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Arbuscular mycorrhizal fungi reduced the ratios of inorganic/organic arsenic in rice grains

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HIGHLIGHTS

- AMF had significant effects on As(III), As(V), DMA and total As conc. in grains.
- AMF reduced the ratios of inorganic/organic As concentrations in rice grains.
- AMF decreased significantly total As and inorganic As conc. in grains of Handao 3.
- As in grains is positively correlated with As in the soil solution of rhizosphere.

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) – *Rhizophagus intraradices* was inoculated to rice to investigate its effects on arsenic (As) uptake, grain As speciation, and rhizospheric As concentration of six rice cultivars grown in As-amended soil (60 mg As kg^{-1} soil). The AMF inoculation induced either positive, neutral or negative responses in rice grown in As contaminated soil, suggesting that functional diversity may exist in AMF symbiosis when As is taken up and transferred. The ratios of inorganic/organic As concentrations in rice grains of all cultivars were significantly reduced by AMF, that involved the transformation of inorganic As into less toxic organic form dimethylarsinic acid (DMA) in rice. AMF decreased significantly total As and inorganic As concentrations in rice grains of Handao 3. Positive correlations ($R^2 = 0.30–0.56$, $P < 0.05$) between As in the rhizospheric soil solution and As in rice grain at different periods were observed. This inferred that the As survey of soil solution can be an effective measure for evaluating As in grains.

1. Introduction

Arsenic (As) toxicity has become of great global concern due to increasing occurrences of water, soil and crop contamination in many regions of the world (Naidu et al., 2006; Tripathi et al., 2007). Rice (*Oryza sativa* L.) plants play an important role in the transfer of toxic As into food chains, leading to serious health risks to humans (Meharg and Rahman, 2003). Rice is the staple food for three billion people, most of who reside predominantly in Asia. It has also been reported that rice grown in severely As contaminated areas can accumulate up to 0.48 mg kg^{-1} inorganic As in grains (Zhu et al., 2008), which substantially exceeds the maximum contaminant level for As in rice grains (0.15 mg kg^{-1} inorganic As, GB2762-2005) (CFSA, 2005). The strategy of development for growing rice safely in the presence of As contamination may help to counteract the detrimental effects of As. Flooding of paddy soil leads to the mobilization of arsenite [As(III)] into soil solution, which in turn enhances As bioavailability in rice plants (Xu et al., 2008). Hence, aerobic rice cultivation is receiving considerable attention as a means of reducing plant exposure to As.

Arsenic can produce inorganic As species [e.g. arsenate (As(V))

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and As(III)] and organic As species [e.g. monomethylarsonic acid, MMA and dimethylarsinic acid, DMA] in the environment. The inorganic As in the form of As(V) is the predominant species in aerobic soils, whereas As(III) predominates under anaerobic environments such as submerged soils (Zhao et al., 2010). As(V), as an analog of phosphate, shares the same transport pathway with phosphate in rice roots and can be reduced to As(III) quickly (Abedin et al., 2002). As(III) is taken up by rice roots via aquaglyceroporins, and shares the same transport pathway as silicon (Si) (Ma et al., 2008). Many archaea, bacteria, fungi, and eukaryotic algae are able to methylate As, producing mono-, di-, tri-, or even tetra-methyl As species (Zhao et al., 2013). The toxicity of As species follows the order of: As(III) > As(V) > MMA > DMA (Abedin et al., 2002). It is therefore essential to take As speciation into account for decreasing As toxicity in rice grain.

Arbuscular mycorrhizal fungi (AMF) are indigenous soil-borne microorganisms, associated with approximately 80% of terrestrial plant species under aerobic conditions, including important crops such as rice. Although phosphorus (P) deficiency severely limits rice production worldwide, arbuscular mycorrhizal symbioses can transfer P to host plants in exchange for organic carbon (C) (Smith and Read, 2008). Rice inoculated with AMF under aerobic soils can be an effective measure to enhance P uptake (Yeasmin et al., 2007). As(V), as the main As species in aerobic soils, is a competitor for the phosphate transporters in plants. The diversity of mycorrhizal plant growth responses in As contaminated soils have been positively shown in white clover (Dong et al., 2008), *Holcus lanatus* (Gonzalez-Chavez et al., 2002), neutrally in basin wildrye (Knudson et al., 2003) and negatively in barley (Grace et al., 2009). Li et al. (2011) also reported that different rice/AMF combinations can bring about positive or negative effects on grain yield and grain As concentration. However, how AMF affects rice grain As speciation and As concentrations of root rhizosphere are still unclear.

Rhizosphere is the zone of soil that lies very close to the roots, where plant uptake of nutrients is taken place. Indirect mechanisms derived from the effects of AMF on rhizosphere properties include changes in pH, Eh, nutrient status, root exudation patterns and heavy metal activities of soil (Laheurte et al., 1990; Li and Christie, 2001), as well as, promoting the biotransformation of As (Jeffries et al., 2003). It has been noted that AMF are involved in the methylation of inorganic As into less toxic organic As in rhizosphere soil (Ultra et al., 2007a, b). Furthermore, the biotransformation process can influence the fate, mobility and bioavailability of As in soils, and As uptake by higher plants (Fitz and Wenzel, 2002). Therefore, studies investigating the As bioavailability in soil of rhizosphere and non-rhizosphere are crucial for a better understanding on the As behavior in soil-plant systems.

There are reports of As species transformation occurring in rice systems (Abedin et al., 2002; Carey et al., 2010), whereas no information is available regarding the activities of As species transformation in rice grain under the effects of AMF. The investigation of As speciation in rice grains involved in soil-fungal-rice interactions is central for gaining a better insight behind the mechanisms of AMF symbiosis.

With the above background, the objectives of the present study were to: 1) investigate the effects of AMF (*Rhizophagus intraradices*) on As uptake by rice, As speciation in rice grain and rhizospheric and non-rhizospheric As concentrations; and 2) determine the relationship between As concentrations in grain and rhizosphere soil solution.

2. Materials and methods

2.1. Plant cultivation

Seeds of six rice (*O. sativa* L.) cultivars including Guinongzhan, TD 71, Xiushui 11, Yuxiangyouzhan (all lowland rice types) and Handao 1, Handao 3 (both upland rice types) were obtained from the Guangdong Rice Research Institute (GDRRI), Guangzhou, and the National Rice Research Institute, Hangzhou, PR China, in March 2011. All seeds were sterilized with H_2O_2 for 1 min and washed thoroughly with deionized water. They were then placed on a plastic mesh, floating on 0.5 mol l^{-1} $CaSO_4$, in a container covered with aluminum foil. After two days, the rice seedlings were transplanted and cultured as stock in basins and supplied with 20% Hoagland-Arnon nutrient solution (Hoagland and Arnon, 1938). After two weeks, the uniform seedlings of rice (height: 15 cm) were used for the pot trial. Plant cultivation was conducted in a temperature-controlled (28/22 °C, day/night) greenhouse, under a random block design. In addition to the natural sunlight, a 12-h photon flux density of 300 μmol m^{-2} s^{-1} was supplied via an assembly of cool-white fluorescent lamps, with a relative humidity of 85%.

2.2. As treatments

Soil was collected from an abandoned farm in Tai Po Wu Kau Tang, Hong Kong in April 2009. The soil (without the addition of As) contained 10% organic matter, 1.58 mg kg^{-1} extractable N, 46.33 mg kg^{-1} extractable P, with a pH value of 5.8 and an average As concentration of 9.8 mg kg^{-1} (Li et al., 2011). Air-dried soil was sieved through a 2 mm mesh to remove stones, roots and rhizomes using a stainless steel sieve. The soil was then autoclaved at 121 °C for 120 min in order for the indigenous AM fungi to be eliminated. The air-dried soil was added either with or without 60 mg As kg^{-1} as $Na_2HAsO_4 \cdot 7H_2O$, and mixed every day and incubated for three months.

2.3. Pot experiment

Two mycorrhizal treatments included the control (without AMF) and *R. intraradices* obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), France. A rhizobag system composed of two compartments was used (Ultra et al., 2007b). The central compartment for rice growing served as the mycorrhizosphere, and the outer compartment as the non-mycorrhizosphere. The two compartments were separated by a 30 μm nylon mesh rhizobag (7 cm in diameter, 8 cm in height). There was 500 g soil (including 50 g sterile or non-sterile AMF inoculants) in the central compartment and 900 g soil in the outer compartment, totaling to 1400 g soil per pot (14 cm in diameter, 13 cm in height). The sampling devices (Rhizon MOM 10 cm length, 2.5 mm OD, Rhizosphere Research Products, Wageningen, Netherlands) were buried diagonally in the middle of the soil of the two compartments for collecting soil solutions.

Uniform rice seedlings (15 cm in height) were transferred into the central compartments, with each pot containing two seedlings and each treatment in replicates of four. Hoagland solution (20%) containing 10% KH_2PO_4 was added to each pot every week for six months. Soil was maintained daily at 80% holding capacity by the supply of distilled water. Soil solutions were collected on day 90, 120, 150 and 180 for the measurement of total As concentrations. After six months, all the plants were harvested and rinsed thoroughly with deionized water to remove any attached soil/substrate particles. Each plant was separated into unpolished grains, straw

and root. The unpolished grains were freeze–dried and then separated into husks and grains, which were stored at −80 °C before total As and As speciation analyses. The washed and cleaned roots were divided into two parts, with one part used for measuring total As concentrations, whilst the other for measuring the colonization rates of roots. Details of estimating the AM fungal colonization rates were described previously (Li et al., 2011).

2.4. Analysis of total As and As speciation

Soil solution was added with two drops of 70% HNO_3 immediately after collection, filtered through a 0.5 μm filter paper and analyzed for total As via the means of inductively coupled plasma mass spectrometry (ICP-MS, Elan 9000; PerkinElmer, Fair Oaks, CA, USA) (Allen, 1989). Each cleaned sample used for measuring total As was oven-dried (60 °C) to a constant weight. The analyzed As in husks, grains, straws and roots was digested by a mixture of HNO_3/$HClO_4$ (85/15, v/v) (Zhao et al., 2003). The temperature of digestion was controlled to be below 120 °C. Blanks and internal standards (1568a rice flour, National Institute of Standards and Technology – NIST, USA) were used to ensure the accuracy of metal determination. The recovery rates of elements were within 90 ± 10%.

Analysis of As speciation in freeze–dried grains was determined by high performance liquid chromatography (HPLC, Agilent Technologies, Santa Clara, CA, USA) coupled with inductively coupled plasma mass spectrometry (ICP-MS), according to the trifluoroacetic acid (TFA) extraction method (Williams et al., 2005; Wu et al., 2011). The detailed method is described in Li et al. (2011). The standard reference material NIST CRM 1568a rice flour was included for As speciation analysis. The mean total recovery [(sum of species recovered from the TFA extraction/total As from acid digestion) × 100%] ranged from 75% to 100%, which was consistent with other studies (Heitkemper et al., 2001; Williams et al., 2005).

2.5. Statistical analyses

All results were tested by two-way ANOVA analysis of variance using the SPSS statistical package and all figures drawn by PC-based Origin program. Duncan's multiple range test at the 5% level of probability was used for post-hoc comparison to separate treatment differences.

3. Results and discussion

R. intraradices appear to have a closer colonization relationship with Guinongzhan and TD 71 than other rice cultivars, both in soil added with and without 60 mg As kg^{-1} (Fig. 1). The results indicate that AMF inoculated with different host plants may possess different nutritional status, symbiotic efficiency and gene expression pattern leading to distinct colonization rates (Feddermann et al., 2010), as well as, the commercially available AM inoculants being viable even at high As concentrations in soil (Ultra et al., 2007a). In As contaminated soil, both mycorrhizal inoculation and rice cultivars had profound effects on grain yield, straw biomass and root biomass (Tables 1 and 2). Inoculation with *R. intraradices* significantly enhanced the grain yield of Yuxiangyouzhan while decreasing the grain yields of Guinongzhan, Handao 3 and Xiushui 11 (Tables 1 and S1). However, the root colonization rates of Yuxiangyouzhan, Guinongzhan, Handao 1 and Xiushui 11 were 71%, 85%, 26% and 48% in As contaminated soil, respectively. Therefore, no close relationship between root colonization and the As tolerance of host plants could be inferred. Hajiboland et al. (2009) suggested that the genotypic difference of host plants in response to the inoculation with AMF is dependent on the nutrients, nutritional status and nutrient efficiency of the genotypes. It is widely recognized that the response of host plants to AMF involves complicated soil-AMF-plant mechanisms, which cannot be simply evaluated by root colonization rates (Wu et al., 2009).

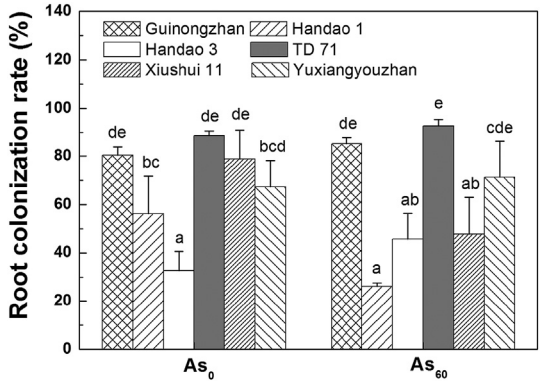

Fig. 1. Root colonization rates of six rice cultivars grown in soil without/with 60 mg As kg^{-1} inoculated with *R. intraradices* (mean ± S.D., n = 4). Different letters indicate a significant difference at $P < 0.05$ (Duncan test).

In the present study, the grain yield of TD 71 increased in soil without the addition of As, with no significant variation in soil being observed when As was added under the influence of AMF. In contrast, the grain yield of Yuxiangyouzhan became enhanced and the grain yields of Guinongzhan, Haodao 3 and Xiushui 11 were reduced in 60 mg As kg^{-1} soil, whereas there were no notable differences seen in soil without adding As (Table 1). Agely et al. (2005) also noted that the presence of AMF increased frond dry mass in *Pteris vittata* at 100 mg As kg^{-1} soil ($P < 0.05$), but no significant effects were apparent at 0 or 50 mg As kg^{-1}. These results suggest that the effects of mycorrhizal inoculation on plant growth are related to soil As concentrations and AMF symbiosis, which will lead to different responses to different As concentrations in soil. *R. intraradices* increased the grain yield of Yuxiangyouzhan while no obvious influence on grain As concentration in As contaminated soil was noted (Tables 1 and S1). This was in line with our previous study on rice which reported that both Guangyinzhan inoculated with *R. intraradices* and Handao 502 inoculated with *Glomus geosporum* can enhance grain yield without any effect on grain As concentrations (Li et al., 2011). Moreover, the results show that *R. intraradices* decreased both the grain yield and grain As concentrations of Handao 3 (Tables 1 and S1). These were different from findings derived from other plants, such as white clover, ryegrass (Dong et al., 2008) and *Medicago sativa* Linn (Chen et al., 2007), which suggests that the "growth dilution effect" lowered As concentrations in shoots, for alleviating As phytotoxicity in order to achieve a better growth of AM plants (Chen et al., 2007). Therefore, the "growth dilution effect" mechanism may be deemed unsuitable for rice colonized by AMF. The reason may be because cereals have more complicated mechanisms relative to other plants (Smith and Smith, 2011).

Although AMF did not exert significant effects on grain As concentrations, it increased the grain yield of Yuxiangyouzhan ($P < 0.05$) in As contaminated soil (Tables 1 and S1). As(V) is the predominant form of As in soils under aerobic conditions, and competes with the major plant nutrient phosphorus (P) in the form of phosphate, not only for sorption sites on mineral surfaces in soil but also for root membrane transporters (Smith et al., 2002; Vetterlein et al., 2007). Thus this positive growth response may be due to the higher grain P/As content molar ratio, via the enhancement of P nutrition to increase grain yield (Xu et al., 2008; Li et al., 2011). However, AMF increased grain As concentrations and decreased grain yields of Guinongzhan and Xiushui 11 (Tables 1 and

Table 1
Grain yield (g pot^{-1}, DW) and As concentrations (conc.) (mg kg^{-1}, DW) in grains of six different rice cultivars, grown in soil without adding As (As_0), and the grain yield, straw and root biomass (g pot^{-1}, DW) and As conc. in grains, husks, straws and roots (mg kg^{-1}, DW) of six different rice cultivars, grown in soil added with 60 mg As kg^{-1} (As_{60}) uninoculated (−F) or inoculated (+F) with *R. intraradices*.

	As_0		As_{60}						
	Grain yield (g pot^{-1})	As in grains (mg kg^{-1})	Grain yield (g pot^{-1})	Straw biomass (g pot^{-1})	Root biomass (g pot^{-1})	As in grains (mg kg^{-1})	As in husks (mg kg^{-1})	As in straws (mg kg^{-1})	As in roots (mg kg^{-1})
Guinongzhan − F	16 ± 1.5 e	0.17 ± 0.03 ab	12 ± 1.0 e	16 ± 0.7 c	4.8 ± 0.8 abc	0.40 ± 0.04 b	1.4 ± 0.2 a	19 ± 2.4 b	199 ± 20 bc
Guinongzhan + F	18 ± 1.5 e	0.27 ± 0.04 c	9.2 ± 0.9 d	11 ± 0.8 b	4.2 ± 0.3 ab	0.69 ± 0.03 cd	2.0 ± 0.1 b	26 ± 1.6 cd	258 ± 26 de
Handao 1 − F	11 ± 0.7 cd	0.38 ± 0.04 d	3.3 ± 1.7 ab	9.7 ± 0.7 ab	9.2 ± 2.6 e	0.74 ± 0.07 d	2.6 ± 0.2 c	25 ± 1.7 c	277 ± 22 ef
Handao 1 + F	9.1 ± 0.6 bc	0.38 ± 0.02 d	2.7 ± 0.8 a	7.0 ± 1.6 ab	6.7 ± 1.3 cd	1.02 ± 0.07 e	3.8 ± 0.3 e	30 ± 2.5 de	300 ± 28 f
Handao 3 − F	8.4 ± 0.5 bc	0.23 ± 0.01 abc	7.9 ± 1.9 cd	9.3 ± 0.4 ab	4.3 ± 0.7 ab	1.03 ± 0.05 e	4.1 ± 0.5 e	50 ± 7.4 f	295 ± 25 ef
Handao 3 + F	7.1 ± 0.2 ab	0.25 ± 0.05 c	4.3 ± 1.3 ab	6.3 ± 1.1 a	2.5 ± 0.01 a	0.67 ± 0.07 cd	2.1 ± 0.2 b	35 ± 0.5 e	228 ± 8 cd
TD 71 − F	12 ± 0.2 d	0.16 ± 0.02 a	14 ± 1.0 ef	23 ± 1.5 ef	3.5 ± 0.1 a	0.61 ± 0.01 c	3.1 ± 0.3 d	27 ± 2.4 cd	270 ± 5 ef
TD 71 + F	17 ± 0.8 e	0.24 ± 0.02 abc	12 ± 0.7 e	18 ± 3.4 cd	2.8 ± 0.7 a	0.68 ± 0.004 cd	2.1 ± 0.3 b	31 ± 0.6 de	289 ± 12 ef
Xiushui 11 − F	5.4 ± 2.1 a	0.24 ± 0.06 abc	5.7 ± 1.7 bc	27 ± 4.7 fg	7.8 ± 0.4 de	0.26 ± 0.02 a	1.1 ± 0.1 a	25 ± 1.5 c	131 ± 09 a
Xiushui 11 + F	6.6 ± 1.7 ab	0.21 ± 0.01 abc	2.6 ± 1.4 a	19 ± 0.7 cde	6.3 ± 0.9 bcd	0.37 ± 0.03 b	1.1 ± 0.1 a	22 ± 1.4 bc	145 ± 21 a
Yuxiangyouzhan − F	22 ± 1.5 f	0.25 ± 0.03 bc	15 ± 1.9 f	23 ± 3.0 def	2.7 ± 0.3 a	0.37 ± 0.03 b	1.1 ± 0.1 a	12 ± 0.01 a	164 ± 22 ab
Yuxiangyouzhan + F	22 ± 1.3 f	0.19 ± 0.04 abc	19 ± 0.6 g	28 ± 1.7 g	4.5 ± 0.8 abc	0.31 ± 0.02 ab	1.0 ± 0.1 a	14 ± 1.5 a	127 ± 13 a

Data are presented as mean values ± SD (n = 4). Means followed by the same letter within the same column are not significantly different determined by the Duncan's multiple range tests at the 5% level.

Table 2
The significance level of two-way ANOVA for the biomass, total As concentrations (conc.) in grains, straws and roots, the ratio of inorganic As conc./organic As conc., As(III), As(V) and DMA conc. in grains, and total As conc. in soil solution of rhizosphere and non-rhizosphere, on day 90, 120, 150 and 180 between mycorrhizal inoculation and rice cultivars in soil added with 60 mg As kg^{-1}.

	Mycorrhizal inoculation	Rice cultivars	Interaction
Grain yield	$P < 0.05$	$P < 0.001$	$P < 0.05$
Straw biomass	$P < 0.01$	$P < 0.001$	$P < 0.01$
Root biomass	$P < 0.05$	$P < 0.001$	NS
Grain As(III) conc.	$P < 0.01$	$P < 0.001$	$P < 0.001$
Grain As(V) conc.	$P < 0.001$	$P < 0.001$	$P < 0.001$
Grain DMA conc.	$P < 0.001$	$P < 0.001$	$P < 0.001$
Grain inorganic/organic As	$P < 0.001$	$P < 0.001$	$P < 0.001$
Grain total As conc.	$P < 0.01$	$P < 0.001$	$P < 0.001$
Husk total As conc.	NS	$P < 0.001$	$P < 0.001$
Straw total As conc.	NS	$P < 0.001$	$P < 0.05$
Root total As conc.	NS	$P < 0.001$	NS
As conc. − R−day 90	$P < 0.001$	$P < 0.001$	$P < 0.001$
As conc. − R−day 120	NS	$P < 0.001$	$P < 0.001$
As conc. − R−day 150	$P < 0.05$	$P < 0.001$	$P < 0.001$
As conc. − R−day 180	NS	$P < 0.001$	$P < 0.01$
As conc. − NR−day 90	$P < 0.001$	$P < 0.001$	$P < 0.001$
As conc. − NR−day 120	$P < 0.001$	$P < 0.001$	$P < 0.01$
As conc. − NR−day 150	$P < 0.001$	$P < 0.001$	$P < 0.001$
As conc. − NR−day 180	NS	$P < 0.001$	$P < 0.001$

NS represents no significance at the 5% level.

Table 3
Arsenic species in grains of six different rice cultivars grown in soil added with 60 mg As kg^{-1} uninoculated (−F) or inoculated (+F) with *R. intraradices* using TFA extraction and HPLC-ICP-MS measurement.

	Total As (mg kg^{-1})	As(III) (mg kg^{-1})	As(V) (mg kg^{-1})	DMA (mg kg^{-1})	MMA (mg kg^{-1})	Inorganic as/organic As[a]	Recovery (%)[b]
Guinongzhan − F	0.40 ± 0.04 b	0.16 ± 0.01 ab	0.15 ± 0.004 f	0.04 ± 0.002 a	ND	8.37 ± 0.46 g	88.71
Guinongzhan + F	0.69 ± 0.03 cd	0.14 ± 0.02 ab	0.19 ± 0.01 gh	0.19 ± 0.01 d	ND	1.79 ± 0.04 ab	74.94
Handao 1 − F	0.74 ± 0.07 d	0.25 ± 0.02 d	0.18 ± 0.02 g	0.18 ± 0.01 d	ND	2.38 ± 0.22 bc	82.23
Handao 1 + F	1.02 ± 0.07 e	0.37 ± 0.01 e	0.13 ± 0.01 e	0.43 ± 0.02 e	ND	1.15 ± 0.06 a	90.83
Handao 3 − F	1.03 ± 0.05 e	0.71 ± 0.01 g	0.18 ± 0.01 g	0.09 ± 0.003 b	ND	10.26 ± 0.43 h	95.06
Handao 3 + F	0.67 ± 0.07 cd	0.38 ± 0.04 e	0.12 ± 0.01 de	0.13 ± 0.01 c	ND	3.91 ± 0.44 d	94.11
TD 71 − F	0.61 ± 0.01 c	0.49 ± 0.01 f	0.09 ± 0.01 c	0.03 ± 0.002 a	ND	20.05 ± 1.35 j	99.19
TD 71 + F	0.68 ± 0.004 cd	0.45 ± 0.04 f	0.11 ± 0.004 cd	0.04 ± 0.002 a	ND	14.55 ± 0.25 i	88.03
Xiushui 11 − F	0.26 ± 0.02 a	0.16 ± 0.01 ab	0.06 ± 0.01 b	0.03 ± 0.003 a	ND	7.41 ± 0.45 f	94.06
Xiushui 11 + F	0.37 ± 0.03 b	0.20 ± 0.02 c	0.06 ± 0.01 b	0.09 ± 0.01 b	ND	2.78 ± 0.27 c	95.12
Yuxiangyouzhan − F	0.37 ± 0.03 b	0.12 ± 0.02 a	0.20 ± 0.02 h	0.05 ± 0.01 a	ND	6.49 ± 0.90 e	100.30
Yuxiangyouzhan + F	0.31 ± 0.02 ab	0.17 ± 0.01 bc	0.03 ± 0.003 a	0.09 ± 0.01 b	ND	2.21 ± 0.06 bc	95.13

Data are presented as mean values ± SD (n = 4). Means followed by the same online letter within the same column are not significantly different determined by the Duncan's multiple range tests at the 5% level.

[a] Inorganic As/organic As = [As(III) + As(V)]/(DMA + MMA).

[b] Recovery (%) = [(species sum)/(total As)] × 100.

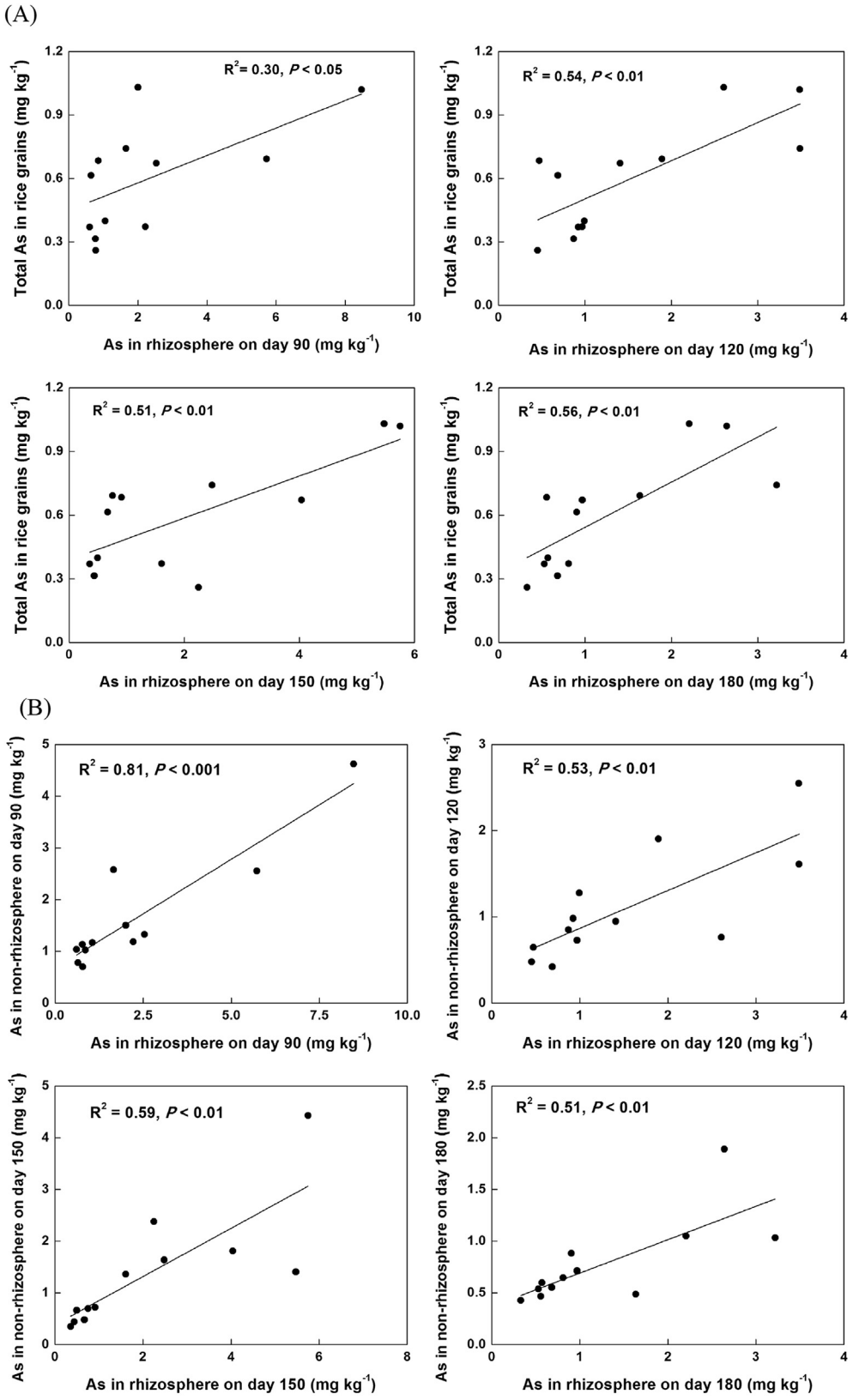

Fig. 2. Correlation between As concentrations in grain/non-rhizosphere soil solution and rhizosphere soil solution of six rice cultivars, grown in soil with 60 mg As kg^{-1} uninoculated/inoculated with *R. intraradices* at different periods. (A) Correlation between As conc. in grain and rhizosphere soil solution. (B) Correlation between As conc. in non-rhizosphere soil solution and rhizosphere soil solution.

S1). Although mycorrhizal roots can enhance P uptake, the induced rice phosphate transporter gene, *OsPT11,* by arbuscular mycorrhizal symbiosis (Paszkowski et al., 2002) may absorp As(V) which is mistaken for P as a P analogue leading to the host plant taking up more As.

There were significant differences in As(III), As(V), DMA, total As and the ratio of inorganic/organic As concentrations in rice grains for both mycorrhizal inoculation and rice cultivars in As contaminated soil ($P < 0.01$) (Table 2). Total As and inorganic As [As(III) and As(V)] in grains of Handao 3 were reduced by AMF markedly (Table S1). Grain DMA concentrations in all genotypes, except TD 71, were found to be elevated due to the influence of AMF (Tables 3 and S1). Furthermore, the ratios of inorganic/organic As concentrations in rice grains of all genotypes were reduced by AMF significantly ($P < 0.05$) (Tables 3 and S1). This suggests that As methylation and speciation in rice grains can be strongly influenced by AMF, which has never been reported before. Many archaea, bacteria, fungi, and eukaryotic algae are able to methylate As, producing mono-, di-, tri-, or even tetra-methyl As species (Zhao et al., 2013). Zhang et al. (2015) suggested that AMF are most likely to be involved in the methylation of inorganic As into less toxic organic DMA in *Medicago truncatula*. More inorganic As was transformed into organic As regardless of the host plants response to AMF. This methylation process happened in the rhizosphere soil before As was transferred into the rice roots, because rice plants lack the As methylation ability (Zhao et al., 2013). Organic As species are taken up by rice roots less efficiently, but are transported to the grain much more efficiently, comparing with inorganic As species (Zhao et al., 2013).

Ultra et al. (2007a, b) demonstrated the presence of methylated As species in the rhizosphere of sunflowers (*Helianthus annuus* L.) when inoculated with *Glomus aggregatum*. In addition, DMA was found in the rhizosphere soil but not in the bulk soil of mycorrhizal plants, nor in the rhizosphere soil of NM plants. Mycorrhizal roots are known to release organic substances as methyl donors for biomethylation by microorganisms or to provide substrates that increase microbial activity (Ultra et al., 2007a). Soil microorganisms can regulate As transformation processes, involving methylation, demethylation, reduction of As(V) and oxidation of As(III) (Gadd, 2004). Zhang et al. (2015) obeserved that AMF potentially played an important role in the reduction of As(V) to As(III) in *M. truncatula*. However, it has been reported that inoculation of *Glomus mosseae* decreased the concentration of As(III) in soil solution of upland rice (Liu et al., 2012). Therefore, AMF may be able to mediate As biomethylation, reduction of As(V) and oxidation of As(III) and release some signal to induce the methylated As in the rhizosphere. Methylated As species have been reported to be less toxic than inorganic As (Bentley and Chasteen, 2002). It has been hypothesized that the chemical behavior and toxicity of As becomes altered in the rice grain, which contributes to better survival of mycorrhizal plants in As contaminated soil (Ultra et al., 2007b).

Mycorrhizal inoculation exerted significant effects on As concentrations in the soil solution of rhizosphere on day 90 ($P < 0.001$) and 150 ($P < 0.05$) and of non-rhizosphere on day 90 ($P < 0.001$), 120 ($P < 0.001$) and 150 ($P < 0.001$) in 60 mg As kg^{-1} soil (Tables 2 and S2). Furthermore, the interaction of rice cultivars and mycorrhizal inoculation had significant effects ($P < 0.01$) on total As concentrations in the soil solutions of rhizosphere and non-rhizosphere (Table 2). Under the influence of AMF, As in the soil solutions of rhizosphere of Handao 3 became decreased, whereas those for Guinongzhan, Handao 1 and Xiushui 11 increased (Table S2). These results show that AMF has a great potential in affecting As availability in soil solution, which is consistent with the results of Ultra et al. (2007b). AMF is known to affect the activities of soil enzymes, such as increasing phosphatase and dehydrogenase enzyme activities (Ultra et al., 2007a), and to induce physical modifications to soil rhizosphere such as soil density, water content, texture and organic matter content (Smith and Read, 2008). Therefore, the variance of As solubilization in the soil adjacent to the root surfaces is as a result of AMF-induced modification of microbial activities, community structures, and physical and chemical soil properties in mycorrhizosphere (Marschner and Baumann, 2003). Our study has demonstrated that there were positive correlations between As in the soil solution of rhizosphere and As in rice grain, and As in the soil solution of non-rhizosphere on day 90, 120, 150 and 180 (Fig. 2). The soluble elements (such as As and nutrients) can be delivered to the roots by diffusion, and through water movement driven by transpirational flow from the soil in the outer compartment, even if root growth happens to be confined to the central compartment (Ultra et al., 2007b). The concentration and speciation of As in the soil solution can reflect its bioavailability in plant roots because roots take up As mainly from soil solution (Xu et al., 2008). The close relationship amongst non-rhizosphere, rhizosphere and plant suggests that the plant As concentrations can be estimated via As bioavailability in soil. This can ultimately provide a simple, fast and effective way to evaluate plant As uptake, in which this method of measuring As bioavailability in soil solution can also be conveniently applied to field trials.

4. Conclusions

The AMF inoculation induced either positive, neutral or negative responses in rice grown in As contaminated soil, suggesting that functional diversity may exist in AMF symbiosis when As is taken up and transferred. Furthermore, both AMF and rice cultivars had significant effects ($P < 0.05$) on the following: grain yield; straw and root biomass; concentrations of As(III), As(V), DMA and total As, and the ratios of inorganic/organic As concentrations in rice grains; and As concentrations in the soil solution of rhizosphere on day 90 and 150; and of non-rhizosphere on day 90, 120 and 150 in 60 mg As kg^{-1} soil. The ratios of inorganic/organic As concentrations in rice grains of all cultivars were significantly ($P < 0.05$) reduced by AMF, that involved the transformation of inorganic As into less toxic DMA and also favored the detoxification of As in rice. AMF decreased significantly total As and inorganic As concentrations in rice grains of Handao 3. Furthermore, there were positive correlations between As in the soil solution of rhizosphere and As in rice grain, and As in the soil solution of non-rhizosphere and rhizophere at different periods, inferring that the As survey of soil solution can be an effective measure for evaluating As in rice grains.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.10.067.

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