00 ± 139.00	Aa		
	10*10	10 µM	3855.00 ± 113.00	Cb	4013.00 ± 33.00 Da	
	0*50	0 µM	2517.00 ± 10.00	Ad	3343.00 ± 47.00 Bc	
	0*50	50 µM	3431.00 ± 24.00	Bc		
	25*25	25 µM	4041.00 ± 68.00	Aa	3502.00 ± 37.00 Cb	
IRGA 424	0*0	0 µM	2932.00 ± 13.00	Dd	3665.00 ± 45.00	Bc
	0*20	0 µM	2427.00 ± 59.00	Be	3929.00 ± 49.00 Ab	
	0*20	20 µM	3846.00 ± 109.00	Bb		
	10*10	10 µM	6436.00 ± 245.00	Aa	4243.00 ± 88.00	Ca
	0*50	0 µM	2646.00 ± 146.00	Ae	3484.00 ± 88.00 Acd	
	0*50	50 µM	3463.00 ± 47.00	Bc		
	25*25	25 µM	3642.00 ± 94.00	Bc	3348.00 ± 51.00	Dd

Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$). Treatment 1 [0/0 mM As]; treatment 2 [0/20 mM As] with half of the root system being exposed to 0 mM As; treatment 3 [0/20 mM As] with half of the root system being exposed to 20 mM As; treatment 4 [0/50 mM As] with half of the root system being exposed to 0 mM As; treatment 5 [0/50 mM As] with half of the root system being exposed to 50 mM As; treatment 6 [10/10 uM] with both halves being exposed to the same concentration; and treatment 7 [25/25 uM] with both halves being exposed to the same concentration.

Regarding the concentration of S in seedlings grown with the split root system (Table 5), IRGA 424 showed an increase in the S concentration in both root halves directly exposed to As compared to the control. This cultivar showed reduced concentrations of S in the root

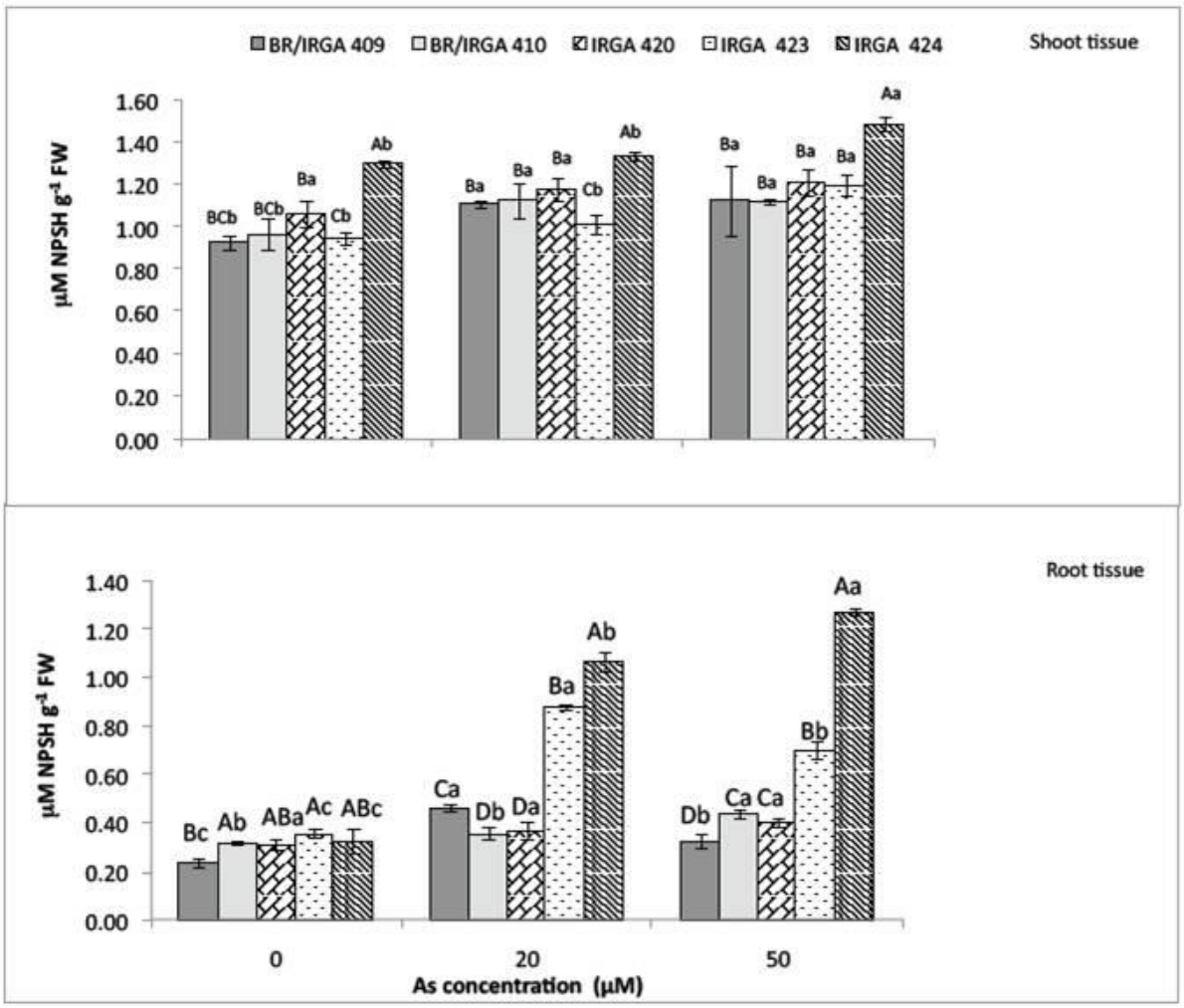
system halves not directly exposed to As (0/20 and 0/50), which reinforces the idea that S content is higher in tissues with greater requirements.

In plants, S metabolism 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 and JEZ, 2012).

Although 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 4). 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 greater increase in the NPSH concentration compared with those of 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 NPSH root concentrations.

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 (NPT) 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.

Figure 4 - Effect of As exposure on non-protein thiol groups (NPSH) concentration in both shoot and root tissues.



Different capital letters indicate significant differences between rice cultivars at the same As level ($p < 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p < 0.05$).

5.4 CONCLUSION

The rice root system showed great plasticity in response to As exposure. A common response among the cultivars was the reduction of total root system length, an increase in the S root tissue concentration and a decrease in the S shoot tissue concentration. However, distinct patterns were also found, such as an increase in the adventitious root number in IRGA 424 and an increase in the average adventitious root length in the halves not direct exposed to As in IRGA 423. BR/IRGA 409 showed the smallest root system during the period prior to As exposure. This genotype also showed a smaller concentration of NPSH during As exposure, whereas IRGA 423 and 424 showed the highest. All of the tested genotypes showed As remobilization; under the treatment 0/20 µM treatment, IRGA 423 showed the highest capacity and BR/IRGA 409 showed the lowest. However under 0/50 µM treatment, an opposite response was noticed. Moreover the cultivar BR/IRGA 409, together with

IRGA 420 e IRGA 424 showed the lowest As concentration in shoot tissues. The cultivar IRGA 420 (with lowest As concentration in shoot) also had the lowest S concentration in these tissues All of the tested genotypes showed some susceptibility to As, and the NPSH results together with those of the root plasticity and As remobilization suggested a higher adaptation to As in IRGA 423 and 424.

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SUPPLEMENTAL DATA

Supplemental figure 1. Experimental conditions.

Seminal root removal (A); Hydroponic experiment with an intact root system (B), without As exposure (D) and with As exposure (F); Hydroponic experiment with a split-root system (C and E).

Supplemental table 1. Effect of the seminal root removal for total root system length of rice plants.

Cultivar	Root system length (cm)	
	Total root system length	Seminal root length
BR/IRGA 409	53.91 ± 5.47 C	21.18 ± 2.62 D
BR/IRGA 410	114.57 ± 3.10 A	47.27 ± 1.67 C
IRGA 420	124.39 ± 18.44 A	57.48 ± 2.10 B
IRGA 423	118.49 ± 8.91 A	59.31 ± 3.04 AB
IRGA 424	101.87 ± 3.60 B	62.98 ± 1.57 A

Means followed by capital letters indicate comparison among cultivars. Tukey test at 5% probability of error.

Supplemental table 2. Effect of the seminal root removal for rice plants exposed to As.

Cultivar	As concentration	Root system	Plant height (cm)	Leaves per plant	Number of roots per plant	Shoot DW (mg plant $^{-1}$)	Root DW (mg plant $^{-1}$)
BR/IRGA 409	0 ! M	with seminal root	19.23 Ab	3.71 Aa	13.87 BCa	0.13 Aa	0.042 Aa
		without seminal root	21.83 Aa	4.12 Aa	14.12 Ba	0.17 Aa *	0.040 Aa
	10 ! M	with seminal root	16.10 Bc	2.87 Bb	11.62 Ca	0.10 Abc	0.047 Aa *
		without seminal root	18.45 ABc	3.00 Bb	14.62 Aa *	0.073 Bcd	0.022 Aa
BR/IRGA 410	0 ! M	with seminal root	18.98 Aa	3.00 Ba	15.50 BCab	0.10 Aa	0.070 Aa
		without seminal root	20.10 ABa *	3.00 Ca	18.50 Aa *	0.18 Aa*	0.070 Aa
	10 ! M	with seminal root	18.70 Aa	3.00 Ba	15.00 Bab	0.11 Aa	0.050 Aa
		without seminal root	18.38 Aa	3.00 Ba	15.62 Aab	0.19 Aa *	0.059 Aa
IRGA 420	0 ! M	with seminal root	17.08 Aab	3.25 Bbc	16.87 Aab	0.22 Aa	0.049 Aa
		without seminal root	17.70 Ba	3.62 ABab	17.80 Aa	0.21 Aa	0.054 Aa
	10 ! M	with seminal root	15.50 Bb	3.00 Bc	20.25 Aa *	0.10 Abc	0.059 Aa
		without seminal root	15.52 Bb	4.00 Aa *	16.87 Aab	0.12 ABbc	0.053 Aa
IRGA 423	0 ! M	with seminal root	19.63 Aa	3.00 Ba	12.62 Cab	0.19 Aa	0.060 Aab
		without seminal root	29.76 Aa *	2.75 Ca	13.25 Bab	0.18 Aa	0.080 Aa
	10 ! M	with seminal root	17.10 Ab	3.00 Ba	14.75 Ba	0.13 Aa	0.036 Aab
		without seminal root	16.76 Bb	2.87 Ba	14.75 Aa	0.14 ABa	0.047 Aab
IRGA 424	0 ! M	with seminal root	18.31 Aa	3.00 Bb	16.25 ABa	0.17 Aa *	0.046 Aa
		without seminal root	18.05 Ba	3.12 BCb	15.62 ABa	0.13 Aab	0.045 Aa
	10 ! M	with seminal root	15.90 Bb	3.87 Aa	15.00 Bab	0.10 Ab	0.044 Aa
		without seminal root	16.53 Bab	4.12 Aa	15.37 Aa	0.15 ABab *	0.059 Aa

Means followed by capital letters indicate comparison among cultivar within the same As level and root system; while lower case letters indicate comparisons between As levels within the same cultivar and root system. Means followed by * indicate comparison of root system within the same cultivar and As level. Tukey test at 5% probability of erro

6 MANUSCRITO 5 - DIFFERENTIAL PARTITIONING OF ARSENIC AND MINERAL NUTRIENTS BETWEEN THE MAIN CULM AND TILLERS OF RICE CULTIVARS

ABSTRACT

The transport mechanisms of arsenic (As) from contaminated soil or irrigation water into roots and subsequently into grain, are critical for assessing health risks imposed by Arsenic contamination as well as to grain nutrient quality. The present study aimed to evaluate the effect of As toxicity on the mineral nutrition of five rice (*Oryza sativa*) cultivars: BR/IRGA 409, BR/IRGA 410, IRGA420, IRGA423 and IRGA 424. Two different experiments were performed, evaluating the rice at the seedling stage and at the end of reproductive cycle. In general, the concentrations of the nutrients in the shoots of seedlings decreased with increasing As concentrations. Our data also suggest that the influence of As on mineral element concentrations in rice was related to the ricegenotype, the plant organ, and the essential element being measured. There was wide variation of As accumulation among the cultivars, which suggests differences in the partitioning of As that is transported to the reproductive organs. The levels of macro and micronutrients found in grains produced by the main culm and by the tillers also differed following exposure to As in the substrate.

Keywords: Arsenic toxicity, heavy metal, nutrient status, *Oryza sativa*, rice grain.

6.1 INTRODUCTION

Rice has being recognized as a major route of arsenic (As) exposure for humans (HEIKENS et al., 2007; MEHARG et al., 2009, CAREY et al., 2015; SIGNES-PASTOR et al., 2016). Several studies have indicated that, in populations not exposed to As-tainted water, rice consumption is the largest contributor to the dietary As intake (TSUJI et al., 2007; MONDAL and POLYA, 2008) as a result of flooded paddy cultivation which lead to arsenite mobilization as well as the inadvertent, yet efficient, uptake of arsenite through the silicon transport pathway (MEHARG, 2008).

Besides the imminent risk to human health, Arsenic has been reported to stimulate the formation of free radicals and reactive oxygen species, leading to oxidative stress (LIN et al., 2008). Occasionally, plants can grow in environments containing As without displaying noticeable symptoms (MEHARG and MACNAIR, 1991). However, the exposure of plants to

this element may cause subtle changes, such as differences in the concentrations of nutrients in plant tissues.

In the case of rice grains, which are considered a staple food for much of the world's population, a slight nutritional change in the grains could have a major impact on human nutrition and affect subsequent crops because these seeds can be used to generate new plants (DWIVEDI et al., 2010).

In species where tillering is common, such as rice, the tillers are considered to be beneficial structures, increasing the number of flowers per unit area and, ultimately, contributing to an increase in grain yield (MILLER, 1991). Furthermore, tillers are known to produce additional tissue than is produced by the main culm and may be linked to a strategy for the dilution and/or translocation of heavy metals in plants to maintain their nutritional status (LI et al., 2003; FARRAG et al., 2012).

Since vegetable foods are an important and increasing source of contamination for humans, this work aimed to define nutritional indicators in rice plants, which reflect the nutritional quality of the rice grains grown under contaminated conditions; and define physiological responses in main culm and tillers tissues.

6.2 MATERIALS AND METHODS

6.2.1 Plant materials and growth conditions

Rice plantlets, *indica* varieties, were obtained from *IRGA* (*Instituto Rio Grandense do Arroz*), RS, Brazil. The seeds of five rice cultivars that are grown in Southern Brazil, BR/IRGA 409, BR/IRGA 410, IRGA 420, IRGA 423 and IRGA 424, were used in the present study. Pre-germinated seeds were grown on filter paper with distilled water under controlled conditions for seven days.

6.2.1.1. *Hydroponic experiment*

Rice seedlings were transferred to plastic pots containing 12 liters of one-half strength Kimura B nutrient solution and maintained in a controlled-environment growth greenhouse. The nutrient solution contained the following concentrations of macronutrients: 0.18 mM $(NH_4)_2SO_4$, 0.27 mM $MgSO_4.7H_2O$, 0.09 mM KNO_3, 0.18 mM $Ca(NO_3)_2.4H_2O$, and 0.09 mM KH_2PO_4; the nutrient solution also contained the following concentrations of

micronutrients: 20 μM NaEDTA-Fe.3H_2O, 6.7 μM $MnCl_2$.4H_2O, 9.4 μM H_3BO_3, 0.015 μM $(NH_4)_6Mo_7O_{24}$.4H_2O, 0.15 μM $ZnSO_4$.7H_2O, and 0.16 μM $CuSO_4$.5H_2O. The pH of the solution was adjusted to 5.5, and the solution was renewed every two days. After seven days of seedling acclimatization, As was added to the nutrient solution as Na_2HAsO_4 at a final concentration of 0 (control), 5, 20 or 50 μM for 10 days. After ten days of As exposure, 35 plants per replicate (each treatment consisted of four replicates) were randomLy harvested and separated into shoots and roots.

6.2.1.2. *Experiment using pots containing sand*

Rice seedlings were transferred to plastic pots containing 6 kg of sand, which received irrigation to the point of saturation with Kimura B nutrient solution containing the following; 0.36 mM $(NH_4)_2SO_4$, 0.54 mM $MgSO_4$.7H_2O, 0.18 mM de KNO_3, 0.36 mM $Ca(NO_3)_2$.4H_2O, 0.18 mM de KH_2PO_4, 40 μM NaEDTA-Fe.3H_2O, 12.14 μM $MnCl_2$.4H_2O, 18.8 μM H_3BO_3, 0.03 μM $(NH4)_6Mo_7O_{24}$.4H_2O, 0.3 $ZnSO_4$.7H_2O μM and 0.32 μM $CuSO_4$.5H_2O. The pH of the solution was adjusted to 5.5 prior to being added to the culture substrate. Daily irrigation with the nutrient solution was used to maintain moisture levels, which were determined through evaluations by weighing.

The experimental unit consisted of one pot containing four plants. Plants were grown in a partially climatized greenhouse. Seven days following transplantation, As was added to the nutrient solution at a final concentration of 0 (control), 5, 20 or 50 μM. When the plants reached the fourth leaf stage, the nutrient solution was supplied to achieve a water depth of approximately 3 cm, which was maintained until the end of the growth cycle. After 133 to 143 days of exposure to As, four plants per replicate (each treatment consisted of four replicates) were randomLy harvested and separated into the main stem and tillers. The grains were processed and polished and subsequently analyzed by atomic absorption spectrometry (ICP-MS) to determine the concentration of the following elements: As, calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), copper (Cu), iron (Fe), manganese (Mn), and Zinc (Zn).

6.2.2 Plant growth analysis

At harvest, the hydroponically grown plants were divided into root and shoots. Roots were rinsed twice with distilled water. Plant biomass was measured on dry weight basis. To

obtain dry weight, roots and shoots were dried at 65 °C to constant weight and weighed. Root length was determined according to Tennant (1975), and shoot length was measured with Vernier calipers, both expressed in cm. After 133 143 days of exposure to As, rice plants cultivate in pots with sand were separated in main culm and tillers. The rice grains were than polished for analysis. Main culm and tillers panicle length were measured with Vernier calipers, both expressed in cm.

6.2.3. Determination of the As and mineral nutrient 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). Heating was performed at 130 °C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The contents of Ca, K, Mg, P, Cu, Fe, Mn, Zn, and As 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.

6.2.4 Multivariate analysis

A principal component analysis (PCA) was used to evaluate the relationships among the variables and to identify patterns in the distribution of the data obtained from different tissues during the rice cultivation cycle.

The data collected were transformed by ranking on a 1–10 scale. The average value for the evaluated parameters corresponded to a value of 5 on the scale, with 1 being the lowest assessed value and 10 being the highest assessed value. The averaged data were analyzed using CANOCO" statistical software (version 4.5, Fa. Biometris, Ithaca, NY).

6.3. RESULTS AND DISCUSSION

6.3.1 Biomass and As concentrations in reproductive and vegetative organs

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

2006; BHATTACHARYA et al., 2010). In plants grown in a hydroponic floating system, BR/IRGA 409 was the only cultivar that had a reduced root dry weight (RDW) at a low As level (5 µM) after five days of exposure (Table 1). In contrast, the RDW of BR/IRGA 410 and IRGA 423 cultivars were only affected starting with 20 µM As, whereas the cultivars IRGA 420 and IRGA 424 were less affected, with a decrease in the RDW being observed only at the highest level of As (50 µM).

Arsenic exposure also had a marked influence on the shoot length of rice plants (Table 1). Interestingly, the cultivar BR/IRGA 409 had the shortest lengths for both the shoot and the root system when compared to the other cultivars after five days of exposure, regardless of the As level. This cultivar also experienced the greatest decreases in shoot biomass production with increasing As levels (Table 2), had the lowest root growth rate following As exposure (50 µM) and had the lowest shoot growth rate without As exposure (Tables 1 and 2).

The marked susceptibility of seedlings to heavy metals may be due to a smaller total biomass, which results in less dilution of the As in the plant tissue, leading to toxic As levels that drastically affect plant development. Conversely, the BR/IRGA 409 cultivar which had a high susceptibility to As and is well known for it susceptibility to Fe (STEIN et al., 2009), did not show higher As tissue concentrations (roots and shoots) when compared to the other cultivars (Table 4). In addition to the biomass reduction, the guttation observed in the early hours ceased 5 days following the addition of high As levels to in seedlings of the BR/IRGA 409 cultivar, which may be related to the translocation and partitioning of As (data not shown).

Panaullah et al. (2009) reported that the rough rice yield progressively decreased across the soil-As gradient, from approximately 7 to 2 t ha^{-1} in 2006 and from approximately 9 to 3 t ha^{-1} in 2007. The reduction in the rice yield was associated with a decrease in the number of productive tillers, which are the grain filled panicles.

In our experiment performed using pots containing sand, there was a distinct separation between cultivars that were sensitive and tolerant to As exposure during the reproductive development stage (Table 3). The cultivars BR/IRGA 409 and BR/IRGA 410 showed the greatest sensitivity to As exposure in terms of biomass and grain production, followed by IRGA 420, which had an intermediate response, and the cultivars IRGA 423 and IRGA 424, which showed a higher tolerance for As exposure (Figure 1).

Interestingly, the cultivar BR/IRGA 409 showed a decrease in shoot length for both the main culm and the tillers with increasing As levels. However, the number of tillers produced per plant was not influenced (Table 3). These data, along with the number of

matured panicles, which also experienced a reduction, showed the drastic negative effect of As on the phenological development stage of this cultivar, which failed to develop normal tillers. Additionally, this cultivar, along with BR/IRGA 410, showed a reduction in grain mass and in the number of grains per plant following As exposure.

Figure 1 - Effect of increasing As level on panicle lengh and grain production of five rice cultivars (BR/IRGA 409, BR/IRGA 410, IRGA 420, IRGA 423 and IRGA 424 exposed to different As levels (0, 2 and 10 μM).

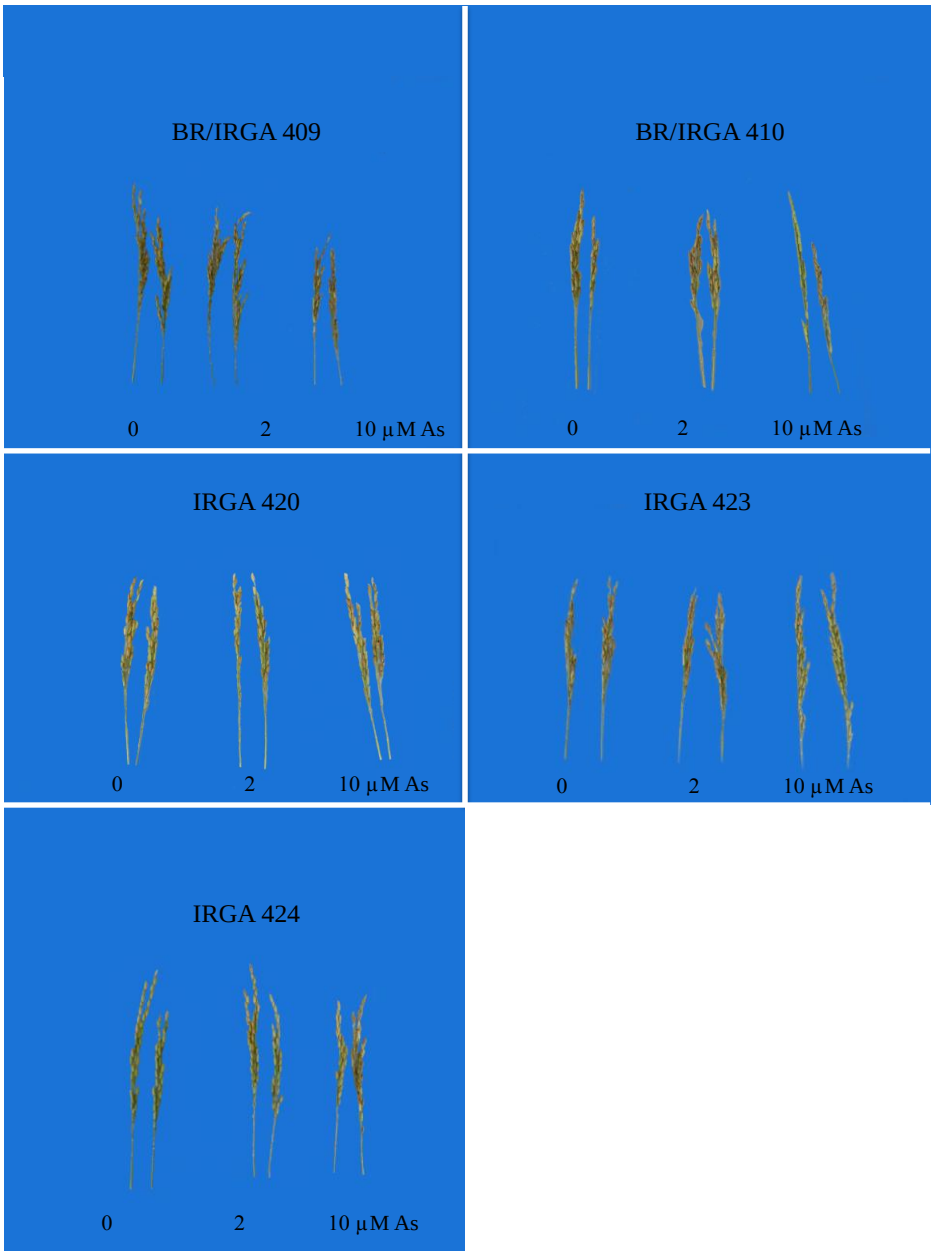


Table 1. Effect of Arsenic exposure on root length, shoot length, number of leaves and plant dry weight.

Cultivar	Arsenic concentration (µM)	Root length (cm plan $^{-1}$)		Shoot DW (mg plan $^{-1}$)		Root DW (mg plant $^{-1}$)		Leaves (per plant)		Shoot length (cm plant $^{-1}$)	
After five days of exposure											
BR/IRGA 409	0	338.69	Aa	37.00	Aa	16.00	Aa	4.00	Aa	17,58	Ca
	5	339.94	Aa	36.00	ABa	13.00	Ab	3,67	Aa	14,53	Cb
	20	212.21	Bb	25.00	Ab	10.00	Bbc	3,75	Aa	14,61	BCb
	50	171.05	Bb	28.00	ABb	9.00	ABc	3,69	Aa	11,38	Bc
BR.IRGA 410	0	401.67	Aa	36.00	Aa	15.00	Aa	4,00	Aa	21,40	Aa
	5	284.24	Bb	31.00	BCa	12.00	Aab	3,88	Aa	16,40	BCb
	20	297.32	Ab	32.00	ABa	11.00	Bb	3,75	Aa	17,17	Ab
	50	222.49	ABb	31.00	Aa	11.00	Ab	3,83	Aa	15,65	Ab
IRGA 420	0	425.84	A a	35.00	Aa	13.00	Aa	4,13	Aa	18,34	BCa
	5	336.20	ABb	26.00	Cb	13.00	Aa	4,08	Aa	16,07	Cb
	20	239.87	ABc	26.00	ABb	10.00	Bab	3,83	Aa	15,10	BCb
	50	189.58	Bc	24.00	Bb	9.00	ABb	3,73	Aa	14,89	Ab
IRGA 423	0	343.60	Aab	35.00	Aa	16.00	Aa	4,00	Aa	19,98	BCa
	5	388.28	Aa	37.00	Aa	14.00	Aab	4,08	Aa	18,65	ABa
	20	277.22	ABbc	29.00	ABa	12.00	Bbc	4,00	Aa	16,58	ABb
	50	252.28	Ac	29.00	Aa	9.00	ABc	4,00	Aa	16,05	Ab
IRGA 424	0	415.88	Aa	34.00	Aa	14.00	Aa	4,38	Aa	20,95	ABa
	5	367.76	ABa	35.00	BCa	12.00	Aab	4,13	Aab	19,58	Aa
	20	208.72	Bb	34.00	Aa	10.00	Bab	3,88	Aab	14,96	BCb
	50	219.05	ABb	18.00	Cb	10.00	ABb	3,75	Ab	13,30	ABb
After ten days of exposure											
BR/IRGA 409	0	818.60	Aa	72.00	Aa	31.00	Aa	4,25	ABa	22,69	Aa
	5	499.28	Ab	73.00	Aa	25.00	Aa	4,00	Bb	20,99	CDab
	20	359.71	Ac	64.00	ABa	17.00	ABb	4,00	Bb	21,12	ABab
	50	139.52	Cd	41.00	Bb	11.00	ABb	4,00	Ab	19,55	Ab
BR.IRGA 410	0	982.44	Aa	94.00	Aa	34.00	Aa	4,25	ABa	26,33	Aa
	5	526.01	Ab	75.00	Aab	20.00	Ab	4,00	Ba	24,13	ABb
	20	395.81	Ab	63.00	ABbc	19.00	ABb	4,00	Ba	21,34	ABc
	50	204.68	ABc	43.00	Bc	16.00	Ab	4,00	Aa	19,33	Ad
IRGA 420	0	815.00	Aa	80.00	Aa	26.00	Aa	4,00	Ba	22,90	Aa
	5	523.46	Ab	69.00	Aa	23.00	Aab	4,00	Ba	20,73	Db
	20	311.52	Ac	46.00	Cb	14.00	Bb	4,00	Ba	19,18	Bbc
	50	178.95	BCd	48.00	ABb	14.00	Ab	4,00	Aa	17,95	Ac
IRGA 423	0	843.54	Aa	85.00	Aa	32.00	Aa	4,75	Aa	24,93	Aa
	5	592.08	Aab	69.00	Aab	25.00	Aab	4,50	Aab	24,53	Aa
	20	375.30	Abc	67.00	Aab	21.00	Aab	4,09	ABb	20,62	ABb
	50	211.92	Ac	60.00	Ab	15.00	Ab	4,00	Ab	20,05	Ab
IRGA 424	0	791.69	Aa	79.00	Aa	32.00	Aa	4,17	ABa	22,30	Aa
	5	618.95	Ab	66.00	Aab	29.00	Aa	4,25	ABa	22,53	BCa
	20	358.38	Ac	50.00	BCbc	14.00	Bb	4,00	Ba	20,99	ABab
	50	199.44	ABd	46.00	Bc	14.00	ABb	4,00	A a	18,53	Ab

Different capital letters indicate significant differences between rice cultivars at the same As level ($p< 0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$).

Table 2. Effect of Arsenic exposure on shoot and root growth.

Cultivar	As concentration (µM)	Shoot growth (mg plant $^{-1}$ day $^{-1}$)	Root growth (mg plant $^{-1}$ day $^{-1}$)
BR/IRGA 409	0	7.00 Ba	3.00 Ba
	5	7.40 Ba	2.40 Ba
	20	7.80 Aa	1.40 Ab
	50	2.60 Cb	0.40 Bb
BR.IRGA 410	0	11.60 Aa	3.80 Aa
	5	8.80 Aab	1,60 Bb
	20	6.20 ABb	1,60 Ab
	50	2.40 Cc	1,00 Ab
IRGA 420	0	9.00 ABa	2.60 Ba
	5	8.60 Aa	2.00 Ba
	20	4.00 Bb	0.80 Bb
	50	4.80 Bb	1.00 Ab
IRGA 423	0	10.00 Aa	3.20 Ba
	5	6.40 Bb	2.20 Bb
	20	7.60 Ab	1.80 Abc
	50	6.20 Ab	1.20 Ac
IRGA 424	0	9.00 ABa	3.60 Aa
	5	6.20 Bb	3.40 Aa
	20	3.20 Cc	0.80 Bb
	50	5.60 Ab	0.80 Ab

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

It is important to note that the tested cultivars had different phenotypic responses. The cultivars considered tolerant to As exposure showed the highest number of matured spikelets and tillers under the control treatment (Table 3), demonstrating greater tillering capacities as compared to the other cultivars. Moreover, the shoot growth in the initial plant stage upon the addition of 50 µM As was higher for these cultivars (IRGA 423 and IRGA 424); additionally, IRGA 423 had the greatest root growth (Table 2).

In rice plants, tillers ensure good grain production by promoting the increased interception of solar light, increasing the number of spikelets and protecting the plant from toxic elements by diluting these elements in plant tissues as a result of larger biomass production, as observed with cases of heavy metal intoxication (FARRAG et al., 2012). In view of this, rice plants could utilize two strategies related to tillering: I) the use of tiller biomass as an escape strategy for the compartmentalization and/or accumulation of toxic substances that occur at high levels in the

environment to ensure the proper development and seed production by the main culm; and II) promoting the accumulation of toxins in the main culm, thus ensuring reduced translocation to the tillers.

Table 3. Effect of Arsenic exposure on main culm and tillers length, number of tillers, weight of 100 unitis of polished grains, panicle length and panicle production.

Cultivar	As concentration µM	Main culm length (cm)	Tillers length (cm)	Number of tillers (per plant)	polished grains (100 units)		panicle length (cm)		number of panicles (per plant)	
					main culm	tillers	main culm	tillers	main culm	tillers
BR/IRGA 409	0	59.34 Aa	33.46 Aa	3.87 Bb	1.71 Bb	1.74 Ba	20.45 Aa	18.75 Aa	1Aa	2.56 ABa
	2	62.15 Aa	38.92 Aa	3.87 Bb	1.76 Ba	1.71 ABb	19.85 ABa	17.46 ABa	1Aa	2.53 Ca
	10	53.63 Ab	18.01 Bb	4.16 Ba	1.63 Bc	1.61 Bc	19.01 Ba	17.63 Aa	1Aa	1.63 Db
BR/IRGA 410	0	60.21 Aa	39.85 Aa	3.62 Bb	1.89 Aa	1.81 Aa	21.25 Aa	19.98 Aa	1Aa	2.30 Ba
	2	58.40 Aa	39.72 Aa	3.50 Bb	1.77 Bb	1.75 Ab	20.61 Aa	18.88 Aab	1Aa	2.16 Da
	10	53.75 Ab	36.43 Aa	4.25 Ba	1.62 Bc	1.62 Bc	18.76 Bb	17.38 Ab	1Aa	2.20 Cb
IRGA 420	0	50.89 Ba	34.70 Aa	4.00 Ba	1.78 Bb	1.70 Bb	15.83 Ca	15.05 Ba	1Aa	2.83 Aa
	2	50.50 Ba	32.53 ABa	4.00 Ba	1.85 Aa	1.70 Bb	15.60 Da	14.40 Ca	1Aa	2.77 Cab
	10	48.32 Aa	35.41 Aa	4.00 Ba	1.82 Aab	1.84 Aa	15.83 Ca	14.01 Ba	1Aa	2.69 Bb
IRGA 423	0	51.68 Bb	33.63 Aa	4.50 Ba	1.56 Cb	1.60 Ca	17.46 Bb	15.33 Bb	1Aa	2.80 Ab
	2	52.04 Bab	37.54 Aa	3.75 Bb	1.62 Ca	1.52 Db	17.71 Cb	16.15 BCb	1Aa	3.21 Ba
	10	56.87 Aa	39.59 Aa	3.50 Bb	1.64 Ba	1.60 Ba	21.78 Aa	19.13 Aa	1Aa	3.26 Aa
IRGA 424	0	51.53 Ba	31.78 Aa	6.92 Aa	1.78 Ba	1.64 Ca	17.73 Ba	16.43 Ba	1Aa	2.80 ABc
	2	45.46 Ba	28.02 Ba	7.25 Aa	1.59 Cb	1.62 Ca	18.33 BCa	16.46 Ba	1Aa	3.89 Aa
	10	45.96 Aa	33.28 Aa	5.25 Ab	1.56 Cb	1.64 Ba	18.46 Ba	17.46 ABa	1Aa	3.16 Ab

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

In both experiments, the arsenic concentration in all tissues increased with increasing levels of external As (Tables 4, 5). In the experiment performed in pots containing sand, differences were not observed in the cumulative final concentrations of As in the substrate at the end of the cycle among cultivars within the same treatment. The concentrations ranged from 2.3-2.8 mg of As at the 2 µM dose and 11-14 mg of As at the 10 µM dose.

The concentration of As in polished rice grains greatly varied among the tested cultivars and tissues (from main culm and tillers), and the values were either similar to the levels reported by other studies (WILLIAMS et al., 2006, SEYFFERTH et al., 2011) or were slightly higher than the "normal" worldwide range of 0.08–0.20 µg g^{-1}, calculated by Zavala and Duxbury (2008) and were similar to those reported for soil-grown rice plants with significant Fe plaque coatings (LIU et al., 2006).

The cultivars BR/IRGA 409 and IRGA 424 had the highest As concentrations in the grains for both the main culm and tillers. The BR/IRGA 409 cultivar had an As concentration in polished grains that was approximately 50% higher than the other cultivars at the 10 µM dose (Table 5).

Table 4 - Effect of Arsenic exposure on Arsenic concentration in root and shoot tissues.

Cultivar	Arsenic concentration µM	As root tissue (µg g^{-1})		As shoot tissue (µg g^{-1})	
		Time of exposure to As			
		5 days	*10 days*	*5 days*	*10 days*
BR/IRGA 409	0	< 8.0	< 8.0	< 8.0	< 8.0
	5	57.36 Cc	83.55 Bc	9.15 Dc	37.77 Bb
	20	181.43 Cb	359.61 Cb	37.29 Da	40.08 Db
	50	332.89 Ca	395.95 Da	30.17 Db	61.55 Ca
BR.IRGA 410	0	< 8.0	< 8.0	< 8.0	< 8.0
	5	64.75 Bc	85.55 Bc	16.47 Cb	29.37 Cc
	20	178.42 Cb	341.60 Cb	42.68 Ca	42.40 Db
	50	289.19 Da	390.00 Da	40.94 Ca	52.78 Da
IRGA 420	0	< 8.0	< 8.0	< 8.0	< 8.0
	5	60.98 BCc	74.05 Bc	31.17 Bc	31.17 Cc
	20	224.87 Bb	482.91 Ba	48.51 Bb	48.51 Cb
	50	399.39 Aa	469.00 Cb	85.45 Aa	85.45 Aa
IRGA 423	0	< 8.0	< 8.0	< 8.0	< 8.0
	5	71.93 Ac	57.91 Dc	27.30 Bc	36.81 Bb
	20	230.24 Ab	341.56 Db	58.26 Aa	65.38 Aa
	50	398.30 Aa	496.26 Ba	47.67 Bb	67.48 Ba
IRGA 424	0	< 8.0	< 8.0	< 8.0	< 8.0
	5	65.72 Bc	131.48 Ac	36.18 Ac	44.26 Ab
	20	169.87 Db	723.34 Ab	42.79 Cb	54.12 Ba
	50	369.63 Ba	769.98 Aa	49.20 Ba	55.48 Da

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

Franco et al. (2011) reported that, in field conditions, the grain production of tillers (per panicle) is equal to or greater than that of the main stem. A rice plant has an average of 3-5 tillers with matured panicles; consequently, in terms of food contamination, the highest impact comes from grains produced by tillers. Thus, the cultivar BR/IRGA 409, in addition to its increased sensitivity to As, has a use restriction due to the greater translocation of As to the tiller grains than the other cultivars.

Table 5 - Effect of Arsenic exposure on Arsenic concentration in external bran and polished grains of main culm and tillers.

Cultivar	Arsenic concentration µM	External Bran		Polished grains	
		Main culm (µg g^{-1})	Tillers (µg g^{-1})	Main culm (µg g^{-1})	Tillers (µg g^{-1})
BR/IRGA 409	0	>0.1	>0.1	>0.1	>0.1
	2	1.05 Ab	1.06 Ab	0.30 Aa	0.30 Ab
	10	1.32 Aa	1.45 Aa	0.72 Aa	0.42 Aa
BR/IRGA 410	0	>0.1	>0.1	>0.1	>0.1
	2	0.61 Bb	0.73 Bb	0.15 Bb	0.21 Aa
	10	0.99 Ba	1.09 ABa	0.28 ABa	0.30 Ba
IRGA 420	0	>0.1	>0.1	>0.1	>0.1
	2	0.84 Aa	0.82 Ba	0.10 Bb	0.16 Bb
	10	0.80 Ca	0.84 Ba	0.26 ABa	0.39 Ba
IRGA 423	0	>0.1	>0.1	>0.1	>0.1
	2	0.64 Bb	0.65 Bb	0.13 Ba	0.21 Aa
	10	0.86 Ca	0.83 Ba	0.19 Ba	0.30 Ba
IRGA 424	0	>0.1	>0.1	>0.1	>0.1
	2	0.77 ABb	0.84 Bb	0.19 Bb	0.25 Ab
	10	1.44 Aa	1.30 Aa	0.45 Aa	0.46 ABa

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

The arsenic was allocated predominantly in the bran layer of the unpolished rice grain (Table 5), which is consistent with the results of other studies showing higher levels of As in brown rice when compared to white (polished) rice, as little As is found in the starchy endosperm (REN et al., 2006; MEHARG et al., 2008; SUN et al., 2008; LOMBI et al., 2009).

The As levels in the bran were much lower than those reported by Lombi et al. (2009) but were comparable to other studies with similar soil conditions (ABEDIN et al., 2002; MEI et al., 2009; CAREY et al., 2010), which may reflect genotypic differences with respect to As translocation in plant fraction. The dramatic decrease in the As concentration from the bran and the grain itself, previously observed by Signes et al. (2008), is likely related to the way nutrients, contaminants and photosynthetates are distributed within the different organs. The concentration of As in the husk, for example, derives from the xylematic transport of this contaminant.

Therefore, the increased As concentration found in the bran fraction compared with polished rice may have two possible causes. First, there could be a physiological barrier in the unloading and uploading process responsible for the transfer of As from the maternal tissues

(ovular vascular system, either phloem or xylem elements) to the filial tissues (aleurone). Second, As, as with many other elements, could accumulate preferentially in the protein-rich aleurone and embryo tissues.

The As concentrations in the root and shoot tissue of the rice changed at the different growth stages, experiencing a cumulative effect of As in tissue over time (Table 4). The concentration of As in the tissue followed the order: root tissue of rice plants exposed to As for ten days > root tissue of rice plants exposed to As for five days > shoot tissue of rice plants exposed to As for ten days > shoot tissue of rice plants exposed to As for ten days > tillers bran of rice grain > main culm bran of rice grain > tillers polished grain > main culm polished grain (Tables 4, 5).

6.3.2 Mineral nutrition of the shoot, root and grain tissues

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).

The results of the present study indicated that rice genotypes had different nutrient partitioning requirements (Tables 6, 7, 8, 9, 10, and 11). The results also indicated that the nutrient status and distribution varied with the amount of As added. For example, in the absence of As, the rice genotypes concentrated approximately 40%, 30%, 55%, and 15% more Ca, K, Mg and P, respectively, in shoot tissues when compared to plants exposed to As for 10 days. The cultivars BR/IRGA 409 (As-susceptible) and IRGA 423 (As-tolerant) showed the lowest concentrations of macronutrient at both high and low As levels. However, as discussed above, in terms of vegetative biomass and grain production, there was a distinct response between these two cultivars. BR/IRGA 409 was more susceptible to As and had a higher As accumulation rate, while IRGA 423 was considered to be tolerant to As and to have reduced As translocation (Tables 3, 5).

The cultivar IRGA 424 had increased macronutrient concentrations in root tissues, an increased tillering capacity, and increased concentrations of Ca, K and Mg in the shoot tissues (Tables 3, 6, 7, 8 and 9) when compared to the other genotypes.

Table 6 - Effect of Arsenic exposure on mineral nutrient concentration of shoot tissue of rice plants after five days of exposure in hydroponic system.

Cultivar	Arsenic concentration (µM)	P (µg g^{-1})	K (µg g^{-1})	Mg (µg g^{-1})	Ca (µg g^{-1})	Zn (µg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})	Cu (µg g^{-1})
BR/IRGA 409	0	5565.90 Eb	12751.48Eb	5250.77 Ca	2670.51 Ea	79.06 Cab	679.13 Db	83.11 Ca	15.10 Aba
	5	3363.96 Ec	7676.06 Ed	2576.47 Ed	1428.87 Ed	51.15 Dc	332.44 Ed	40.95 Cb	8.52 Dc
	20	6905.42 Ca	13207.4 Ea	4684.40 Ab	2596.56 Cb	71.97 Cb	760.70 Ca	55.85 Cb	14.03 Ba
	50	5610.30 Db	11805.83Ec	3325.31 Ac	2123.80 Cc	87.38 Aa	583.73 Bc	54.18 Bb	11.28 Bb
BR.IRGA 410	0	7315.23 Ab	15308.64 Cb	5317.54 Bb	3370.82 Ab	106.82 Ab	774.02 Cb	82.80 Ca	16.81 Aab
	5	8094.10 Aa	14136.73 Dc	5372.73 Ba	3665.80 Aa	126.47 Ba	618.30 Dd	78.00 Aba	18.03 Aa
	20	7359.38 Bb	16146.24 Da	4465.30 Dc	2748.47 Bc	89.51 Bc	921.34 Aa	93.54 Aa	16.17 Ab
	50	6521.41 Bc	12383.08 Dd	3243.77 Bd	2323.09 Ad	69.54 Bd	623.07 Ac	72.45 Ba	16.38 Aab
IRGA 420	0	5910.75 Dc	14562.56 Dd	4966.92 Da	2812.80 Ca	72.65 Bb	680.33 Db	94.28 Bca	14.91 Ba
	5	6272.45 Da	20608.52 Aa	4632.46 Db	2616.38 Db	57.48 Dc	707.85 Aa	70.58 Bb	12.28 Cb
	20	5987.82 Db	18075.36 Ab	3676.05 Ec	2485.91 Dc	88.10 Ba	588.60 Ec	53.14 Cb	10.21 Cc
	50	5492.48 Ed	17576.06 Ac	2578.77 Cd	1910.38 Ed	63.66 Bc	508.91 Dd	60.27 Bb	8.97 Cc
IRGA 423	0	6889.15 Cb	18686.57 Ab	5024.29 Ea	2845.70 Ba	95.20 Bb	778.34 Bb	117.20 Bb	14.74 Ba
	5	7052.92 Ca	18789.68 Ba	4805.24 Cb	2837.52 Ca	221.35 Aa	642.71 Cc	86.70 Abc	15.06 Ba
	20	6855.68 Cb	17230.00 Bc	4570.90 Bc	2797.17 Ab	91.42 Bb	836.20 Ba	92.19 Abc	12.63 Bb
	50	6401.04 Cc	16906,25 Bd	3312.02 Ad	2083.17 Dc	87.08 Ab	579.74 Bd	202.78 Aa	9.82 BCc
IRGA 424	0	7199.47 Bc	17664.34 Ba	5406.52 Ab	2753.06 Dc	102.96 ABb	796.99 Aa	166.09 Aa	15.65 ABb
	5	7372.36 Bb	16918.27 Cc	5781.23 Aa	3232.07 Ba	107.77 Cb	680.51 Bc	94.84 Ab	18.42 Aa
	20	7695.04 Aa	17075.00 Cb	4517.34 Cc	2815.51 Ab	151.63 Aa	700.61 Db	68.79 BCc	13.15 Bc
	50	6806.90 Ad	15032.86 Cd	3353.27 Ad	2275.32 Bd	92.97 Ac	559.15 Cd	71.21 Bc	10.30 BCd

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$).

The exposure of the rice plants to As increased the root P concentration in all the tested cultivars, except for IRGA 424. The P availability and the arsenic-induced physiological requirements of a plant (CARBONELL et al., 1998; GAO and MUCCI, 2001; TU and MA, 2005) may be the reasons for the increased P uptake.

The formation of Fe plaque is considered to be a consequence of the oxidation of Fe from ferrous (II) to ferric (III) and the precipitation of Fe oxide on the root surface (ZHANG et al., 1999). During the present study, rice cultivars showed Fe plaque formation in the form of reddish brown coating on the root surface (data not shown), and the Fe root concentration significantly varied among the genotypes, which demonstrated that rice varieties differed significantly with respect to Fe plaque formation. Fe plaque is commonly formed on rice roots due to the release of oxygen and oxidants into the rhizosphere (LIU et al., 2006); thus, the differential abilities of rice genotypes, in terms of oxygen evolution from the roots, leads to variable Fe plaque-forming abilities and, subsequently, to a variable tendency to accumulate metals and metalloids.

Due to the high adsorption capacity of the functional groups on Fe hydroxides, Fe plaque sequesters a number of metals and metalloids by adsorption or co-precipitation (LIU et al., 2007). Dwivedi et al. (2010) described the sequestration of many elements in the iron

plaque in an order of Fe>Zn>P>As>Se. Other studies have also demonstrated that Fe plaques can adsorb P (ZHANG et al., 1999; BATTY et al., 2002), Zn, Pb, Ni, Cu (GREIPSSON and CROWDER, 1992), and Cd (LIU et al., 2007). In our study, in general, As exposure increased Fe, P and Zn concentrations in the root tissue, with the exception of the IRGA 424 cultivar, which had the opposite response (Tables 7 and 9).

Table 7 - Effect of Arsenic exposure on mineral nutrient concentration of root tissue of rice plants after five days of exposure in hydroponic system.

Cultivar	Arsenic concentration (µM)	P (µg g^{-1})	K (µg g^{-1})	Mg (µg g^{-1})	Ca (µg g^{-1})	Zn (µg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})	Cu (µg g^{-1})
BR/IRGA 409	0	1780.00 Aa	1650.10 Dc	1147.00 Aa	495.05 Bd	37.00 Bc	47.59 Bd	364.78 Dc	14.79 Ca
	5	1315.76 Dc	2001.52 Ca	1060.94 Bb	594.53 Cd	57.05 Cb	68.63 BCc	379.08 Dc	15.15 Da
	20	1328.73 Cc	1923.70 Db	494.34 Bc	649.43 Cb	52.05 Cb	125.17 Bb	493.48 Db	13.35 Aab
	50	1602.16 Bb	984.21 Dd	459.88 Bc	831.55 Aa	78.33 Aa	160.55 Aa	870.34 Da	12.18 Abb
BR.IRGA 410	0	1775.77 Ab	892.34 Ec	898.27 Db	480.00 Bd	58.31 Ac	46.08 Bd	393.67 Cb	14.21 Cb
	5	1770.60 Ab	1267.19 Eb	1006.05 Ca	642.99 Bb	68.40 Bb	70.54 Bc	551.48 Ac	17.57 Bca
	20	1848.93 Aa	1711.42 Ea	527.42 Bc	762.06 Ba	79.88 Aa	129.05 Ba	643.23 Ab	13.89 Ab
	50	1841.05 Aab	626.10 Ed	409.18 Cd	512.82 Ec	31.52 Cd	114.60 Bb	908.10 Ca	11.86 Abc
IRGA 420	0	1481.17 Cc	3703.80 Bc	1037.47 Ba	508.62 Bc	44.50 Bc	57.12 Ad	391.54 Cd	24.96 Aa
	5	1563.91 Bb	4451.52 Aa	864.98 Db	676.81 Ab	56.91 Cb	75.13 Ac	438.23 Cc	20.05 Ab
	20	1827.73 Aa	3876.14 Ab	503.02 Bc	793.60 Aba	66.76 Ba	202.23 Aa	644.48 Ab	15.22 Ac
	50	1885.42 Aa	2688.37 Cd	421.17 BCd	657.12 Cb	55.86 Bb	102.46 Cb	1031.10 Ba	13.24 Ad
IRGA 423	0	1564.56 Bb	2175.76 Cc	959.97 Cb	501.82 Bc	43.69 Bb	38.26 Cd	557.96 Bb	15.07 Cb
	5	1444.27 Cc	1873.39 Dd	1185.87 Aa	663.70 ABb	79.84 Aa	66.31 Cc	400.07 Dd	19.19 Aba
	20	1575.78 Bb	2638.75 Cb	594.79 Ac	797.51 Aa	81.42 Aa	201.16 Aa	523.01 Cc	11.38 Bc
	50	1665.89 Ba	3148.66 Ba	421.59 BCd	768.02 Ba	83.07 Aa	102.42 Cb	1062.53 Aa	10.45 Bc
IRGA 424	0	1497.17 Bca	5400.20 Aa	1065.89 Ba	1037.26 Aa	57.00 Cb	44.12 Bd	734.62 Ab	17.83 Ba
	5	1209.68 Eb	3361.90 Bb	1103.61 Ba	520.80 Dd	57.26 Cb	59.53 Dc	488.76 Bd	17.04 Ca
	20	1137.11 Dc	3211.13 Bc	406.04 Cc	824.71 Ab	87.48 Aa	109.73 Cb	555.09 Bc	10.80 Bb
	50	1476.43 Ca	3402.51 Ab	560.21 Ab	559.50 Dc	40.58 Cc	118.57 Ba	892.07 Cda	10.93 Bb

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$).

In the plantlets grown in a hydroponics floating system, As exposure promoted an increase in the Ca concentrations in the root tissue after five and ten days for all the tested genotypes, with the exception, again, of IRGA 424 (Tables 7 and 9). IRGA 424 showed a decrease in the root Ca concentrations with increasing As levels. As exposure had the opposite affect in shoot tissues, with the exception of the IRGA 424 genotype, which only showed a decrease in the shoot Ca concentration after five days with 50 µM As and after ten days with 20 µM As (Tables 6, 7, 8, and 9).

Interestingly, in processed grain tissues, the Ca concentration differs slightly following As exposure. Only BR/IRGA 409, IRGA 424 (in main culm) and BR/IRGA 410 (in tillers)

responded positively to As levels, with respect to the Ca concentrations in the grains (Table 10).

Table 8 - Effect of Arsenic exposure on mineral nutrient concentration of shoot tissue of rice plants after five days of exposure in hydroponic system.

Cultivar	Arsenic concentration (μM)	P (μg g^{-1})	K (μg g^{-1})	Mg (μg g^{-1})	Ca (μg g^{-1})	Zn (μg g^{-1})	Mn (μg g^{-1})	Fe (μg g^{-1})	Cu (μg g^{-1})
BR/IRGA 409	0	4961.72 Db	11051.59 Eb	5218.64 Aa	2774.93 Ca	69.92 Ca	572.52 Ea	121.88 Aa	15.72 Ca
	5	5277.49 Ea	11818.18 Ea	4581.73 Eb	2359.92 Eb	69.53 ABa	565.63 Eb	76.09 Ab	15.13 Ba
	20	4643.50 Ec	10680.27 Ec	2830.98 Ec	1812.34 Ec	53.65 Db	535.92 Dc	46.45 Ac	8.72 Bb
	50	4107.41 Dd	5987.34 Ed	2021.35 Cd	1560.60 Cd	59.88 Ab	394.63 Dd	60.58 Bbc	8.27 ABb
BR.IRGA 410	0	5870.93 Ab	26643.22 Bb	4695.59 Db	3032.99 Ab	83.11 Ba	665.11 Ba	125.17 Aa	21.36 Aa
	5	6954.25 Aa	24575.66 Bc	5073.20 Ba	3468.21 Aa	60.87 BCb	614.81 Db	101.67 Aa	18.67 Ab
	20	5386.24 Cc	32207.66 Ba	3053.36 Dc	2175.88 Dc	84.25 Ca	554.35 Cc	68.46 Ab	11.06 Ac
	50	4998.73 Cd	20567.37 Bd	2324.87 Bd	1825.09 Bd	41.19 Bc	437.84 Bd	71.36 Bb	9.58 Ac
IRGA 420	0	5910.75 Ac	34029.85 Aa	4966.92 Ca	2812.80 Ba	72.65 Cb	680.33 Ab	94.28 Ba	14.91 CDa
	5	6272.45 Ba	32320.71 Ab	4632.46 Db	2616.38 Db	57.48 Cc	707.85 Aa	70.58 Bb	12.28 Cb
	20	5987.82 Ab	29223.39 Cc	3676.05 Ac	2485.91 Bc	88.10 Ca	588.60 Bc	53.14 Ab	10.21 ABc
	50	5492.48 Ad	22969.21 Ad	2578.77 Ad	1910.38 Ad	63.66 Ac	508.91 Ad	60.27 Bb	8.97 Ac
IRGA 423	0	5156.66 Cb	14732.67 Dc	4469.16 Eb	2814.05 Ba	104.17 Ab	637.26 Db	110.27 Aba	13.46 Da
	5	5902.78 Ca	15302.58 Db	4744.27 Ca	2773.57 Cb	72.54 Ac	702.60 Ba	84.24 Abb	12.74 Ca
	20	5153.68 Db	15787.91 Da	3546.91 Bc	2534.36 Ac	116.58 Aa	701.44 Aa	50.76 Ac	9.69 ABb
	50	4152.69 Dc	9709.36 Dd	1898.05 Dd	1453.16 Dd	63.59 Ad	344.49 Ec	49.91 Bc	6.79 ABc
IRGA 424	0	5749.68 Ba	21728.46 Cb	5149.57 Ba	2805.25 BCa	92.22 Ba	642.71 Cb	121.42 Aa	19.10 Ba
	5	5744.50 Da	20794.67 Cc	5175.49 Aa	2822.15 Ba	66.80 ABb	667.55 Ca	83.85 ABb	15.44 Bb
	20	5501.15 Bb	33228.57 Aa	3413.06 Cb	2283.57 Cb	99.04 Ba	587.22 Bc	52.48 Ac	9.95 ABc
	50	5282.04 Bc	18262.38 Cd	2284.69 Bc	1910.53 Ac	41.70 Bc	411.11 Cd	102.67 Aab	7.70 Bd

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

Similarly, tissue K concentrations, in general, had the same pattern of response as the Ca concentrations in vegetative organs, with increased concentrations in roots and decreased concentrations in shoot tissues after ten days of As exposure and with no distinct response in grain tissues (Tables 6, 7, 8, 9 and 10). After five days of exposure, there was a significant decrease in the root tissue concentration of K for BR/IRGA 409, BR/IRGA 410 and IRGA 420 with 50 μM of As when compared to the other treatments.

In the experiment using pots containing sand as the substrate, K and Mg concentrations in the grains of the main culm and tillers of IRGA 424 were not affected by any As level. Similarly, in IRGA 420 and BR/IRGA 409, the Ca and K concentrations were not affected by As (Table 10). Ca and K concentrations in the grains from tillers, in general, showed less sensitivity to As exposure. In contrast, tissue Mg and P concentrations were less affected in grains from the main stem. There were also clear differences between the tillers and the main stem and among the cultivars. The cultivars BR/IRGA 409, BR/IRGA 410 and

IRGA 424 concentrated macronutrients primarily in the main stem, while the other cultivars concentrated macronutrients primarily in the tillers (Tables 10 and 11).

Table 9 - Effect of Arsenic exposure on mineral nutrient concentration of root tissue of rice plants after five days of exposure in hydroponic system.

Cultivar	Arsenic concentration (µM)	P (µg g^{-1})	K (µg g^{-1})	Mg (µg g^{-1})	Ca (µg g^{-1})	Zn (µg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})	Cu (µg g^{-1})
BR/IRGA 409	0	1831.02 Bd	1276.17 Dd	1002.03 Cb	728.00 Cd	150.68 Bb	78.45 Cc	375.38 Bd	18.64 Cb
	5	2400.47 Ba	2288.11 Dc	2077.87 Ba	1105.75 Bb	194.85 Ba	98.59 Cb	415.07 Cc	16.42 Dc
	20	1909.86 Cc	3505.49 Da	782.07 Dd	978.45 Cc	77.31 Dd	96.88 Cb	493.61 Bb	18.47 Cb
	50	2133.84 Bb	2718.62 Cb	866.81 Bc	1141.86 Ba	101.33 Bc	108.82 Da	547.76 Ca	22.44 Ba
BR.IRGA 410	0	1931.00 Bd	1179.65 Dd	1010.05 Cb	740.00 Cd	172.44 Bb	80.70 Cc	382.00 Bd	20.00 Cb
	5	2450.63 Ba	2178.10 Dc	2060.70 Ba	1030.95 Bb	200.05 Ba	103.99 Cb	405.05 Cc	15.40 Dc
	20	2009.80 Cc	3315.40 Da	779.03 Dd	957.33 Cc	87.00 Dd	85.80 Cb	509.60 Bb	21.07 Cb
	50	2103.84 Bb	2730.50 Cb	856.80 Bc	1167.860 Ba	105.30 Bc	110.00 Da	540.75 Ca	22.67 Ba
IRGA 420	0	1624.82 Cc	1405.63 Cd	1167.68 Bb	822.85 Bd	88.81 Cc	118.38 Bc	348.59 Cd	27.68 Bb
	5	1666.10 Cbc	2862.73 Bc	1576.13 Ca	929.18 Cc	118.05 Ca	132.29 Bb	399.72 Cc	29.98 Ba
	20	1988.09 Ba	4633.75 Ba	895.60 Cc	1041.91 Bb	100.96 Cb	105.20 Bd	470.95 Bb	26.64 Bb
	50	1696.53 Cb	2985.56 Bb	697.17 Cd	1087.17 Ca	105.77 Bb	147.85 Ba	541.08 Ca	18.28 Cc
IRGA 423	0	1433.34 Dc	3044.75 Bb	739.27 Dc	416.61 Dd	76.97 Dc	42.02 Dd	215.78 Dd	14.84 Dc
	5	1639.89 Cb	2545.13 Cd	1364.83 Da	698.50 Dc	123.77 Cb	83.46 Db	524.15 Bb	20.54 Ca
	20	1750.34 Da	4277.11 Ca	953.87 Bb	926.63 Da	135.06 Ba	73.94 Dc	482,.41 Bc	18.47 Cb
	50	1688.63 Cab	2931.61 Bc	712.97 Cc	867.52 Db	85.84 Cc	117.34 Ca	775.20 Ba	14.25 Dc
IRGA 424	0	3616.57 Ab	3371.05 Ac	2283.06 Ab	2449.96 Aa	443.24 Aa	165.07 Ab	842.76 Ac	53.07 Aa
	5	3533.60 Ac	5061.98 Ab	3426.26 Aa	1888.12 Ac	237.36 Ab	143.65 Ac	786.33 Ad	33.54 Ab
	20	4211.76 Aa	9632.06 Aa	1798.20 Ac	1854.48 Ad	192.51 Ad	163.42 Ab	2330.67 Aa	32.03 Ac
	50	3578.86 Abc	5110.21 Ab	1409.95 Ad	2203.14 Ab	219.10 Ac	320.10 Aa	1319.35 Ab	26.33 Ad

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

The tissue P concentrations varied considerably among the genotypes, with IRGA 420 and IRGA 423 showing a continuous increase in P concentrations in the root tissue with increasing As levels after five days of exposure (Tables 7 and 9). On the tenth day of exposure, although the smallest concentrations of P were observed in the control tissues, both cultivars showed a decrease in P concentrations at the 50 µM As dose when compared to the 20 µM As dose. After five days of As treatment both BR/IRGA 409 and BR/IRGA 410 genotypes showed either a decrease or no change in the P concentrations with increasing As levels, but after ten days, both showed increasing P root tissue concentrations (Tables 7 and 9). In contrast to the other cultivars, IRGA 424, a genotype with a unique response to As intoxication, showed a significant decrease in the P concentration upon the addition of As for ten days. In shoot tissues, the macronutrient concentrations in all cultivars were affected by As levels, resulting in decreased concentrations.

As(V), a chemical analog of P, is taken up and transported via the phosphate transporter

(MEHARG, 2008). The competition of uptake and the translocation between phosphate and As(V) has been shown in many plant species. The competition of uptake and the translocation between P and As(V) proposes a potential strategy for reducing As uptake and accumulation in grains. However, under flooded conditions, the competition between P and As(V) becomes more complicated because P will compete with As(V) for the adsorption of soil minerals. Studies have shown that the presence of P can strongly suppresses As(V) adsorption, thus increasing the As mobility in soil.

Table 10 - Effect of Arsenic exposure on macronutrients of external bran and polished grains of main culm and tillers.

Cultivar	Arsenic (µM)	External bran		Polished grains		External bran		Polished grains	
		Main culm	Tillers	Main culm	Tillers	Main culm	Tillers	Main culm	Tillers
		phosphorous (µg g^{-1})				*potassium (µg g^{-1})*			
BR/IRGA 409	0	16573.33 Da	20969.00 Ca	2413.00 Ba	2785.00 Aa	9292.07 Aa	11140.66 Aa	414.80 Ba	553.30 Ba
	2	18891.67 Ca	18631.33 Db	2665.67 Ba	2523.00 Bb	8668.23 Ab	10510.00 Aab	472.50 Ba	378.00 Bc
	10	16118.00 Ca	18630.00 Ab	2069.33 Cb	1477.33 Dc	8573.00 Bb	9752.67 Ab	489.22 Ca	473.70 Bb
BR/IRGA 410	0	15375.00 Eb	15117.00 Db	2411.00 Bb	1540.00 Cc	9487.00 Aa	8760.67 Bb	542.40 Ab	515.80 Ba
	2	21073.67 Ba	19017.33 Ba	2457.00 Cb	2864.00 Ab	9142.65 Aab	9968.66 Aa	910.30 Aa	427.00 Ba
	10	14163.67 Db	14784.00 Db	2626.00 Aa	3026.00 Aa	8853.66 Bb	8433.00 Bb	1117.67 Aa	515.10 Aba
IRGA 420	0	21416.00 Ab	24190.33 Aa	1317.00 Cb	2156.33 Bb	10610.00 Aa	9485.35 Ba	602.50 Aa	874.20 Aa
	2	29169.00 Aa	13701.00 Ec	806.00 Ec	2178.00 Bb	11100.00 Aa	9719.00 Aa	508.00 Bb	461.00 Bc
	10	21302.33 Bb	17327.33 Bb	2159.00 Ba	2974.00 Aa	10130.00 Aa	9905.25 Aa	460.67 Cb	597.50 Ab
IRGA 423	0	17413.67 Cb	20873.00 Ca	2982.00 Ab	2235.00 Bb	9364.67 Ab	10270.00 ABa	473.40 Bc	553.40 Bb
	2	18622.67 Db	21326.00 Aa	4047.00 Aa	2975.00 Aa	10580.00 Aa	10530.50 Aa	578.70 Bb	1267.67 Aa
	10	27297.00 Aa	12013.67 Eb	509.67 Ec	2205.00 Cb	8137.00 Bb	8928.23 Bb	736.00 Ba	474.50 Bb
IRGA 424	0	19437.67 Ba	23855.67 Ba	2372.00 Ba	881.00 Db	10070.00 Aa	10130.67 ABa	384.10 Ba	496.90 Ba
	2	18304.33 Ea	18804.67 Cb	2295.00 Da	1491.00 Ca	10550.00 Aa	9257.67 Ab	407.80 Ba	432.00 Ba
	10	12637.00 Eb	16153.67 Cb	1319.67 Db	1432.67 Da	8261.00 Bb	8205.00 Bb	430.00 Ca	438.70 Ba
		magnesium (µg g^{-1})				*calcium (µg g^{-1})*			
BR/IRGA 409	0	6183.00 Aa	6978.40 Aa	108.40 Bb	177.00 Ba	652.40 Aa	739.00 Aa	62.40 Bb	60.17 Ca
	2	5964.67 Cb	6849.00 Aa	179.90 Ba	130.00 Bb	585.33 Ba	686.80 Da	67.53 Ab	67.80 Ba
	10	5738.00 ABb	6262.30 Aa	92.80 Bc	113.70 Ab	680.66 Ba	699.20 Aa	83.30 Aa	54.28 Ba
BR/IRGA 410	0	5930.00 Aa	5442.00 Aa	148.90 Ac	116.00 Bb	639.93 Aa	662.00 Ba	111.40 Aa	53.49 Cb
	2	5960.67 Ca	6081.67 Aa	354.30 Ab	76.50 Cc	693.86 Ba	718.66 Ca	72.28 Ab	58.50 Bb
	10	5814.22 Aa	5574.00 Aa	705.20 Aa	168.90 Aa	665.20 Ba	681.00 Aa	105.40 Aa	75.18 ABa
IRGA 420	0	6465.00 Aa	5704.33 Aa	144.60 Aa	291.90 Aa	961.90 Ab	777.00 Bb	70.90 Ba	73.33 Ba
	2	7387.50 Aa	6094.00 Aa	146.40 Ba	201.00 Bb	1436.66 Aa	1457.70 Aa	79.45 Aa	74.80 Ba
	10	6081.70 Aa	5904.00 Aa	142.70 Ba	162.90 Ac	846.60 Ab	914.50 Ab	66.30 Ba	67.66 Aa
IRGA 423	0	6134.00 Aa	6601.67 Ab	157.70 Ab	242.30 Ab	896.73 Ab	1015.00 Aa	84.40 Ba	95.92 Aab
	2	6405.67 BCa	7228.50 Aa	256.00 Aa	586.80 Aa	1659.33 Aa	1019.00 Ba	79.33 Aa	107.20 Aa
	10	5111.70 Bb	5457.30 Ac	259.20 Ba	183.40 Ac	706.7 ABb	850.50 Ab	73.10 ABa	81.93Ab
IRGA 424	0	6383.50 Aa	6641.70 Aa	105.70 Ba	158.20 Ba	736.10 Aa	581.00 Bb	64.40 Bb	58.22 Ca
	2	6583.00 Ba	5703.33 Ab	91.50 Ca	128.30 Bb	839.10 ABa	717.40 Ca	66.04 Ab	67.90 Ba
	10	4841.67 Bb	4939.00 Ab	125.60 Ba	108.00 Ab	648.10 Ba	645.40 Aa	93.60 Aa	56.89 Ba

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

Hossain et al. (2008) found that the addition of P fertilizer to flooded paddy soil increased As accumulation in rice. However, Wu et al. (2011) found that overexpressing OsPT8 in rice plants did not significantly change As accumulation in rice grains. This result implies that, under flooded conditions, As(V) is reduced to As(III), which is taken up by aquaporin nodulin 26-like intrinsic proteins; thus, P transporter regulation is unlikely to significantly affect As accumulation in rice grains. Therefore, the processes of As and P interaction is growth condition dependent, and P management should vary according to soil and environmental factors.

Table 11 - Effect of Arsenic exposure on micronutrients of external bran and polished grains of main culm and tillers.

Cultivar	As(μM)	External bran		Polished grains		External bran		Polished grains	
		Main culm	Tillers	Main culm	Tillers	Main culm	Tillers	Main culm	Tillers
		zinc (μg g^{-1})				*manganese (μg g^{-1})*			
BR/IRGA 409	0	262.50 Bb	236.90 Bb	20.42 ABa	22.59 Bca	197.50 Bb	223.30 Aa	17.17 Ba	14.23 Ba
	2	465.50 Aa	460.20 Aa	19.58 Ba	19.94 ABab	172.90 Ab	182.40 Bb	13.08 Aa	10.72 Bb
	10	305.20 Bb	414.20 Aa	16.13 Bb	17.84 Bb	248.90 Aa	189.40 Bb	18.89 Ba	10.40 Bb
BR/IRGA 410	0	175.93 Bb	362.70 Aba	17.61 Bb	16.72 Ca	164.80 Bb	170.70 Bb	33.09 Aa	10.33 Ca
	2	260.60 Bb	207.20 Bb	26.62 Aa	16.51 Ba	186.10 Ab	203.60 ABa	14.24 Ac	9.91 Ba
	10	457.20 Aa	203.90 Bb	24.82 Aa	17.50 Ba	210.80 Aba	190.30 Bb	24.31 Ab	12.24 Aa
IRGA 420	0	440.60 Aa	568.60 Aa	21.55 Aa	24.16 Ba	209.40 Abb	158.60 Bb	13.38 Ca	16.44 Aa
	2	429.80 Aa	458.20 Aa	18.09 Ba	20.89 ABa	288.00 Aa	297.20 Aa	12.22 Aa	13.40 ABb
	10	304.60 Bb	281.60 Bb	20.42 Aa	21.59 Aa	222.70 Ab	232.60 Aa	12.42 Ba	11.37 ABb
IRGA 423	0	256.30 Bb	454.50 Aa	20.85 Aa	48.00 Aa	268.50 Ab	281.80 Ab	14.16 Bca	14.50 ABb
	2	536.70 Aa	424.90 Aa	19.52 Ba	27.23 Ab	276.60 Aa	320.70 Aa	16.45 Aa	19.00 Aa
	10	230.20 Cb	232.00 Bb	20.74 Aa	18.60 ABb	229.80 Ab	253.50 Ab	12.68 Bb	13.56 Ab
IRGA 424	0	217.20 Bb	255.50 Ba	18.38 Ba	17.60 Ca	190.20 Bb	134.40 Bb	10.28 Ca	11.51 Ba
	2	324.90 Aba	204.80 Ba	17.89 Ba	17.39 Ba	229.90 Aa	175.40 Ba	11.84 Aa	10.94 Ba
	10	306.40 Ba	224.50 Ba	17.73 Aba	13.87 Bb	185.00 Bb	152.50 Ba	10.78 Ba	9.40 Ba
		iron (μg g^{-1})				*copper (μg g^{-1})*			
BR/IRGA 409	0	110.10 Bb	118.60 ABb	19.94 Aa	8.54 BCb	12.21 ABa	11.75 Aa	3.64 Aa	3.80 Aa
	2	110.80 Ab	111.90 Bb	13.91 Bb	19.39 Aa	10.83 Aa	12.24 Aa	3.52 Aa	3.27 Aa
	10	153.60 Aa	147.60 Aa	9.48 Bb	12.57 ABb	12.89 Aa	11.35 Aa	3.31 Ba	3.41 Aa
BR/IRGA 410	0	110.10 Bb	107.60 Bb	8.69 Bb	13.29 Ba	10.93 Ba	11.26 Aa	3.11 ABb	3.28Aa
	2	141.60 Aa	145.70 Aa	21.14Aa	9.87 Bb	11.04 Aa	12.01 Aa	3.43 Ab	3.07Aab
	10	147.00 Aa	138.50 Aa	20.12 Aa	7.46 Bb	11.64 Ba	13.54 Aa	4.24 Aa	2.83 Bb
IRGA 420	0	139.20 Ab	150.60 Aab	8.47 Bb	11.49 Ba	13.23 Aa	10.44 Ba	3.76 Aa	3.68 Aa
	2	261.60 Aa	189.67 Aa	9.64 Bb	10.98 Ba	8.12 Ab	10.52 Ba	3.93 Aa	3.15 Aa
	10	122.40 Ab	114.60 ABb	15.7 ABa	11.19 Aa	8.69 Cb	10.85 Aa	3.74 ABa	3.05 ABa
IRGA 423	0	132.30 Aab	110.30 Bab	18.83 Aa	21.84 Aa	12.05 Ba	12.37 Aa	2.97 Bb	3.69 Aa
	2	140.40 Aa	130.20 ABa	12.28 Bb	16.10 ABb	10.80 Aa	12.24 Aa	3.50 Aa	3.91 Aa
	10	95.20 Bb	99.00 Bb	10.43 Bb	14.07 Ab	11.26 Ba	10.69 Ab	3.05 Bab	3.21 Aa
IRGA 424	0	119.50 Ab	160.10 Aa	11.73 Bb	5.44 Cb	12.83 Aa	11.41 Aa	3.15 ABa	2.92 Bb
	2	234.20 Aa	116.10 Bb	19.22 Aa	11.59 Ba	12.46 Aa	13.60 Aa	2.89 Ba	3.95 Aa
	10	99.80 Bb	98.80 Bb	15.49 ABab	10.57 Aa	12.14 Aa	10.80 Aa	3.55 Ba	2.72 Bb

Different capital letters indicate significant differences between rice cultivars at the same As level ($p<0.05$). Different lowercase letters indicate significant differences between As levels in the same rice cultivar ($p<0.05$)

The tissue micronutrient concentrations in early vegetative plants appeared to have a pattern of response to As exposure that was more standardized and related to that observed in rice grains. Overall, Zn, Mn and Cu concentrations decreased with increasing As levels in shoots and in rice grain tissue. Conversely, Fe decreased in the shoots of young plants but increased in grains with As exposure. Cu, Fe and Mn had the same response in roots for all cultivars at the maximum As dose after five days of exposure (Tables 7, 8, 9, 11). In plants exposed to As for ten days, a decrease in the tissue concentration of Cu was observed, whereas Fe and Mn increased even at ten days.

In root tissue, the cultivar IRGA 424 had the highest micronutrient concentration regardless of the As level added. However, in shoot tissue this cultivar was not significantly different from the other cultivars (Tables 6, 7, 8 and 9).

One of the most important factors of the effects of toxic elements (heavy metals and metalloids) on plants is their relationships with other mineral nutrients and their effects on the metabolic system (SIEDLECKA, 1995). Symeonidis and Karataglis (1992) divided the plant responses to metals into three basic groups: additive, antagonist or synergistic. Interestingly, the responses are dependent not only on the metal or heavy metal species but also on the degree of exposure.

The capacity of the tissue to promote a correct homeostasis can be described as an adaptive response. In this view, the Cu concentration and the Fe concentration in root tissues changed during the As exposure (Tables 7 and 9). For instance, after five days of exposure, IRGA 420 showed an increase of 639.56 μg in Fe root tissue concentration with increasing As levels (50 μM); however, after ten days this increase was only 192.5 μg (Tables 7 and 9).

In shoot tissues, all cultivars tested had significant reductions in Mn concentrations after five days of As treatment. Cu, Fe and Zn tissue concentrations over all were depressed upon the addition of As, with the exception of BR/IRGA 410, in relation to Cu and Fe, and BR/IRGA409 and IRGA 423, in relation to Zn. Interestingly, after ten days of exposure, all cultivars tested had reduced micronutrient concentrations in the shoots, with the exception of IRGA 424, in relation to Fe.

The concentration of nutrients in the grains of the main stem and tillers varied widely among the cultivars (Tables 10 and 11). The cultivar IRGA 424 was the only genotype that was not affected by As levels in the grains of the main stem. However, in the grains of the tillers there was an increase in the Fe concentration and a decrease in the Zn concentration.

Comparing the response of the main stem and the tillers, BR/IRGA 410 was the cultivar with the greatest contrast; the As treatment stimulated Cu, Fe and Zn concentrations

in main stem, but these micronutrients decreased in tiller tissues, with the exception of Zn. The Cu concentration was not affected at any As level in grain tissues of BR/IRGA 409. Conversely, the cultivar IRGA 423 had a significant increase in grain tissue at 2 μM As, with no significant difference between the control and 10 μM As.

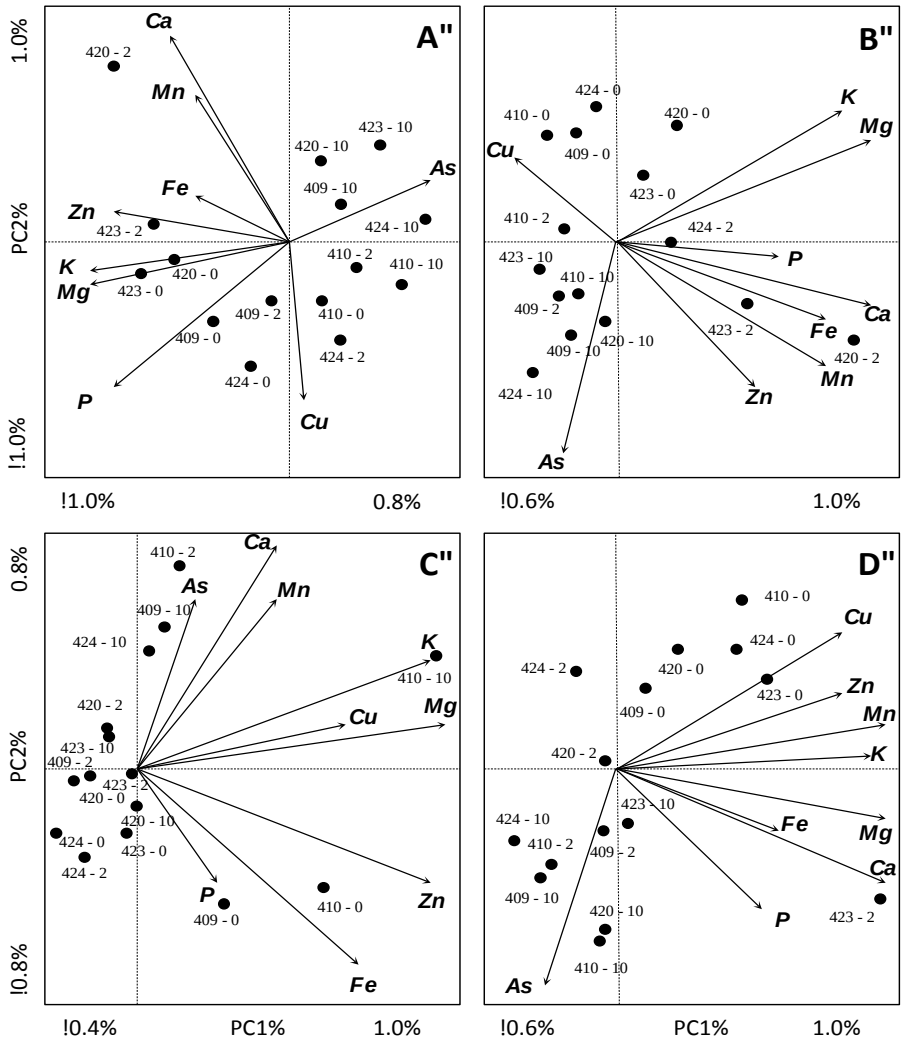
Similar to the Fe concentration, the Mn data also suggests two different groups, where the cultivars BR/IRGA 409, BR/IRGA 410 and IRGA 424 had a continuous content increase with As exposure, while IRGA 423 and IRGA 420 had a significant reduction from the 20 to 50 μM As level. The Zn concentration varied greatly among cultivars and As doses. In plants cultivated hydroponically at ten days of As exposure (Tables 6, 7, 8, and 9), IRGA 424 showed significantly higher roots micronutrient concentrations (Fe and Mn) than others cultivars for the control treatment and the maximum As dose. However, in the remaining cultivars, the micronutrients concentrations had similar responses to As exposure.

In shoot tissues, after five days of As treatment (Tables 6 and 8), most micronutrients had lower concentrations upon the addition of As, with the exception of the Fe concentration in IRGA 423. However, with the continuous exposure to As, all cultivars showed significant reductions in micronutrients concentrations. In grain tissues (Tables 10 and 11), the cultivars IRGA 424 and IRGA 423 appeared to be less affected by As exposure when compared to the other cultivars. These cultivars showed increases in the micronutrient Fe, Cu and Mn concentrations in response to As treatment, while in BR/IRGA 409, BR/IRGA 410 and IRGA 420 these micronutrients were repressed.

6.3.3 Plant clustering by multivariate analysis

The results of a PCA performed on a correlation matrix for the nutrient levels in the rice tissues suggest that the concentrations of nutrients in polished rice grains from the main culm are not good predictors of As toxicity in this experiment; conversely, there was a distinct cluster formation for As contamination in tissues from tillers (Figure 2).

Figure 2 - Biplot graphic of scores and weights (loadings) for the fisrt two principal componentes (PC1 and PC2) for mineral evaluation in rice plants exposed to different As levels.



The graphics represent the effect of As on macronutrients Ca, K, Mg, P; micronutrients Cu, Fe, Mn, Zn and As concentration in bran of polished grains (A from main culm; B from tillers) and of polished grains (C from main culm; D from tillers) of five rice cultivars (BR/IRGA 409, BR/IRGA 410, IRGA 420, IRGA 423 and IRGA 424 exposed to different As levels (0, 2 and 10 μM).

Interestingly, in bran tissues, all samples exposed to high As levels (10 μM) were related to As levels. However, polished grains from the main culm that were exposed to As were also related to Mn, Ca, K, Mg and Cu. These data support the previous results described in the present study, with a dramatic decrease in the As concentration from the bran and the grain itself (Tables 10 and 11). The manner in which contaminants and photosynthetates are distributed among the different organs and the accumulation of photosynthetates and minerals through the phloem (TANAKA et al., 2007) also explain the co-occurrence of As and some minerals in the endosperm.

The larger concentration of As in the bran fraction compared with the polished rice may have two possible causes. First, there could be a physiological barrier in the unloading and uploading process responsible for the transfer of As from the maternal tissues. As, as with many other elements, could accumulate preferentially in the protein-rich aleurone and embryo tissues, which would correspond to the formation of a distinct cluster related to As in bran tissue but not polished grains.

There was no consistent pattern of the responses among the tested minerals to As toxicity or among the different tissues. However, some similarities were observed. Regardless of the sample tissue tested, pairs of minerals, such as Zn and Fe, Ca and Mn, and Mg and K, were always clustered together.

6.4. CONCLUSIONS

Exposure to As levels higher than 2 μM was toxic to rice plants. Additionally, the nutrient concentrations in the tissue were strongly affected at higher As concentrations.

The mineral parameters were highly variable among the cultivars, with cultivars that had a high tillering capacity showing less sensitivity to excess As. However, the pattern of this variation among the cultivars was not sufficient to separate into clusters using PCA analysis. Furthermore, there were significant differences among the genotypes, suggesting differences in mineral acquisition efficiency.

Our data suggest that tissue from tillers should be used to study As toxicity in rice plants. However, further studies at the molecular level are required to clarify the mechanisms involved in the interactions among mineral nutrition, As uptake and translocation between the main culm and tillers.

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7 MANUSCRITO 6 - RELATIONSHIPS BETWEEN WATER MANAGEMENT AND ARSENIC ACCUMULATION IN RICE GRAINS UNDER DIFFERENT NUTRICIONAL LEVELS

ABSTRACT

The transport mechanisms of arsenic (As) from contaminated soil or irrigation water into roots and subsequently into grain, are critical for assessing health risks imposed by As contamination as well as to grain nutrient quality. In an effort to minimize As uptake by rice grains, field experiments were conducted to investigate As accumulation in rice grains of two rice cultivars under different water management practices and phosphorus fertilization (P). Results indicated that As concentrations in rice grains was cultivar-dependent and influenced by water management when grown aerobically but with no effect of water suppression during short periods.

Key words: arsenate, contamination, grains, irrigation, phosphorus

7.1 INTRODUCTION

Arsenic (As) is listed as one of the top health hazards by the International Agency for Research on Cancer, which is linked to bladder, lung, skin, and prostate cancers (NATIONAL RESEARCH COUNCIL, 2001). Human exposure to As is primarily through the intake of drinking water and foods, such as rice grains with elevated As contents (MARIN et al., 1993).

In addition to As accumulation in the tissues and grains, high As levels in soil and irrigation water would also hinder root growth, decrease plant height, and reduce grain yields (ABEDIN et al., 2002). As a result, As accumulation in rice plants would have profound adverse impacts on the quality, security, marketability, and profitability of rice products.

The interactions of As species with naturally occurring minerals in soil have strong influences on the As bioavailability and mobility in the environment. Understanding such interactions would be critical to minimize As uptake by rice plants in the fields. In contrast to most recent studies focusing only in inorganic As, this study was to investigate and compare As uptake by rice cultivars in the soils with different levels of phosphorus (P), iron (Fe) and water managements and its relationship with grain yield.

Knowledge of the processes of transformation of the organic fractions of this element, considering the effects and interactions of cultures and managements adopted contributes to the understanding of soil-plant dynamics in various cropping systems and to identify the mechanisms involved in phosphorus dynamics.

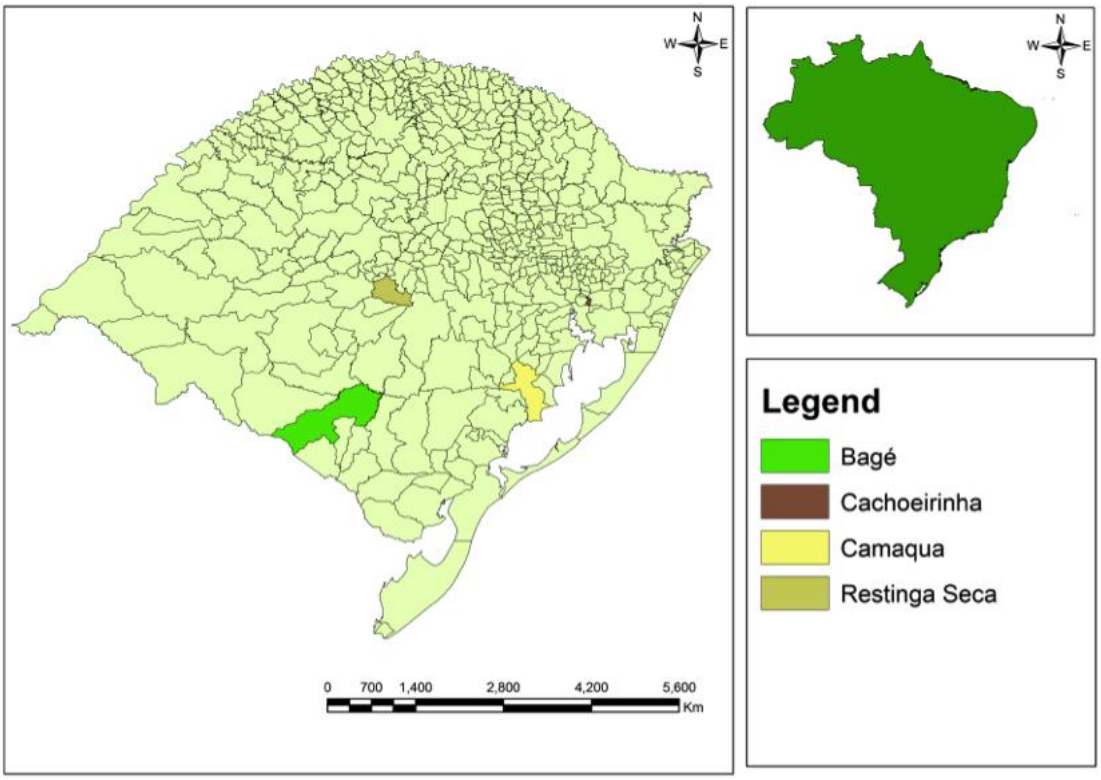
Given the above, this study aimed to evaluate the impact of water management and P fertilizer in As accumulation as well as to evaluate the physiology of two contrasting cultivars in terms of Fe susceptibility against different levels of this nutritional disorder and soil fertility in different localities.

7.2 MATERIALS AND METHODS

7.2.1 Experimental system

The experiment was conducted at four locations in Southern Brazil at the State of Rio Grande do Sul (Figure 1). Two different systems were tested: I) water management through water suppression in three different areas with phosphorus levels; II) water management trough the use of irrigation with water blade formation or center pivot system.

Figure 1 - Experiment locations in Southern Brazil. Experiment I Cachoeirinha, Camaquã and Restinga Seca; experiment II Bagé.



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7.2.1.1 Experiment I

This experiment was carried in three locations, Cachoeirinha, Camaquã and Restinga Seca (Figure 2), varying the Fe content in soil. Restinga Seca with high available content Cachoeirinha and Camaquã, intermediate levels (Table 1).

The following treatments (Figure 2) were used: T1 - continuous irrigation with 5cm blade height beginning at V3-V4 growth stage until R6 growth stage; T2 - irrigation with a water suppression between the V6-V8 stages, with the restoration of the blade until the R6 stage; and T3 - irrigation with two water suppressions, the first between the V6-V8 stadiums reestablishing the water blade and starting the second suppression between V8-V10, with restoration of irrigation until the R6 stage.

It was used two contrasting cultivars regarding Fe toxicity tolerance: IRGA 425 (tolerant) and BR-IRGA 409 (susceptible). The phosphorus fertilization was made in increasing doses of 0, 75, 150, 600, 1200 Kg P_2O_5 ha^{-1}. Nitrogen fertilizer in form of urea (350 kg ha^{-1}) was applied 5% on planting, 50% as topdressing fertilizer for promoting tillering (applied at V3-V4 stadiums) (Counce et al., 2000), 45% for promoting panicle initiation (applied at V9 or R0) (Counce et al., 2000; Sosbai, 2010).

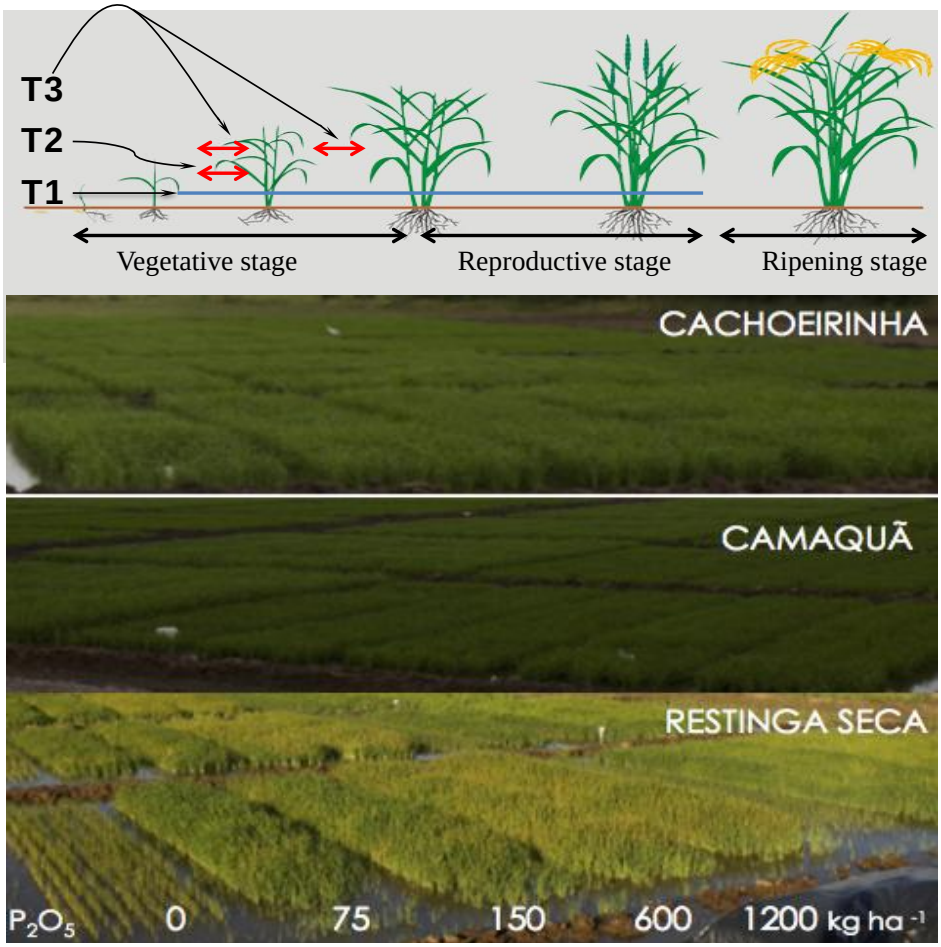
All P and potassium (K), respectively fertilizer were used during the seeding. The experimental design was a randomized blocks in sub-sub-divided plots with three replications. Irrigation treatments were the main plots (10 x 17m - 170 m^2), the sub-plots cultivars (10 x 8.5 m - 85 m^2 each) and sub-sub-plots, the P_2O_5 doses (10 x 1.7 m - 17 m^2 each). Each crop frame was isolated with individualized levees for the irrigation treatment. The water used for irrigation has been provided by gravity, with side leads to the experimental units, in order to irrigate portions according to the proposed treatments. The irrigation with blade formation started during V3-V4 stages in all treatments, after the first nitrogen fertilization. At the end of the cycle rice grains were harvest and the results of grain yield were adjusted for 13% moisture.

7.2.1.2 Experiment II

The following treatments were used: T1 - continuous irrigation with 5cm blade height from V3-V4 growth stage until R6 growth stage; T2 - continuous irrigation with the maintenance of 70-80% of field capacity trough center pivot system during the whole plant cycle with 60 Kg P_2O_5 ha^{-1}. It was used the cultivar BR-IRGA 409. Nitrogen fertilizer in form

of urea (340 kg ha^{-1}) was applied 5% on planting, 50% as topdressing fertilizer to promote tillering (applied at V3-V4 stadiums) (Counce et al., 2000), 45% to promote panicle initiation (applied at V9 or R0) (Counce et al., 2000; Sosbai, 2010). All P and K fertilizers were added during the seeding.

Figure 2 - Practical scheme of irrigation systems used and overview of three localities evaluated



At the top of the figure, a practical scheme of irrigation systems used: Continuous irrigation (T1), one water suppression between V6-V8 stages (T2) and two water suppressions between V6-V8 V8-V10 (T3). Below an overview of three localities evaluated.

7.2.2 Soil analysis

The particle size distribution of the soil constituents was analyzed using the pipette method (EMBRAPA 1997). The soil pH in water was determined using a 1:1 ratio according to the methodology of Tedesco et al. (1995). The soil OM content was analyzed by wet oxidation using potassium dichromate in a sulfuric acid medium (0.4 N) followed by titration

with 0.1 N ammonium ferrous sulfate according to EMBRAPA (1997).

Poorly crystalline iron oxides were extracted in the dark using ammonium oxalate {[$(NH_4)_2$ $C_2O_4.H_2O$]} at 0.2 mol/L adjusted to pH 3.0. Following the removal of the supernatant, the iron concentration was determined by atomic absorption spectrometry (TEDESCO et al., 1995). Soil K and plant-available P concentrations were extracted using Mehlich 1 (0.05 mol/L HCl + 0.0125 mol/L H_2SO_4) in a soil/solution ratio of 1:10. The P concentration was determined using the Murphy and Riley (1962) method, and the K concentration was determined by flame spectrometry (B262 Micronal; Micronal S.A., São Paulo, Brazil). Exchangeable cations (Ca^{2+}, Mg^{2+}, and Al^{3+}) were extracted with a 1 mol/L KCl solution (EMBRAPA, 1997). The concentration of Al^{3+} was determined by an acid–base titration with a 0.0125 mol/L NaOH solution (TEDESCO et al., 1995).

Table 1 - Chemical and physical properties of soils used for rice production.

Parameters	Cachoeirinha	Camaquã	Restinga Seca	Bagé
Clay (%)	17.00	18.00	21.00	20.00
pH H_2O	5.00	7.00	5.20	5.80
P mg dm^3	27.00	30.5	2.90	35.00
K mg dm^3	35.00	125.00	60.00	88.00
MO (%)	1.5	0.8	1.9	2.1
Al $cmol_c$ dm^3	0.6	0.0	1.7	1.2
Fe oxalate mg kg^{-1}	13.00	9.00	115.00	11.00

7.2.3 Tissue elements analysis

For As speciation freeze-dried milled rice was weighed accurately to a weight of 0.1g into 50mL polypropylene centrifuge tubes to which 2mL of 1% conc. Aristar nitric acid was added and allowed to sit overnight. Batches of up to 48 samples were prepared which also include d 2 blanks and 2 rice CRM (NIST 1568b Rice flour) that has the arsenic species As_i and dimethylyarsonic 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 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 10mL with deionized distilled water and centrifuged at 3,500 rpm for 15 min.. A 1 mL aliquot was transferred to a 2mL polypropylene vial and 10µl 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^{o}C in 5 min. hold for 10 min. to 135^{o}C then hold for 10 min., to 180^{o}C then hold for 30 min.) was employed, with 2mL of concentrated Aristar nitric acid and 2mL hydrogen peroxide added and left to soak overnight before microwaving. 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 analysed.

7.2.4 Biochemical Analysis

At the begging of the reproductive phase (R1), it was collected whole rice plants. For each block it was collected 9 plants totaling 27 rice plants per cultivar/treatment. These analyses were only performed for the location of Restinga Seca at the P doses of 0, 75 and 1200 P_2O_5 ha^{-1}. The samples were separated into root and shoot, and the shoot subdivided into main culm and tillers. The flag leaves were immediately frozen in liquid N_2 and then stored at -20 °C. The roots were collected in the form of blocks with soil. The soil block was partially air-dried, than the soil from rhizosphere was collected and stored in frezer at -20 °C.

7.2.4.1 Acid Phosphatases in soil

The determination of the acid phosphatase activity was performed according to method of Tabatabai and Bremner (1969) described below: Soil samples (1.0 g) placed in duplicate into 50 mL Erlenmeyer flask using a control which was only added to the substrate after incubation. Then were added 4,0 mL MUB solution of pH 6.5 and 1.0 mL of ρ-nitrophenyl phosphate substrate 0.05 M. flasks were closed and incubated at 37 ° C for one hour. After incubation, was added 1.0 mL of 0.5 M CaCl2, 4.0 mL 0.5 M NaOH and 1.0 mL of ρ-nitrophenyl phosphate 0.05 M (only for controls), proceeding then the filtering on filter paper No. 02 at 37 ° C for one hour. After incubation, was added 1.0 mL of 0.5 M CaCl2, 4.0 mL 0.5 M NaOH and 1.0 mL of ρ-nitrophenyl phosphate 0.05 M (only for controls), proceeding then the filtering on filter paper No. 02.

The intensity of the yellow color of the filtrate was determined in a spectrophotometer at 410 nm. The amount of ρ-nitrophenol formed in each sample was determined based on a standard curve prepared with known concentrations of ρ-nitrophenol (0, 10, 20, 30, 40, 50 mg of ρ-nitrophenol mL-1). Enzyme activity was expressed in g of ρ-nitrophenol released per hour per gram of soil (g ρ-nitrophenol h-1g-1solo).

7.2.4.2 Acid phosphate (EC 3.1.3.2) in leaf tissue flags

The acid phosphate activity (APA) was determined in flag leaves according to Tabaldi et al. (2007). Leaves were frozen in liquid nitrogen and stored at -20 °C. Subsequently, leaves were manually ground in liquid nitrogen and put in the reaction medium consisted of 3.5 mM NaN 3, 2.5 mM NaCl and 100 mM citrate buffer (pH 5.5) to a final volume of 200 uL. The inorganic phosphate was measured at 630 nm in a spectrophotometer SF325NM (Bel Engineering, Italy) using malachite green as a reagent and KH_2PO_4 colorimetric standard calibration for the curve.

7.2.4.3 Lipid peroxidation

Lipid peroxidation of plant tissues was measured in reactive substances thio-barbituric acid (TBARS) using the method described by El-Monshaty et al. (1995). A sample was mixed solution 1/2 thiobarbituric acid (5%) and 1/2 of trichloroacetic acid (20%). The mixture was heated in a water bath at 95 ° C for 30 min and the reaction was stopped by abrupt placed on an ice bath. The mixture was centrifuged at 10,000g for 10 min and the absorbance of the supernatant was read at 532nm and 600nm lengths.

7.2.5 Statistics

The grain yield results were divided between local and irrigation schemes, and subsequently submitted to regression analysis, and adjusted according to the highest coefficient of regression. The statistical significance of the correlation coefficients to a level of 5% was considered. The best-fitted model was sigmoidal with three parameters. The biochemical parameters were submitted to a tree way anova.

7.3 RESULTS AND DISCUSSION

7.3.1 Grain yield

It was observed three double interactions for the grain yield parameter in experiment I. The observed interactions were: Local x Irrigation. Local x Cultivars and Local x P_2O_5 dose

(Tables 2, Table 3 and Table 4), but no difference occurred in grain yield between water treatments on experiment II (Figure 4).

Cachoeirinha was the only location where the irrigation system with one or two suppressions resulted in an increase of grain yield (Table 3; Figure 3). Interestingly, Camaquã and Restinga Seca had no response to water management (Table 1; Figure 3). This response to land use with water suppression was more pronounced for IRGA 425 cultivar, which had its highest productivity in Cachoeirinha (Table 2); and with the exception of Camaquã city, it had higher grain yield production as compared to BR-IRGA 409 (Table 2; Figure 3).

Table 2 - Effect of cultivar on rice grain yield in three locations of Rio Grande do Sul state.

Local	BR-IRGA 409	IRGA 425
Cachoeirinha	7.39 Bb	10.28 Aa
Camaquã	8.61 Aa	8.79 Ab
Restinga Seca	4.44 Bc	6.62 Ac

Means followed by lowercase letters indicate comparison among locations within the same cultivar, whereas capital letters indicate comparison between cultivars for the same location. Tukey, $\alpha = 0.05$.

Water stress as a result of water deprivation can affect plants in anatomical, morphological and physiological levels, impacting on aspects of growth and development (KUCHAKI and SOLTANI, 1997). Water deprivation reduces the number of tillers, leaf area, dry matter accumulation, grain number filled per panicle, the 1000 seeds and finally due to the reduction of solutes and transport of nutrients, it reduces photosynthesis rate (REZAEE and NAHVI, 2007). However, this study did not cause situations of water deprivation, occurring only the removal of the water blade, while maintaining adequate soil moisture.

Thus, these anaerobic soil conditions favor the accumulation of organic matter (OM) because of the slower rate of decomposition of organic substrates (WITT et al., 2000; SAHRAWAT, 2004). The efficient use of accumulated OM can be profitable by improving rice production, since the removal of the blade of water for rice cultivation results in acceleration of the decomposition of the OM, and greater supply of mineralized nutrients to plants (BIRCH, 1958; VENTURA and WATANABE, 1978; KUNDU and LADHA, 1995).

The effect of drying the soil, followed by re-wetting it, with intermittent irrigation on rice growth, is more important when the rice crops are in continuous submerged conditions, or are poor drainage and high in OM content (VENTURA and WATANABE, 1978; KANKE

and KANAZAWA, 1986). In this regard it should be noted that the soil collected in Camaquã showed about half OM as compared with other localities, and neutral pH (Table 1) and it may lead to a lower management system impact on plants cultivated in this locality.

Table 3 - Effect of water management on rice grain yield in three locations of Rio Grande do Sul state.

Local	Water management		
	Continuous irrigation	One water suppression	Two water suppressions
Cachoeirinha	5.91 Bb	10.18 Aa	10.42 Aa
Camaquã	8.75 Aa	8.63 Ab	8.74 Ab
Restinga Seca	5.18 Ac	5.66 Ac	5.75 Ac

Means followed by lowercase letters indicate comparison among locations within the same irrigation system, whereas capital letters indicate comparison of the irrigation systems for the same location. Tukey, $\alpha = 0.05$.

Aside from the above, the water suppression can also favor the production of rice by suppressing the toxicity of elements such as Fe and increasing the available nutrients, such as P, to rice plants (van BREEMEN MOORMAN, 1978; DOBERMAN, 2004) and reducing the uptake and accumulation of toxic elements in grains such as As (ref). Some lowland soils are described as acids, with high amounts Fe and with low available P, thus resulting in a high potential for Fe toxicity to rice plants grown under continuous submerged conditions (VIZIER, 1990) as observed in Restinga Seca field (Figure 2).

Table 4. Effect of phosphorus increment on rice grain yield in three locations of Rio Grande do Sul state.

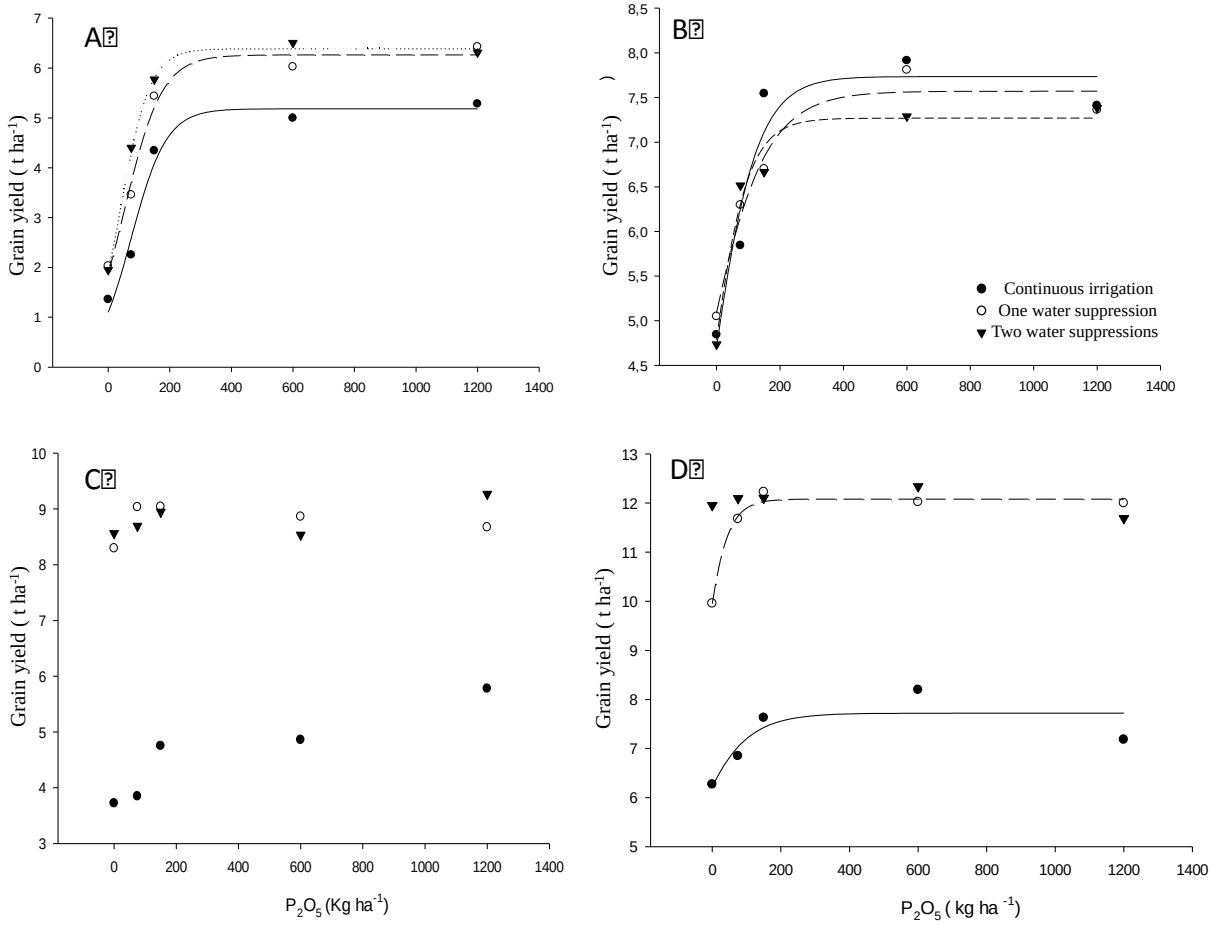
Local	P_2O_5 Kg ha^{-1}					
	0	75	150	600	1200	
Cachoeirinha	8.14 a	8.70 a	9.11 a	9.13 a	9.10 a	yield = 8.1219+1.0162*(1-exp(-0.0137*P dose))
Camaquã	8.55 a	8.53 a	8.82 a	9.06 a	8.57 a	
Restinga Seca	3.33 b	4.79 b	6.76 b	6.76 b	6.70 b	yield = 3.2742+3.4838*(1-exp(-0.0091*P dose))

Means followed by lowercase letters indicate comparison among locations within the same dose of P_2O_5. Tukey, $\alpha = 0.05$.

It should be emphasized that although classified as belonging to the same class of soil, Planosol Haplic, some peculiarities among the locations were crucial to the production of grains. Among them, besides the toxicity of history by Fe Restinga Seca, this location presented the P content in the soil prior to installation of the experiment 10 times lower than that observed in other locations, also having the highest values of Al content. Moreover

Camaquã showed the highest content of P and potassium concentration (K), more than two fold higher than in other locations and with no Al detected (Table 1).

Figure 3 - Rice grain yield under different water managements and locals, A, B Restinga Seca; C, D Cachoeirinha, and cultivars A, C BR-IRGA 409 and B, D IRGA 425.

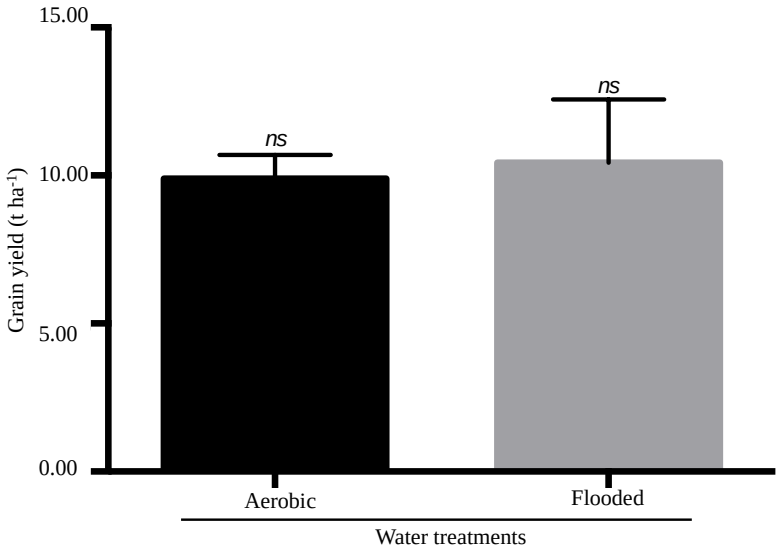


There are many factors that determine the productivity of a culture. The genetic material and soil fertility are decisive, but their effectiveness are modulated by secondary factors such as pH, climate and presence of potentially toxic elements (BIELESKI, 1973; MARSCHNER, 1995). For this same purpose, the productive potential is reached on ideal conditions, where the nutritional requirements are achieved and no external stresses occur. Thus, as the lower compliance of the nutritional requirement and greater stress is generated, greater is the response observed with the production of any of these attenuation factors. In the case of Fe toxicity, where it has a strong relationship with the plant nutritional status and the

whole P availability in both plant cell metabolism and soil (BIELESKI, 1973; MARSCHNER, 1995), this response is even more striking.

In this scenario, when comparing two different locations such as Restinga Seca and Cachoeirinha, one with low P and high Fe and another with higher levels of P and low Fe, the highest response observed is probably the limiting factor with the greatest impact. In Cachoeirinha irrigation it was crucial for the production while in Restinga Seca this parameter was not significant thus occurring a continuous response to increasing P (Table 3, Table 4, Figure 1, Figure 3).

Figure 4 - Rice grain yield under different water managements, aerobic, with center pivot irrigation; and Flooded.

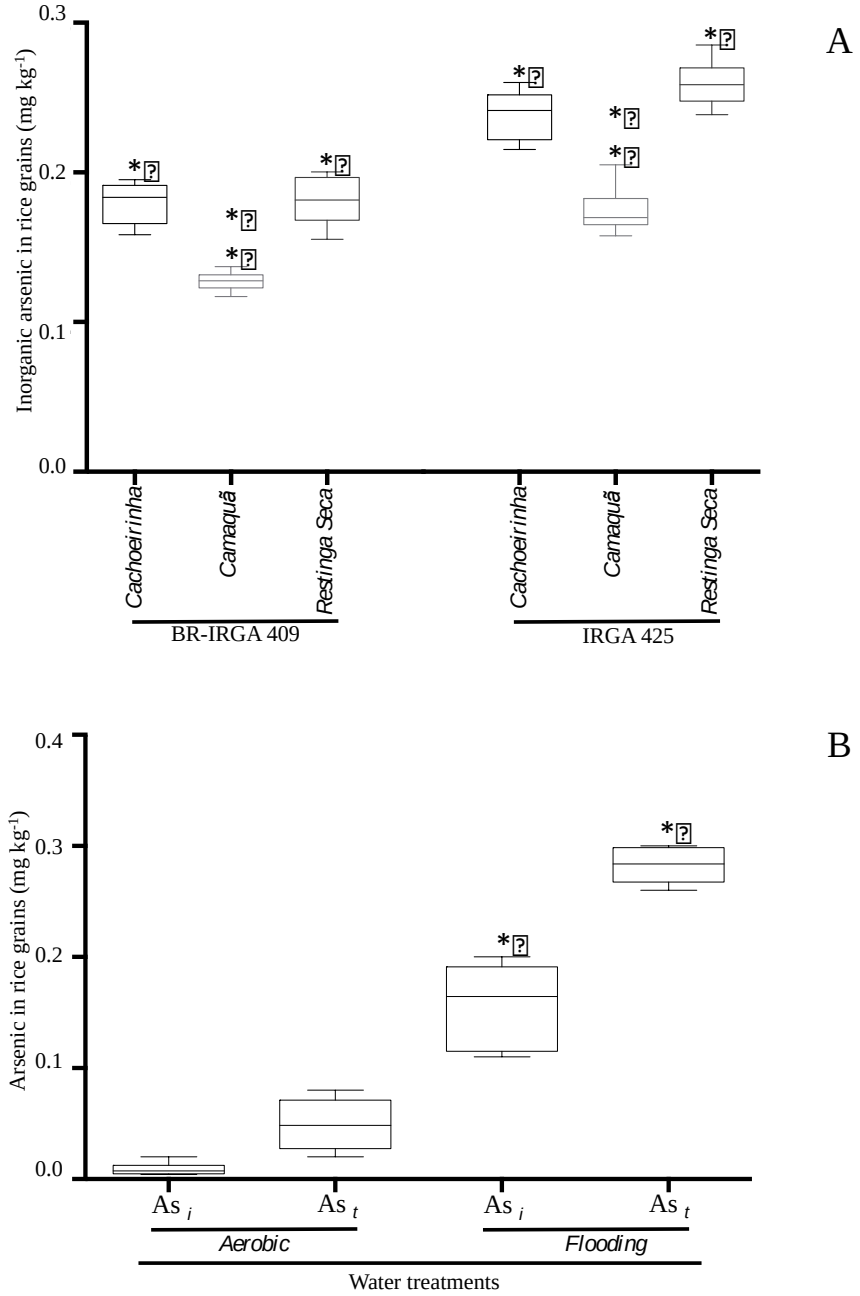


7.3.2 Arsenic in rice grains

Works have shown that flooding treatment increase the concentration of As in rice grain and straw (XU et al., 2008; NORTON et al., 2013; HU et al., 2014; MORENO-JIMÉNEZ et al., 2014). This occurs because under flooding conditions the redox potential of the soil decreases and the As concentration in the soil solution increases (MASSCHELEYN, et al., 1991; TAKAHASHI et al., 2004; HAMON et al., 2004). However, in the present study, water suppression did not altered As concentration in rice grains (Figure 5) under the tested

treatments on experiment I; conversely aerobic treatment reduce As concentration in 15 fold in experiment II as compared to the flooding treatment (Figure 5).

Figure 5 - Arsenic in rice grains of experiment I and II



Experiment I (A), under different locals, Cachoeirinha, Camaquã and Restinga Seca; and cultivars BR-IRGA 409 and IRGA 425. *significant difference between cultivars and ** significant difference among cultivars and locations. Experiment II (B), under different water treatments, aerobic, with center pivot and flooded.* significant difference between water treatments.

Two aspects have to be consider for these responses: I) development stage of rice plants during the water suppressions and II) water suppression duration. In our work in experiment I both treatments of water suppression occurred during vegetative stage and only for a short period of time (Figure 1). Since As translocation in rice grains mainly occur after the R2 stage (CAREY et al., 2010; CAREY et al., 2011) and previous works showed As reduction in grains after 3 weeks of intermittent water management (NORTON et al., 2013; HU et al., 2014; MORENO-JIMÉNEZ et al., 2014), it is possible to say that our treatments weren't suitable for this proposal. On the other hand, the irrigation with center pivot of experiment II allowed an aerobic condition during the whole plant cycle thus resulting in a major reduce of As in grain as described in previous reports (XU et al., 2008).

An important data from this study, was the cultivars differences in As accumulation in grains, even though BR-IRGA 409 cultivar showed a lower rice yield as compared to IRGA 425 (Figure 3), the fact that this cultivar translocate less As to grains show how important tool plant breeding can be for the achievement of food security. Other interestingly aspect was the P increment, which although with contrasting doses, the P fertilizer had no significant difference in grain yield in fields with prior high P levels (Camaquã and Cachoeirinha, with 30.5 and 27.0 mg P dm^3 $soil^{-1}$ respectively), though with differences in Restinga Seca (2.9 mg P dm^3 $soil^{-1}$) field which had the lowest P values and highest Fe values prior the experiment (Table 1, Table 4). Thus there was no differcences in As accumulation in grains among the tested P levels (Figure 5).

Studies with As speciation data in the soil solutions suggests that arsenite would be the main form of As taken up by flooded rice, and arsenate the main form taken up for aerobic rice (XU et al., 2008; NORTON et al., 2013). This is consistent with the finding that addition of phosphate, which is expected to inhibit the uptake of arsenate but not arsenite, had no significant effect on As accumulation by flooded rice in pot experiments (ABEDIN et al., 2002).

Rice roots release oxygen to the rhizosphere, resulting in the oxidation of ferrous iron and the formation of the iron plaque on the root surface, which has a substantial capacity to retain As. Much of the adsorbed As on the rice iron plaque appears to be arsenate (LIU et al., 2006). Thus, oxidized species, arsenate, is much more strongly adsorbed by the iron plaque than arsenite (CHEN et al., 2005); consequently, arsenite that remains unoxidized in the rhizosphere solution may be more readily taken up by rice roots. The lack of a significant correlation between the amount of iron plaque formed and As accumulation by different rice genotypes (LIU et al., 2006) suggests that the ability of roots to take up arsenite and/or root to

shoot translocation may be the critical steps controlling As accumulation in flooded rice.

7.3.3 Biochemical analysis

The application of P can improve the nutritional quality of rice (HAO et al., 2009) as well as physical parameters (CAO et al., 2001; HU et al., 2005; WANG et al., 2010) protein content and cooking quality (HAO et al., 2009). Conversely, in our study P increment affected acid phosphatases (Apase) in flag leaf tissues (Figure 6), acid phosphates in rhizosphere soil (APA) (Figure 7) and lipid peroxidation (TBARS) (Figure 8) in flag leaf tissues; but no changes in As concentration in rice grains as prior described.

Research has linked the onset of symptoms of Fe toxicity to nutrient deficiency, such as P, K, Ca, Mg and Zn (FAGERIA et al., 2008; NAGAJYOTI, 2010). The enhancement of the appearance of toxicity symptoms due poor mineral nutrition of plants may occur by the collapse of the oxidative capacity of the roots (BENCKISER et al., 1984). In this sense, an adequate supply of elements such as P may help through increasing the roots Fe exclusion capacity (WIN and LUNT, 1967).

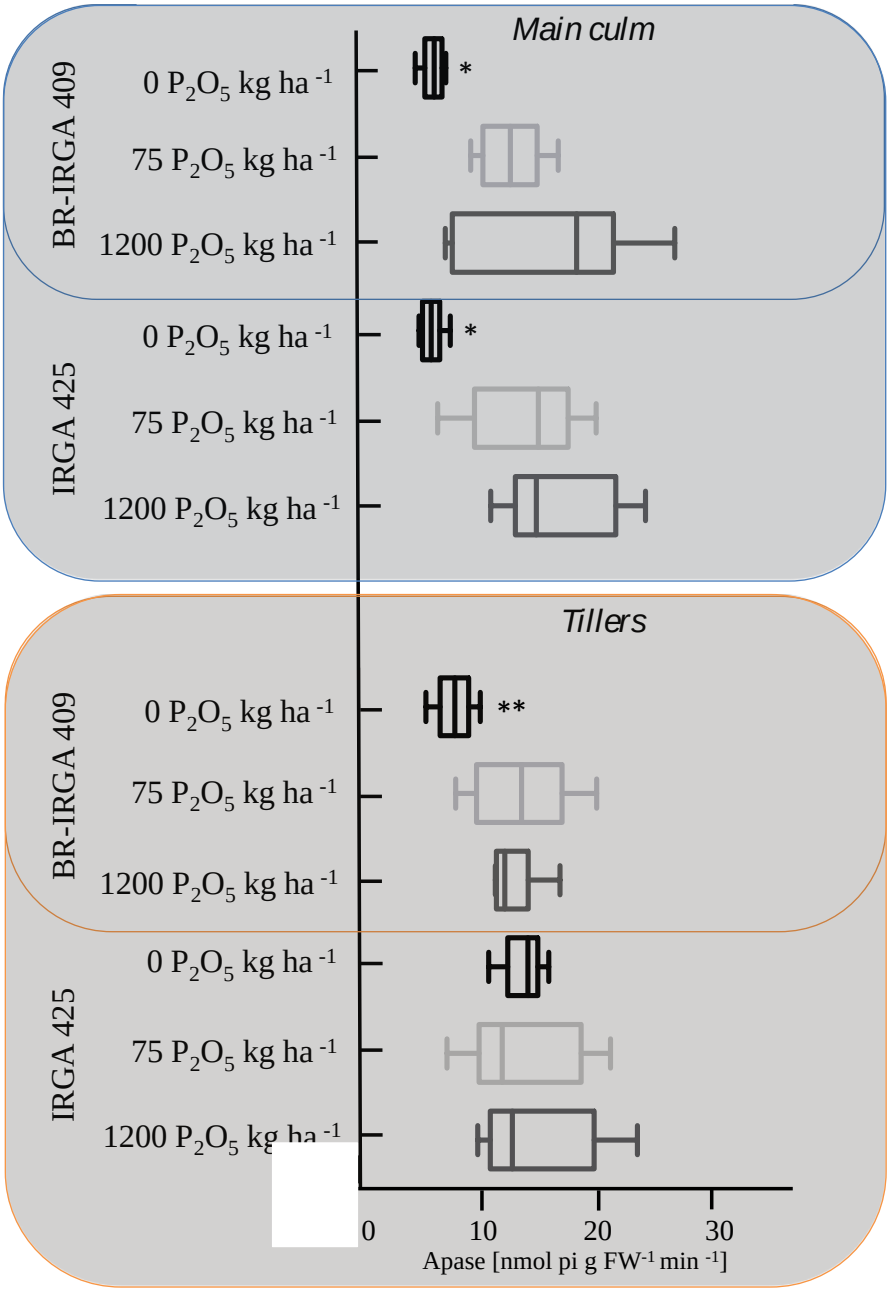
The Apases activity on flag leaves, overall, increased with P addition in main culm leaves of IRGA 425 and both main culm and tillers leaves of BR-IRGA 409 (Figure 6), therefore, the grain yield (Figure 6, Figure 9). It suggests that in flag leaf tissue this enzyme may be linked to metabolic activity and production of photosynthates, even though other works relate it with P mobilization under P starvation (DUFF et al., 1994).

Unlike what was observed in the Apase activity, in soil samples our study is in agreement with the literature (CONTE et al., 2002) regarding APA activity, with higher activity in treatments with no P added in all locations tested regardless of the irrigation system (Table 7); but with a higher activity under one and two water suppressions as compared to continuous irrigated system.

When a soil under flooding system is drained oxidation of reduced constituents occurs as well as changes in pH and Fe chemestry, decreasing the solubility of both the native soil P and the applied P (SAYAL and De DATTA, 1991), thus impacting the biological activity in plant and soil. Tsujimoto et al. (2010) observed an increase in N uptake by rice plants as well as substantial increases in biomass production in soil where he was promoted drying soil in the period prior to planting, in relation to soil permanently flooded. Intermittent irrigation can increase microbial activity and decomposition of MO, followed by an increase in N

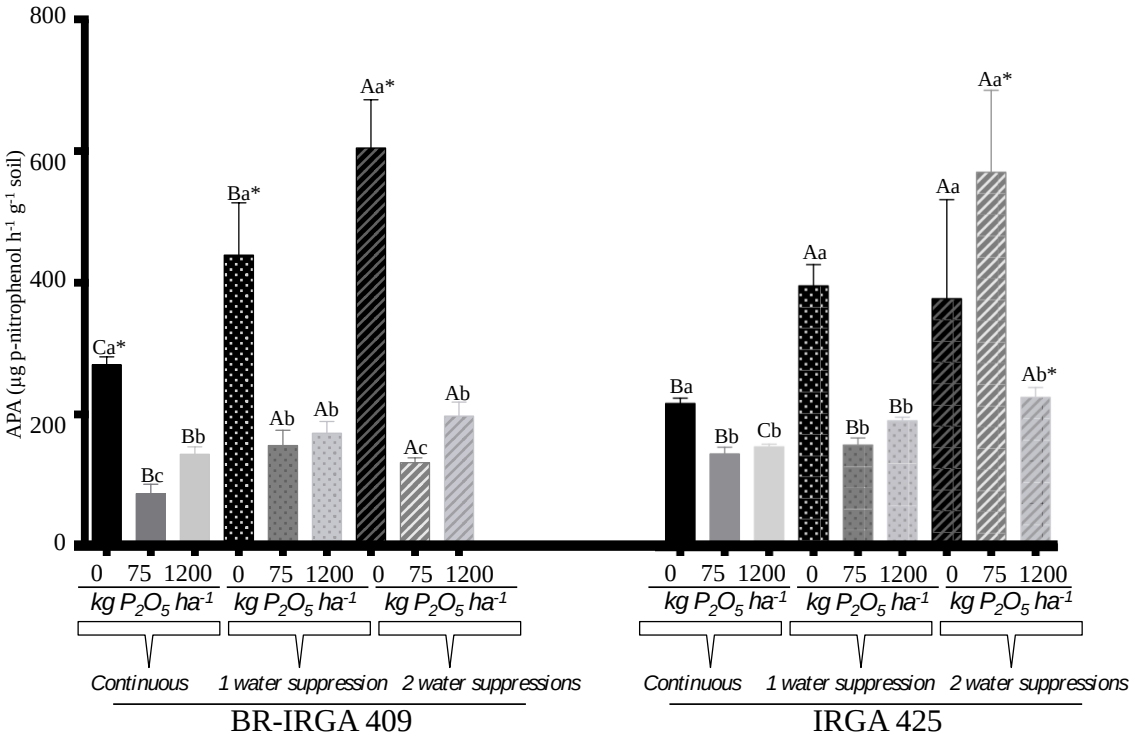
mineralization (BIRCH, 1958; MARUMOTO et al., 1977). This correlation was also observed in crops without fertilizer (RUSSELL et al., 2006).

Figure 6 - Acid phosphatase activity (Apase) in main culm and tillers flag leaves under different phosphorus levels and cultivars BR-IRGA 409 and IRGA 425.



*significant difference among phosphorus levels and ** significant difference among phosphorus levels and cultivars.

Figure 7 - APA activity under different phosphorus levels, irrigation system of the cultivars BR-IRGA 409 and IRGA 425.



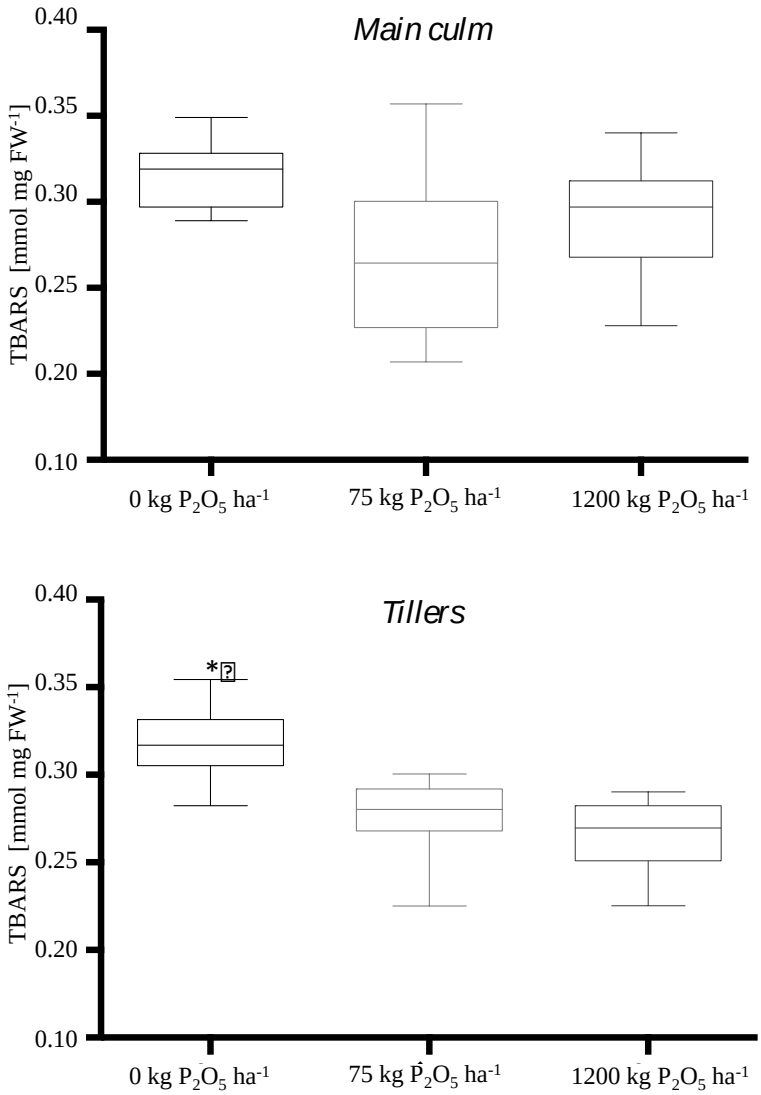
Means followed by capital letters indicate comparison among irrigation system within the same phosphorus level and cultivar, whereas lowercase letters indicate comparison among phosphorus level, within the same irrigation systems and cultivar. And * compare cultivars with the same irrigation system and phosphorus level. Tukey, α = 0.05.

Drying intermittent irrigation of soil and also have positive effects for the absorption of other nutrients. Increases in uptake of P in soil drying relative to continually flooded soils are also described in the literature (TURNER and HAYGARTH 2001, 2003). Sparling et al. (1985) and Turner and Haygarth (2001) described a linear relation between the microbial soil P levels and P increases water soluble, with the soil-drying effect. Simonsson et al. (1999) reported a significant increase in extractable content of Si with ammonium oxalate after drying and rewetting the soil. Increases in the concentration of such soluble and extractable components were related to the mineralization of the lysed microbial cells and disrupting organic material coverings, clay and mineral surfaces caused by physical and biological

damage during soil drying and rewetting process (MARUMOTO et al., 1977;. CABRERA, 1993; TURNER and HAYGARTH, 2001).

As for the oxidative damage, measured by thiobarbituric reactive substance (TBARS), there was a higher concentration tiller tissues under low P as compared to the other treatments (Figure 8), with a negative relationship with grain yield (Figure 10).

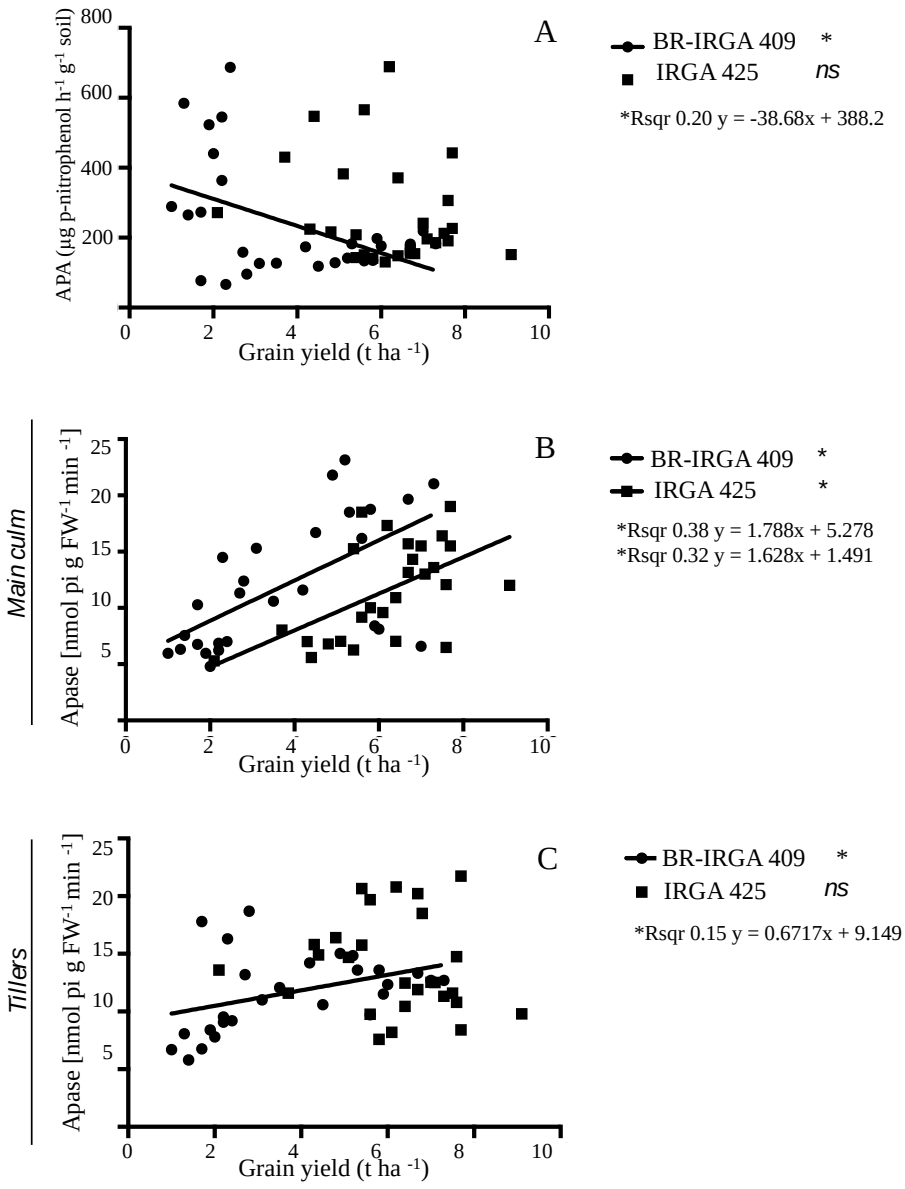
Figure 8 - TBARS concentration in main culm and tillers flag leaves under different phosphorus levels.



*significant difference among phosphorus levels.

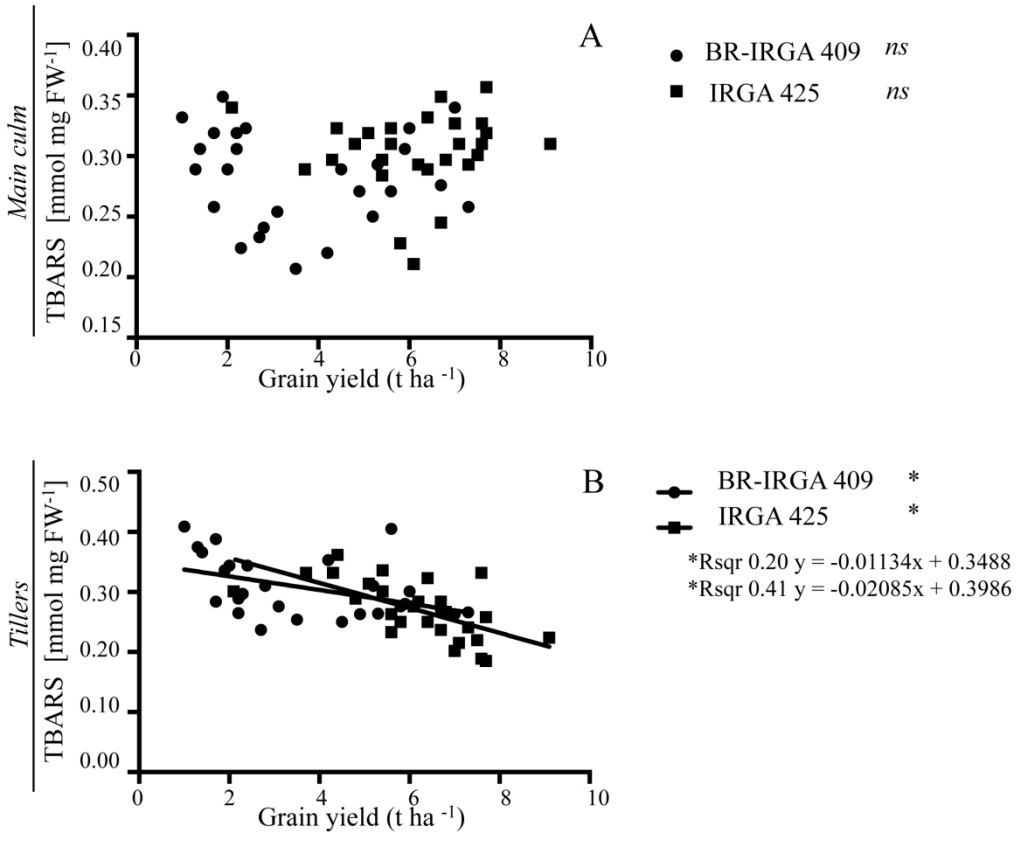
Contrary to expectations, there was no difference in TBARS among the evaluated locals, and P levels in main culm tissue, and no relation with grain yield was observed (Figure 10). Similarly, Stein (2009) found that susceptible cultivars, including BR-IRGA 409, and tolerant Fe showed no increase in the concentration of TBARS in areas with Fe excess as compared to control areas. However, it was reported a significant increase in protein oxidation in plants grown at high Fe relative to control plants. These data suggest that assessment Fe, being suitable primarily for evaluation of protein oxidation.

Figure 9 - Rice grains yield relationship to phosphatase activity in soil (A), Main culm flag leaf (B) and tillers flag leaf (C).



Another factor to be taken into account is the plant tissue used for this study. The last three expanded leaves in rice plants, especially the flag leaf had the highest contribution to grain yield (RAY et al., 1983;. MISRA, 1986). Greater translocation of carbohydrates from vegetative parts of spikelets (MISRA, 1986; SONG et al., 1990) and greater leaf area index (LAI) during the grain filling period. Flag leaf has an important role in rice production, increasing grain yield by 40%. The leaf senescence during the reproductive stages and maturity is directly related to biomass production and yield of rice crop grain (RAY et al., 1983;. MISRA et al., 1997). In addition, during leaf senescence, chlorophyll content also decreased, but the rate of decline is much slower than the content of Rubisco. In cases of abiotic stress, rice plants the can abort some leaves or even tillers, while keeping the leaves flags which are also those that remain green longer during the growing season (MAKINO et al., 1983;. DILNAWAZ et al. , 2001).

Figure 10 - Rice grains yield relationship to TBARS in Main culm flag leaf (A) and tillers flag leaf (B).



These physiological aspects help explain why we did not observe standards of linear responses at concentrations of Apase and TBARS in response to the level of fertility and availability of Fe in the flag leaf tissues. Because one has to consider all involved metabolism.

7.4 CONCLUSIONS

Apparently the local effect, ie the soil used and the set of environmental factors was higher than the other factors tested for grain production in experiment I. Thus, the response to water managements and fertilization was dependent on the local factor.

Moreover intermittent irrigation and P fertilizer affect Apase, APA and TBARS, however it didn't resulted in changes on As concentration in rice grains. On the other hand aerobic growth had a massive impact reducing As concentration in grains.

The two tested cultivars showed distinct response in grain yeld and As translocation to grains, with the heist grain yield production observed for 425 cultivar and the lowest As translocation in BR-IRGA 409.

Even though the water suppressions did not altered As concentration in grains, this management may be important for better use of water, special in southern Brazil where quite often drought occurs at during the rice growing season, considerably reducing water sources from rivers and lakes. It is also important to reinforce that P increment over the agricultural indication, overall didn't altered both As in grain and grain yield, thus showing that this element should be use with caution and without abuses.

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8 DISCUSSÃO

No presente trabalho, entre as amostras testadas, o Brasil foi o único país da América do Sul com concentrações de As_i acima do limite da UE, para alimentos destinados à bebês e crianças pequenas (0,10 mg As_i kg^{-1}). No entanto, houve variação considerável nas concentrações entre as regiões avaliadas (Figura 3, manuscrito 1). As regiões Norte e Nordeste apresentaram 100% de amostras dentro dos limites enquanto a região sul, principal região produtora, teve apenas 80% das amostras apropriadas. Conforme discutido anteriormente, a toxicidade do As depende de sua forma química. Para As_i, a dose letal para 50% dos ratos (DL50) variou de 15 a 293 mg kg^{-1} peso corpóreo (VALLEE, 1960). Para os seres humanos, 70-80 mg de DMA foi reportado como fatal (APOSHIAN, 2006).

A via oral é a principal via de exposição ao As_i. Neste sentido, no Brasil, o arroz é considerado alimento base, sendo uma importante fonte de exposição. O consumo médio de arroz no Brasil é de 70 g dia^{-1} $pessoa^{-1}$ (FAO, 2008), dos quais 80% é o arroz polido, entretanto esta grandeza pode variar é até quatro vezes, dependendo do status social e região avaliada (IBGE, 2010). Outro fator a ser considerado é que o arroz é apenas uma das diversas fontes possíveis de contaminação alimentar na America Latina, onde estudos relataram a água com importante contaminante (BUNDSCHUH et al., 2000). Esta exposição crônica reforça a importância de estabelecer limites nas diferentes variedades de alimentos, para garantir a segurança alimentar a nível local e global, uma vez que muitos países latinos também exportam cereais para outros continentes.

Mas uma vez que é constatada a contaminação, bem como uma amplitude de resposta entre regiões, que caminho podemos seguir na busca da segurança alimentar? Uma alternativa importante é a caracterização genotípica de diferentes cultivares, de forma a definir padrões de resposta que ajudem na escolha do material genético. Outra opção é buscar alternativas podendo ser útil a identificação de marcadores fisiológicos que possam resultar em menores concentrações de As nos grãos de arroz e ainda que possam vir a ser ferramentas para o melhoramento de cultivares futuras. Para tal, a escolha de parâmetros para caracterizar a tolerância / susceptibilidade ao agente estressor é extremamente importante. No entanto, muitas vezes os parâmetros escolhidos são incapazes de diferenciar de forma eficiente os genótipos. Um exemplo claro desse padrão é a avaliação do sistema radicular realizado no manuscrito dois. Este estudo levantou muitas questões como (i) o que define uma cultivar como sensível? (ii) A redução da partição de biomassa ou mesmo a redução da sua produção pode sempre ser considerada relacionada à suscetibilidade? (iii) Poderia essa redução de

biomassa ser uma estratégia de tolerância ou adaptação destinada a deixar descendentes? Estas questões têm recebido cada vez mais atenção na comunidade científica. Portanto, o presente estudo procurou analisar diferentes parâmetros para responder a essas perguntas. Destes parâmetros, foram avaliados o número de raízes adventícias por planta, que ao contrário dos parâmetros acima mencionados, mostraram padrões distintos entre as cultivares (Figura 1, manuscrito 2). Para este parâmetro observou-se três padrões: (i) Redução do número de raízes adventícias por planta (cultivar BR - IRGA 409 e IRGA 420), com um aumento de As na solução (II) Nenhuma mudança no número de raízes adventícias (BR - IRGA 410 e IRGA 423), independentemente da exposição ao As; e (iii) aumento do número de raízes para cultivar IRGA 424.

Outro dado interessante foi em relação aos grupos tióis não-protéicos (NPSH). Embora trabalhos têm descrito a importância dos NPSH para plantas (GUPTA et al.,2013), alguns estudos têm relatado diferenças nas concentrações destes compostos. No presente estudo, observou-se duas situações diferentes, em resposta à toxicidade de As: o primeiro foi a diferença observada entre a concentração na parte aérea e raízes, e o outro é uma grande discrepância entre os cultivares em relação ao sistema radicular (Figura 4, manuscrito 2). As alterações na concentração de NPSH foram muito mais pronunciadas em raízes do que em parte aérea com adição de As. Em ambos os tecidos a cultivar IRGA 424 mostrou um maior incremento na concentração NPSH em comparação aos demais cultivares. Curiosamente, sob condições de controle (sem a adição de As), a concentração de NPSH foi semelhante entre as cultivares.

É importante notar que as cultivares testadas também tiveram respostas diferentes fenotípicas (manuscrito 3) com a exposição ao As. As cultivares tolerantes apresentaram maior capacidade de perfilhamento, em comparação com as outras cultivares (Tabela 3, manuscrito 3). Além disso, o crescimento da parte aérea na fase inicial de plantas na maior dose de As foi maior para essas cultivares (IRGA 423 e IRGA 424) (Tabela 2, manuscrito 3).

Em plantas de arroz, os perfilhos garantem uma boa produção de grãos, promovendo o aumento da intercepção da luz solar, o aumento do número de espiguetas além de proteger a planta de elementos tóxicos, através da diluição destes elementos em tecidos, como resultado da maior produção de biomassa (FARRAG et al., 2012). Em vista disto, as plantas de arroz podem utilizar duas estratégias relacionadas com a perfilhamento: i) a utilização da biomassa do perfilho como uma estratégia de para a compartimentalização e / ou acumulação de substâncias tóxicas que ocorrem em níveis elevados no ambiente para assegurar o desenvolvimento correto e produção de sementes pelo colmo principal; e ii) promover a

acumulação de toxinas no colmo principal, assegurando, assim, reduzida translocação para os perfilhos.

A agricultura moderna tem pretensões muito maiores do que uma boa produtividade. Cada vez mais a pressão por produtos de qualidade e sem resíduos químicos tem tomado espaço; adicionalmente a sociedade clama por uma agricultura consciente. Neste sentido, a utilização de produtos finitos como o P e, consequentemente, melhorias na eficiência da nutrição de P em culturas podem trazer mudanças na tecnologia de fertilizantes, melhorias na exploração de biologia do solo, e melhores práticas de gestão de fertilizantes, bem como o melhoramento genético. No manuscrito 4, buscamos entender um pouco mais as respostas das diferentes cultivares à eficiência de uso e resposta ao P, bem como sua interação com o As. A necessidade de melhorias na nutrição de P, bem como o requerimento da produção agrícola global, resultou em vários estudos explorando estes aspectos (HINSINGER 2001; McNEILL e PENFOLD 2009; RICHARDSON et al., 2009;. RYAN et al., 2009;. McLAUGHLIN et al., 2011; SIMPSON et al., 2011). Nossos resultados ajudam a elucidar o papel do P no controle dos efeitos do As no arroz, com uma separação clara entre os genótipos para PUE, sendo a cultivar mais eficiente a IRGA 424 (Figura 4, manuscrito 4).

Outro dado importante deste estudo, em relação a diferenças entre cultivares, foi a acumulação de As em plantas cultivadas a campo. Embora a cultivar BR-IRGA 409 tenha um rendimento de grãos menor em comparação com IRGA 425 (Figura 3, manuscrito 5), o fato desta cultivar translocar menos As aos grãos demonstra o quão importante o melhoramento de plantas pode ser para o alcance da segurança alimentar. Outro aspecto interessante foi o incremento de P, que, embora com doses contrastantes, não teve diferença significativa no rendimento de grãos em áreas com níveis elevados de P (Camaquã e Cachoeirinha, com 30,5 e 27,0 mg P dm^{-3} de solo, respectivamente), embora com diferenças em Restinga Seca (2,9 mg de P dm^{-3} de solo) (Tabela 1, Tabela 4, manuscrito 5).

Neste trabalho também observamos que as supressões de água, provavelmente devido ao tempo de duração e estágio de desenvolvimento, não foram suficientes para reduzir o As em grãos; mas, assim como o P, a água também têm sido uma questão delicada na agricultura atual e, como a mesma não alterou a produtividade negativamente, talvez possa ser uma estratégia para anos com possibilidade de estiagem. Também é importante reforçar que o incremento de P, com doses acima da indicação agrícola, em geral não alterou a produção de grãos, mostrando assim que este elemento deve ser usado com precaução e sem abusos.

Finalmente, quando coletamos em propriedades agrícolas, em diferentes regiões do estado do Rio Grande do Sul (manuscrito 6), tivemos a grande oportunidade de caracterizar o

manejo ocorrente em cada região e avaliar o efeito do mesmo na qualidade de grãos de arroz. Acreditamos que o tamanho da área cultivada foi um fator determinante para escolher o manejo de uso do solo. Por exemplo, o manejo da produção de arroz seguido por dois ou três anos de pousio (Figura 1, manuscrito 6) foi observado em grandes propriedades rurais, em geral localizadas na região da fronteira. Nestas áreas, após a produção de arroz, azevém é semeado durante o inverno; e, juntamente com as gramíneas espontâneas que ocorrem nessas áreas, é utilizado para a produção de gado. Durante todo este período, o solo permanece sob condições aeróbicas. Mesmo que os produtores fiquem dois ou três anos sem a produção de arroz, eles ainda têm outra fonte econômica importante, a pecuária no sistema de engorda. No entanto, em uma pequena área essa dinâmica poderia ser pouco rentável.

A rotação de culturas é uma prática de manejo utilizada para evitar o esgotamento do solo, melhorando a propriedades biológicas do solo além da física, química e ajudando a controlar as pragas e doenças (EMBRAPA, 2004). Esta prática de manejo também pode evitar o esgotamento ou a concentração de elementos específicos causados por monoculturas (como o uso contínuo de um mesmo grupo de defensivos). Embora outros estudos demosntrem reduções significativas das concentrações de Cd e Pb em plantas cultivadas sob um sistema de rotação de culturas (WU et al., 2011), no presente estudo não foram detectadas diferenças significativas nas concentrações de As nos grãos de arroz cultivado em rotação de cultura com a soja (Figura 1; Figura 3, manuscrito 6).

Em nosso estudo, um número significativo de amostras de solo estavam acima do limite das concentrações máximas admissíveis de As em solos agrícolas (15-20 mg kg^{-1}) (KABATA-PENDIAS e MUKHERJEE, 2007). Além disso, 48% das amostras de arroz apresentaram concentrações de AS_i acima de 0,1 mg kg^{-1}, que é considerado não adequado para bebês e crianças jovens, de acordo com documento da UE 2015. Por outro lado, as amostras da região da fronteira tiveram valores baixos ou ainda abaixo do nível de detecção para As_i (0,001 mg kg^{-1}). A identificação de amostras com baixos níveis de As_i, pode gerar um benefício direto ao fornecedor, assim como para os países onde o arroz é importado, e, em última análise, uma compreensão do germoplasma de arroz, biogeoquímica do solo, gestão de campo e as condições climáticas.

Este estudo é encerrado com a avaliação do potencial genotóxico do As e efeito do alecrim com amenizador desta exposição (manuscrito 7). Embora trabalhos anteriores descrevam a genotoxidade do As, existe uma carência de trabalhos buscando alternativas a contaminação alimentar dentro da própria fonte de alimentos. A anormalidade mais comum causada pela exposição ao arsênico neste estudo foi a ocorrência de micronúcleos, o que já foi

relatado por outros estudos (YI et al., 2007;. BANERJEE et al., 2013.). Banerjee et al. (2013) relataram a associação entre a frequência de micronúcleos em células uroteliais de homens e o conteúdo de As em arroz cozido para o consumo, demostrando uma forte correlação positiva entre a presença de As na urina com o conteúdo de As no arroz. Este estudo demonstrou que o extrato de *Rosmarinus officinalis* tem potencial antimutagênico, reduzindo o dano ao DNA e a peroxidação lipídica, resultante do tratamento com exposição ao As, podendo ser uma importante alternativa entre os fitoterápicos, em áreas de contaminação por As.

9 CONCLUSÕES

Primeiramente, os dados referentes às amostras comerciais de arroz da América Latina evidenciam que a contaminação de grãos de arroz vai muito além de países asiáticos e EUA. Estes resultados têm um impacto global uma vez que o consumo deste material não se restringe a área produzida, pois existem inúmeros são os contratos de exportações/importações entre continentes.

Adicionalmente, os manuscritos aqui apresentados reforçam a idéia de que a variabilidade genética pode gerar padrões de respostas completamente diferentes frente a pressões de estresses. Entretando, para as cultivares testadas observou-se um limite de resposta, sendo o fator ambiental conjuntamente com o manejo determinantes para a concentração de As em grãos.

Vale lembrar que tratam-se de cultivares com algumas linhagens ancestrais comuns e logo com características comuns tanto em nível genético como no fenológico. O interessante, e também o que buscamos fazer, foi avaliar o quanto que estas amplitudes entre comportamentos distintos é determinante do ponto de vista fisiológico da planta, ou seja, o quanto esta pequenas diferenças impactam a capacidade da planta em deixar descendentes e passar por todos processos anteriores a isso.

Através de nossos resultados podemos fazer alguns apontamentos, sendo os principais:

I) Existe um variabilidade considerável entre países/regiões da América Latina a qual deve ser considerada para que se alcance de fato o status de segurança alimentar. Dentre os países avaliados, os valores mais elevados form encontrados na região sul do Brasil.

II) Aparentemente a cultivar BR-IRGA 409 apresenta uma maior suscetibilidade ao As, apresentando também uma menor translocação

para os grãos, enquanto que cultivares com comportamentos tolerantes apresentaram um maior acúmulo deste elemento. A eficiência de uso de fósforo e a concentração de tióis não proteicos parecem estar relacionadas a tolerância ao As sendo altamente pronunciadas em cultivares tolerantes.

III) Tanto o As quanto o Fe têm impacto sobre a nutrição mineral de plantas de arroz. Ainda neste sentido plantas mais eficientes na aquisição e uso de elementos como o P apresentam uma capacidade adaptativa maior sob situações de excesso destes elementos.

IV) Observou-se dois pontos cruciais no ciclo fenológico de plantas de arroz em resposta ao As. Primeiro durante a emissão de raízes adventícias, quando plantas com maior número de raízes apresentam maior capacidade adaptativa, e durante o perfilhamento, onde plantas com maior capacidade de perfilhamento conseguem produzir mais grãos sob estresse de As. Podendo ocorrer uma resposta cruzada destes fatores em relação a tolerância ao Fe.

V) Em solos pobres em P, a toxidez por Fe é mais eminente, reforçando a correlação entre estes elementos. Adicionalmente, ainda que a fertilidade de P seja corrigida, a ocorrência de alto Fe em pH baixo é pouco amenizada, havendo diferenças genotípicas em resposta ao P.

VI) Embora existam diferenças genéticas importantes entre as cultivares avaliadas, o manejo do solo parece ser o principal fator determinante na translocação de As para os grãos; sendo manejo de pousio em geral relacionado com menores teores de As no grão e maiores teores de Mo no solo.

VII) Como trabalhos anteriores, fica aqui elucidado o impacto em termos de genotoxidade e estresse oxidativo do As, e, interessantemente o efeito protetor de extrato de alecrim sobre este, planta encontrada abundantemente na América Latina, a qual pode ser incluída como tempero na alimentaçãoo diária ou ainda como chá.

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