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Uptake kinetics of different arsenic species in lowland and upland rice colonized with *Glomus intraradices*

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ABSTRACT

The effect of *Glomus intraradices* on four As species (arsenate; arsenite; dimethylarsinic acid, DMA; and monomethylarsonic acid, MMA) uptake by lowland rice and upland rice were investigated based on two experiments: (1) high (0–0.05 mM) and low (0–2.5 mM) – affinity uptake kinetics of four As species in the short-term, and (2) As speciation in rice treated with 1 mM arsenate, arsenite, DMA or MMA solutions. The results showed that mycorrhizal roots of two rice cultivars reduced the arsenate uptake significantly ($P<0.001$) in low-affinity uptake system, and decreased the uptake of arsenite and MMA noticeably ($P<0.05$) in high or low-affinity uptake systems. The four As species influx have significant differences ($P<0.05$) between two rice varieties in low-affinity uptake systems. In the arsenate treatment, the ratio of arsenate/arsenite reduced in shoots while increased in roots because of *G. intraradices* presence.

1. Introduction

Arsenic (As) is a class 1 carcinogen, which affects hundreds of millions of people worldwide [1]. Arsenic contamination in drinking water and irrigation water has attracted much attention. In Bangladesh, well water containing high arsenic level is used both for irrigation and drinking, leading to many hazardous diseases, even cancer [2]. Plant-based foods such as rice are an important source of As intake. Rice is a big problem regarding the entry of As into the food chain and it is also the staple diet for 3 billion people, predominantly in Asia. Up to 1.8 mg kg^{-1} of As was detected in the rice grain of the As affected areas of Bangladesh [3]. Therefore, there is an urgent need to reduce As uptake by rice.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in the rhizosphere, which have a significant contribution on inducing the formation of large lateral rice roots [4] and increasing P uptake by rice in flooded conditions [5]. Zhang et al. [6] found that *Glomus mosseae* inoculation has significantly reduced Pb and Cd translocation from root to shoot on upland rice cultivar "277" under the combined soil contamination, compared with nonmycorrhizal roots. Li et al. [7] also reported that in As contaminated soil, lowland rice (Guangyinzhan) inoculated with *Glomus intraradices* and upland rice (Handao 502) inoculated with *Glomus geosporum* enhanced their As tolerance, grained P content, grained yield and molar ratio of grain P/As content, whereas the ratio of grain/straw As concentration was decreased. However, the mechanisms of AMF involved in As uptake by rice is not yet clear. Therefore, the effects of AMF on As speciation and accumulation in rice are investigated in this study.

Arsenic forms inorganic and organic complexes in the environment. Arsenate (As(V)) is the main species in aerobic soils, while arsenite (As(III)) dominates in anaerobic environments such as flooded paddy soils [8]. As(V) and As(III) are inorganic arsenic which are interconvertible, depending on the redox status of the environment. The main route of As(V) uptake in plants is through the phosphate transporters as a phosphate analogue [9], whereas As(III) is transported in the neutral $As(OH)_3$ form through aquaglyceroporins [10]. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are both organic arsenic and have been found in paddy soils [11]. They may have been derived from microbial and algal biomethylation. We speculate that AMF may influence As speciation and uptake by rice in As contaminated soil and water, leading to either an increase or reduction of total As accumulation in rice. There are a number of studies investigating As kinetics of different plant species, such as rice [12], *Brassica carinata* [13], *Pteris vittata* [14], maize [15], but there is a lack of information on the uptake mechanism of As species in plants colonized by AMF, except maize [16]. Hence, due to increasing severity of As toxicity in rice, a good understanding of the dynamics of different As speciation and uptake in rice inoculated with AMF is needed.

There are two uptake systems described by additive Michaelis–Menten functions. One is high-affinity uptake system, at lower substrate concentrations, and the other is low-affinity uptake

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system, at high substrate concentrations. Michaelis–Menten functions are used in this kinetic study. The main objectives of this experiment were to investigate: (1) the effects of AMF on uptake kinetics of the four As species (As(V), As(III), DMA, MMA) by lowland rice and upland rice, and (2) the effects of AMF on As accumulation and speciation in lowland rice and upland rice, under four different As species treatments.

2. Materials and methods

2.1. Plant cultivation

Seeds of two rice cultivars (*Oryza sativa* L.) – lowland rice (Guangyinzhan) and upland rice (Handao 502) were obtained from Guangdong Rice Research Institute (GDRRI), Guangzhou, China, in May 2010. The rice seeds were sterilized with H_2O_2 and washed thoroughly with deionized water. They were then placed on a petri dish with 0.5 mol L^{-1} $CaSO_4$ solution. After 2 days, the germinated seedlings were transplanted and cultured in 20% Hoagland–Arnon nutrient solution [20%-strength composition 1.0 mM $Ca(NO_3)_2$, 1.0 mM KNO_3, 0.4 mM $MgSO_4$, 0.2 mM KH_2PO_4, 10 μM Fe(II)-EDTA, 9 μM H_3BO_4, 0.2 μM $ZnSO_4$, 0.1 μM $CuSO_4$, 2 μM $MnSO_4$, 0.02 μM $(NH_4)_6Mo_7O_{24}$] [17]. After 2 weeks, the uniform seedlings of rice (15 cm) were used for the pot trails.

2.2. Plant inoculation

Soil samples were collected from an abandoned farm in Tai Po Wu Kau Tang, Hong Kong in May 2009. After air-dried for 2 weeks, they were sieved through a 2 mm mesh to remove stones, roots and rhizomes. Then soil and uncontaminated river sands were autoclaved at 120 °C for 120 min for the elimination of indigenous AM fungi. The uniform seedlings were transferred into the pots (diameter: 15 cm; height: 20 cm) with soil–sand (5:1) combination. Two mycorrhizal treatments included control (without AMF) and *G. intraradices* (E31V) were obtained from International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). Eighty percentage water holding capacities of soil were maintained daily by supplying distilled water. Hoagland–Arnon nutrient solution (20%) was added to each pot every week in order to maintain adequate soil nutrient levels. After a growth period of 7 weeks, all the plants were washed thoroughly by deionized water to remove any soil/substrate particles attached and used for subsequent treatments.

A subsample of fresh roots was stained with 0.05% Trypan Blue (w/w) according to the method of Phillips and Hayman [18], and AMF colonization was quantified on one hundred 1 cm long root segments by the slide length method, expressing as a percentage of AM colonization [19]. There were four replicates for each subsample.

2.3. Uptake kinetics of As(V), As(III), MMA and DMA

One part of washed rice plants was excised at the basal node. The excised roots of rice seedlings (0.3–0.6 g fresh weight) were incubated in aerated test solution (containing 5.0 mM MES and 0.5 mM $Ca(NO_3)_2$, adjusted to pH 5 using KOH) for 30 min at 25 °C, and then transferred into aerated test solutions containing different concentrations of As(V)/As(III)/DMA/MMA. There were four replicates for each treatment. Test solution concentrations of As(V), As(III), DMA, and MMA for high-affinity uptake experiments ranged between 0 and 0.0532 mM, and for low-affinity uptake experiments with As(V), As(III), DMA, and MMA concentrations were 0–2.5 mM. Stock solutions of As(V), As(III), DMA, and MMA were prepared from sodium As(V) (Aldrich Chemical Co.), sodium As(III) (Aldrich Chemical Co.), dimethylarsinic acid sodium salt (Aldrich Chemical Co.) and monomethylarsonic acid (Wako Pure Chemical Industries, Ltd.), respectively. All test solutions containing 5.0 mM MES and 0.5 mM $Ca(NO_3)_2$ were adjusted to pH 5 using KOH. After terminating As exposure for 20 min, the roots were rinsed in ice-cold phosphate solution containing 1.0 mM K_2HPO_4, 5.0 mM MES and 0.5 mM $Ca(NO_3)_2$ for 10 min to remove the adsorbed As species from the root free space and stop further root activity. Fresh roots were weighed, oven-dried at 70 °C for 2 days, and ground to powder for total As concentration determination.

2.4. Plant As uptake and speciation

The other part of washed plants was rinsed into 4 solution treatments, i.e., 1 mM As(V), As(III), DMA or MMA, respectively. There were four replicates for each treatment. After treatments for 20 min, the roots of plants were rinsed in ice-cold phosphate solution containing 1.0 mM K_2HPO_4, 5.0 mM MES and 0.5 mM $Ca(NO_3)_2$ for 10 min to remove the adsorbed As species. Then the plants were washed with deionized water and separated into shoots and roots. All the samples were frozen in liquid nitrogen, freeze-dried, and ground to powder for analysis of As speciation and total As concentrations.

2.5. Plant analysis for total As

The oven-dried root tissues were digested by a mixture of $HNO_3/HClO_4$ (85/15, v/v) [20] and the total As concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan 9000; PerkinElmer, Fair Oaks, CA, USA) [21]. The temperature of digestion was controlled under 120 °C to avoid As volatilization [22]. The 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%.

2.6. Plant analysis for As speciation

Analysis of As speciation in freeze-dried shoots and roots 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 [23–25]. The standard reference material NIST CRM 1568a rice flour was included for As speciation analysis, which was also used to validate the method [24,26].

2.7. Statistical analysis

All results were tested by three-way ANOVA analysis of variance using the SPSS statistical package. All figures were drawn by PC-based Origin program. Duncan test at 5% of probability was used for post hoc comparisons to separate treatment differences.

3. Results

3.1. Root colonization rate

The soil used for rice growth contained 10% organic matter, 1.58 mg kg^{-1} extractable N, and 46.33 mg kg^{-1} extractable P, with a pH value of 5.8. No mycorrhizal colonization was detected in non-mycorrhizal roots, whereas the colonization rates of Guangyinzhan

Table 1
Analysis of covariance for arsenate, arsenite, DMA and MMA influxes for high-affinity and low-affinity experiments.

	Arsenate		Arsenite		DMA		MMA	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
High-affinity								
Rice variety	5.99	<0.05	0.75	0.39	3.81	0.06	2.45	0.13
AMF treatment	1.73	0.20	7.68	<0.05	0.01	0.94	5.78	<0.05
Concentration	44.66	<0.001	36.20	<0.001	27.79	<0.001	55.80	<0.001
Rice × AMF	29.25	<0.001	1.00	0.33	0.02	0.88	0.26	0.62
Rice × Conc.	2.90	<0.05	0.58	0.68	1.01	0.42	0.91	0.47
AMF × Conc.	0.77	0.56	2.88	<0.05	0.23	0.92	1.23	0.32
Rice × AMF × Conc.	5.86	<0.01	0.46	0.76	1.95	0.13	0.73	0.58
Low-affinity								
Rice variety	6.24	<0.05	10.80	<0.01	21.48	<0.001	15.90	<0.001
AMF treatment	33.26	<0.001	96.40	<0.001	1.72	0.20	11.67	<0.01
Concentration	16.95	<0.001	113.16	<0.001	21.93	<0.001	66.99	<0.001
Rice × AMF	4.45	<0.05	12.92	<0.01	0.50	0.48	1.04	0.31
Rice × Conc.	1.33	0.27	16.84	<0.001	3.44	<0.05	5.28	<0.01
AMF × Conc.	3.71	<0.01	21.71	<0.001	0.66	0.66	4.22	<0.01
Rice × AMF × Conc.	1.08	0.39	15.33	<0.001	0.40	0.85	0.42	0.83

and Handao 502 inoculated with *G. intraradices* were 42% and 58%, respectively. Table 1 indicates that mycorrhizal colonization had significant effects on As(III) ($P < 0.05$) and MMA ($P < 0.05$) uptake in the high-affinity uptake experiment, and As(V) ($P < 0.001$), As(III) ($P < 0.001$) and MMA ($P < 0.01$) uptake in the low-affinity uptake experiment.

3.2. High-affinity uptake kinetics of As(V), As(III), DMA and MMA

As(V) and As(III) influx in Guangyinzhan and Handao 502 inoculated with and without *G. intraradices* suggested a hyperbolic increase with increasing concentrations of As(V) and As(III) (Fig. 1). Furthermore, the concentration dependent influx data fit better to Michaelis–Menten functions using nonlinear curve fitting than to linear regressions (Table 2). The kinetic parameters for both the As species differed between mycorrhizal and nonmycorrhizal roots of Guangyinzhan and Handao 502. In Guangyinzhan, the average V_{max} for As(V) uptake by mycorrhizal roots (1305 nmol g^{-1} f wt h^{-1}) was 2-fold higher compared to nonmycorrhizal roots (571 nmol g^{-1} f wt h^{-1}), while the corresponding K_m values in mycorrhizal roots (0.0345 mM) was lower than nonmycorrhizal roots (0.0649 mM), which indicated the uptake carriers had a higher affinity. This was in contrast with Handao 502, the average V_{max} for As(V) uptake by mycorrhizal roots (1593 nmol g^{-1} f wt h^{-1}) was lower than nonmycorrhizal roots (2498 nmol g^{-1} f wt h^{-1}), while the corresponding K_m value in mycorrhizal roots (0.0918 mM) was higher than nonmycorrhizal roots (0.0894 mM). These suggested the rice cultivars had significant influence on As(V) uptake in high-affinity uptake system (Table 1). For As(III) uptake, both the V_{max} and K_m values in nonmycorrhizal roots were higher than mycorrhizal roots of Guangyinzhan and Handao 502 (Fig. 1). In addition, Table 1 and Fig. 1 show that in the low As concentrations, the As(V) uptake of both two rice cultivars was considerably higher than As(III) uptake.

Table 1 shows that DMA and MMA influx was concentration dependent ($P < 0.001$), with no significant difference between the rice varieties ($P > 0.05$) in high-affinity uptake systems. Concentration-dependent influx isotherms for both DMA and MMA in mycorrhizal and nonmycorrhizal roots of two rice cultivars fit well to an additive Michaelis–Menten function, except DMA uptake by mycorrhizal roots of Handao 502 (Table 2). At lower substrate concentrations, the influx data for DMA uptake by mycorrhizal roots of Handao 502 fit better to a linear model ($R^2 = 0.983$) rather than a nonlinear model ($R^2 = 0.981$). DMA and MMA uptake by mycorrhizal roots of Guangyinzhan all had a lower V_{max} and K_m than control (Table 2). Results presented in Table 1 and Fig. 2 suggested that mycorrhizal inoculation remarkably inhibited MMA uptake by two rice cultivars ($P < 0.05$).

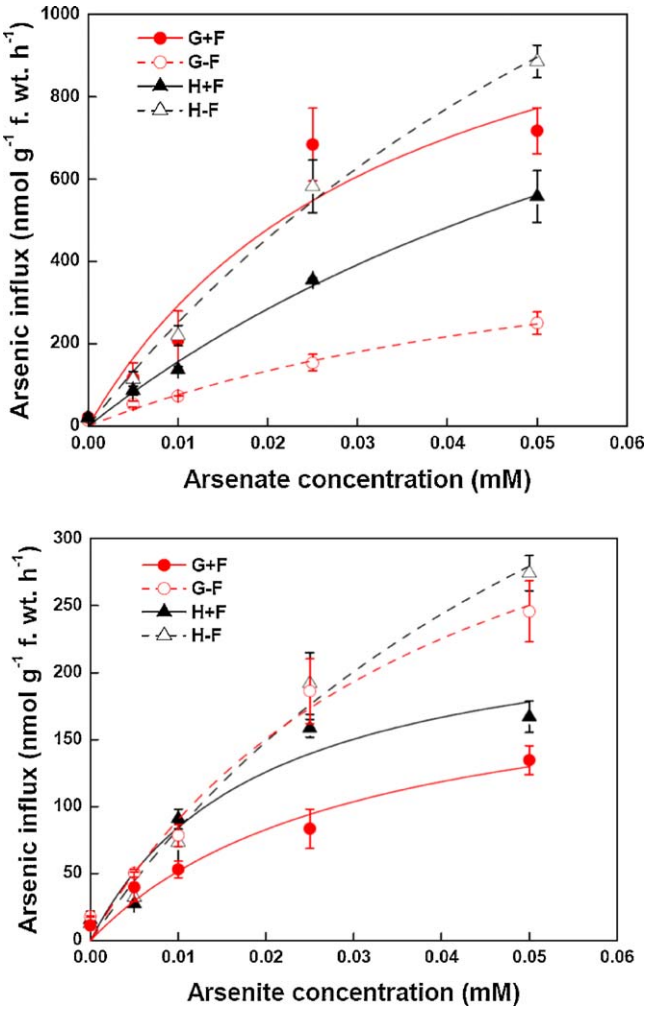


Fig. 1. Concentration-dependent kinetics for high-affinity root arsenate and arsenite influx of Guangyinzhan (G) and Handao 502 (H) inoculated with *Glomus intraradices* (+F) or uninoculated (−F). Each point is an average of four replicates. Error bars are ±S.D. of the replicates.

Table 2
Kinetic parameters for arsenate, arsenite, DMA, and MMA influx into roots of lowland rice (Guangyinzhan) and upland rice (Handao 502) inoculated with *Glomus intraradices* (+F) or uninoculated (−F). Kinetic parameters were calculated from mean As influx ($n = 4$) using Michaelis–Menten function (nonlinear regression) and linear regression model.

As conc.	Rice cultivars	As speciation and AMF	Nonlinear regression			Linear regression		
			V_{max} (nmol g^{-1} f wt h^{-1})	K_m (mM)	R^2	a (slope)	b (intercept)	R^2
High affinity	Guangyinzhan	Arsenate − F	571 ± 106	0.0649 ± 0.0188	0.992	4630	26	0.990
		Arsenate + F	1305 ± 379	0.0345 ± 0.0136	0.951	14,842	82	0.836
		Arsenite − F	449 ± 65	0.0397 ± 0.0106	0.990	4636	32	0.946
		Arsenite + F	208 ± 39	0.0302 ± 0.0115	0.974	2299	23	0.973
		DMA − F	295 ± 83	0.0722 ± 0.0307	0.985	2273	11	0.984
		DMA + F	120 ± 24	0.0173 ± 0.0085	0.940	1485	22	0.887
		MMA − F	828 ± 99	0.0764 ± 0.0136	0.998	6292	25	0.988
		MMA + F	461 ± 111	0.0524 ± 0.0211	0.980	4190	26	0.967
	Handao 502	Arsenate − F	2498 ± 472	0.0894 ± 0.0225	0.996	17,675	46	0.977
		Arsenate + F	1593 ± 268	0.0918 ± 0.0219	0.997	10,875	36	0.985
		Arsenite − F	678 ± 153	0.0714 ± 0.0245	0.990	5452	19	0.963
		Arsenite + F	246 ± 42	0.0192 ± 0.0077	0.966	3121	36	0.792
		DMA − F	170 ± 21	0.0179 ± 0.0055	0.977	2100	30	0.886
		DMA + F	1038 ± 982	0.2406 ± 0.2669	0.981	3459	7	0.983
		MMA − F	472 ± 110	0.0406 ± 0.0172	0.974	4939	29	0.903
		MMA + F	446 ± 75	0.0557 ± 0.0153	0.991	4082	18	0.953
Low affinity	Guangyinzhan	Arsenate − F	6232 ± 377	0.3632 ± 0.0677	0.987	1892	1553	0.799
		Arsenate + F	2008 ± 222	0.1605 ± 0.0705	0.933	634	722	0.662
		Arsenite − F	4848 ± 1443	0.5615 ± 0.4504	0.803	1314	899	0.447
		Arsenite + F	4175 ± 913	3.0257 ± 1.0510	0.982	708	182	0.981
		DMA − F	1392 ± 74	0.4622 ± 0.0706	0.990	415	294	0.798
		DMA + F	1633 ± 186	1.178 ± 0.2856	0.983	412	165	0.885
		MMA − F	37,801 ± 17,511	14.08 ± 7.507	0.996	2212	194	0.997
		MMA + F	7306 ± 1661	2.3450 ± 0.9143	0.972	1412	413	0.958
	Handao 502	Arsenate − F	3341 ± 502	0.1779 ± 0.1026	0.895	985	1176	0.477
		Arsenate + F	1564 ± 112	0.0781 ± 0.0296	0.962	332	818	0.292
		Arsenite − F	28,394 ± 15,774	15.7519 ± 9.9357	0.995	1509	128	0.997
		Arsenite + F	3806 ± 850	2.4693 ± 0.9313	0.973	722	195	0.960
		DMA − F	608 ± 47	0.3744 ± 0.0878	0.976	182	150	0.772
		DMA + F	548 ± 37	0.4036 ± 0.0815	0.982	163	132	0.818
		MMA − F	5734 ± 930	1.5947 ± 0.5009	0.974	1328	412	0.926
		MMA + F	4107 ± 655	2.6700 ± 0.7020	0.988	750	192	0.978

3.3. Low-affinity uptake kinetics of As(V), As(III), DMA and MMA

In the high concentration range, both As(V) and As(III) uptake can be described satisfactorily by the Michaelis–Menten equation better than linear curve, with the exception of As(III) uptake by nonmycorrhizal roots of Handao 502 (Fig. 3). However, the R^2 of the nonlinear curve (0.995) was very close to linear curve (0.997) in nonmycorrhizal roots of Handao 502. The V_{max} and K_m for As(V) uptake by nonmycorrhizal roots in Guangyinzhan and Handao 502 were two times higher than mycorrhizal roots (Table 2). The V_{max} for As(III) uptake by mycorrhizal roots was lower than nonmycorrhizal roots of two rice cultivars (Table 2). Table 1 and Fig. 3 show that As(V) and As(III) influx was concentration dependent ($P < 0.001$) with significant difference between the rice varieties ($P < 0.05$) in low-affinity systems.

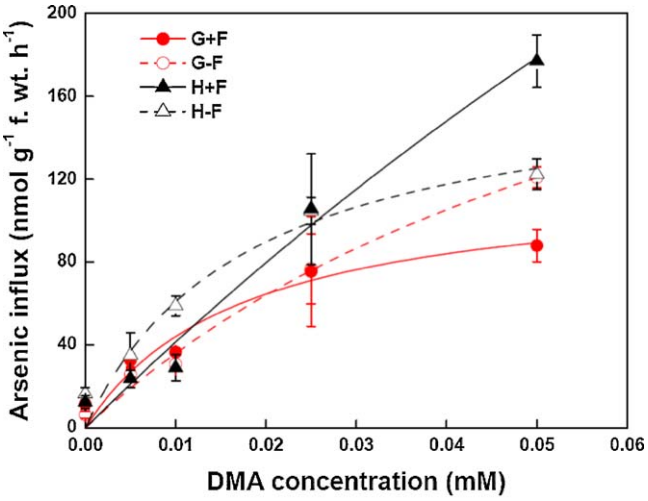
In the high concentration range (0–2.5 mM), short-term (20 min) uptake of MMA by nonmycorrhizal roots of Guangyinzhan was linear in relation to the external concentration (Fig. 4), but the other DMA and MMA uptake data exhibited a clear hyperbolic pattern. Table 2 indicates that V_{max} values for DMA uptake increased when Guangyinzhan was inoculated, whereas reduced when Handao 502 was inoculated. In addition, K_m values for DMA uptake raised in both of two mycorrhizal rice roots, which suggested uptake carriers in mycorrhizal rice tended to have lower-affinity combined with substrate. Both of the two rice cultivars had lower V_{max} for MMA uptake by mycorrhizal roots (Table 2). Results presented in Table 1 and Fig. 4 suggested that DMA and MMA influx was concentration dependent ($P < 0.001$) with significant difference between the rice varieties ($P < 0.001$) in low-affinity systems. Furthermore, the influx rate of DMA was much lower when compared with As(V), As(III), MMA in both two rice cultivars under high As concentration range (Table 2).

3.4. As species treatments

In the As(V)/As(III)/DMA/MMA treatments, the total As concentrations of shoots were 2.1–11; 4.5–6.2; 5.3–9.2; 1.2–4.8 mg kg^{-1}, and 44–94, 33–81, 41–75, 55–120 mg kg^{-1} in roots. Furthermore, the mean total recovery rates [(sum of species recovered from the TFA extraction/total As from acid digestion) × 100%] of shoots ranged from 84 to 102% and ranged from 83 to 103% in roots. In the As(V) treatment, the ratio of As(V)/As(III) in shoots of both two rice cultivars reduced because of the presence of AMF, while the ratio of As(V)/As(III) in roots increased (Fig. 5). Fig. 5 also shows that As(V) was the predominate As speciation in shoots of two rice cultivars in the As(III) treatment. In MMA treatment, a large amount of MMA was found in shoots of Guangyinzhan, however, no MMA was detected in shoots of Handao 502. Furthermore, in the DMA and MMA treatment, As(III) was detected in roots of Guangyinzhan rather than Handao 502. These differences between two cultivars suggested genotype variation exerted a notable effect on As speciation in host plants. In addition, the total As concentration in roots decreased significantly because the presence of AMF in the As(III) and MMA treatments.

4. Discussion

We studied two (high-affinity and low-affinity) uptake systems of As(V), As(III), DMA and MMA in mycorrhizal and nonmycorrhizal roots of lowland rice (Guangyinzhan) and upland rice (Handao 502).



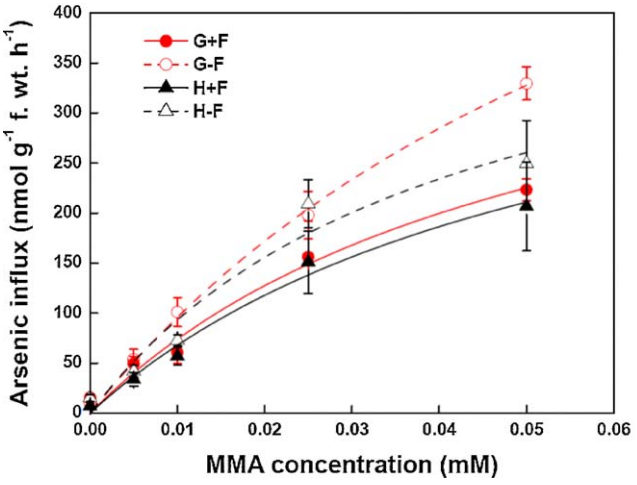
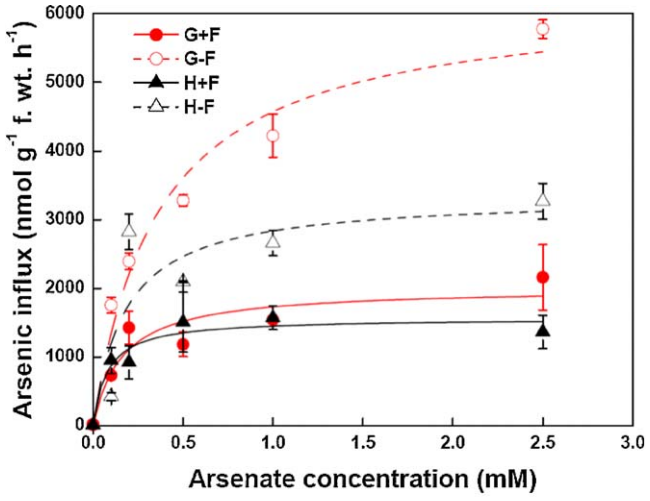


Fig. 2. Concentration-dependent kinetics for high-affinity root DMA and MMA influx of Guangyinzhan (G) and Handao 502 (H) inoculated with *Glomus intraradices* (+F) or uninoculated (−F). Each point is an average of four replicates. Error bars are ±S.D. of the replicates.



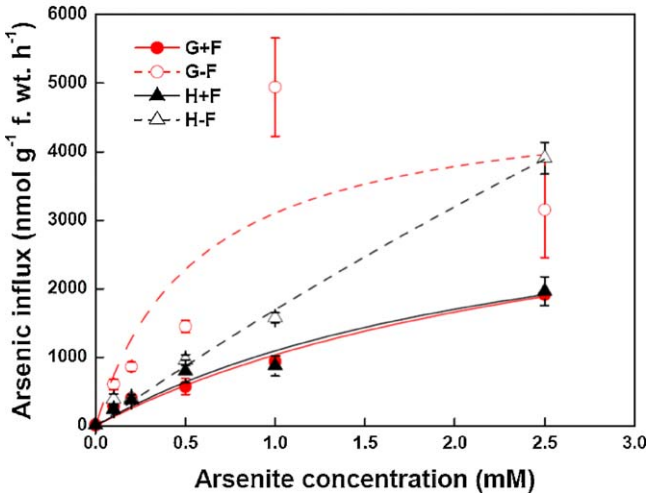


Fig. 3. Concentration-dependent kinetics for low-affinity root arsenate and arsenite influx of Guangyinzhan (G) and Handao 502 (H) inoculated with *Glomus intraradices* (+F) or uninoculated (−F). Each point is an average of four replicates. Error bars are ±S.D. of the replicates.

Concentration-dependent influx isotherms for the most of four As species uptake in mycorrhizal and nonmycorrhizal roots fit better to Michaelis–Menten function than linear regression model in the short term (Table 2). This suggests that most of the four As species uptake by mycorrhizal and nonmycorrhizal rice roots are an active process, which needs selective binding sites and energy supply as driving force. Abedin et al. [12] also reported that the uptake of As(V), As(III) and MMA by rice in high-affinity systems fit better to an additive Michaelis–Menten function, while the uptake of As(V) and As(III) fit better to a linear model in low-affinity systems that is different from this study, possibly due to the response of host plants with different genotype.

This study shows that the uptake of As(V), As(III), DMA and MMA by mycorrhizal and nonmycorrhizal rice roots was all concentration dependent in high or low-affinity uptake systems (Table 1). This agreed with previous studies performed in rice [12,27] and maize [15]. This may be due to the fact that substrate concentration is the most important factor affecting As uptake. Fig. 5 shows that a large amount of MMA was found in shoots of Guangyinzhan, while no MMA was detected in Handao 502 in MMA treatment, and As(III) was detected in roots of Guangyinzhan rather than Handao 502 in the DMA and MMA treatment. Furthermore, there were significant differences ($P < 0.05$) between two rice varieties in low-affinity uptake systems concerning the four As species influx. The reason was due to varietal differences existed in some physiological (transporter concentrations) or morphological (root length, root diameter and root hairs) attributes of root systems [12]. In this study, Handao 502 tend to possess a lower As(V), DMA, MMA influx than Guangyinzhan in the low-affinity uptake system (Table 2, Figs. 3 and 4). Therefore, kinetic uptake characteristics can be regarded as one of the important criteria for selecting a variety to use in areas contaminated by high As concentration.

The present results suggest *G. intraradices* had a significant effect on As(III) ($P < 0.05$) and MMA ($P < 0.05$) uptake by rice in high-affinity uptake experiments, and As(V) ($P < 0.001$), As(III) ($P < 0.001$) and MMA ($P < 0.01$) uptake in low-affinity uptake systems (Table 1). AMF colonization influencing the architecture of the host root system has been reported in Price et al. [28] and Paszkowski and Boller [29]. Furthermore, Gutjahr et al. [4] further demonstrated that *G. intraradices* preferentially colonizes large lateral rice roots and induces the formation of large lateral rice roots. Hence, the changes of architecture of rice root systems in response to AMF colonization may influence As species uptake and As uptake kinetics profoundly.

Mycorrhizal inoculation exerts a significant effect on As(V) uptake in low-affinity uptake system ($P < 0.001$) (Table 1 and Fig. 3).

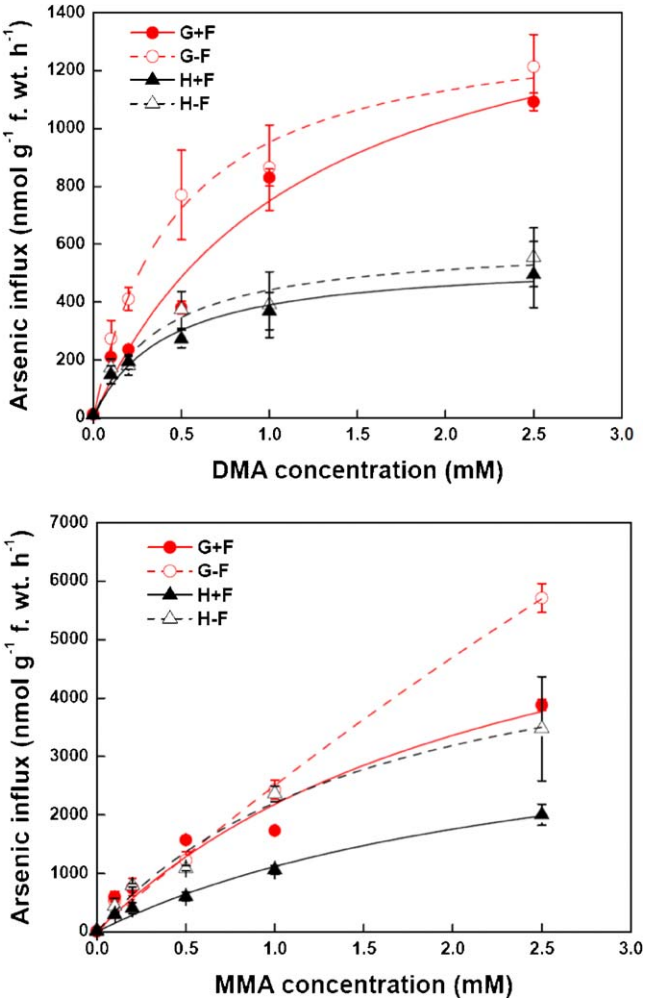


Fig. 4. Concentration-dependent kinetics for low-affinity root DMA and MMA influx of Guangyinzhan (G) and Handao 502 (H) inoculated with *Glomus intraradices* (+F) or uninoculated (−F). Each point is an average of four replicates. Error bars are ±S.D. of the replicates.

This is in accordance with the result of Yu et al. [16], which reported that mycorrhizal inoculation (*G. mosseae*) remarkably inhibited As(V) uptake in maize. Moreover, Leung et al. [30] have showed that the synergistic effect of mycorrhiza and P rock affected As subcellular distribution of *P. vittata*. Arsenate, which is a phosphate analogue, is taken up by the phosphate transport system, with phosphate competing much more effectively for transport sites in plants [31]. *Medicago truncatula* Pi transporters (MtPT4) indicated the presence of mycorrhiza-specific Pi uptake system in vascular plants [32]. Furthermore, Paszkowski et al. [33] proved that the AMF symbiosis with rice can induce the phosphate (P) transporter gene (*OsPT11*) which responsible for absorption of P. However, the extra P is provided by the AMF pathway which does not transport As(V) or has a higher selectivity for P than As(V) [34]. In addition, arbuscular mycorrhizal colonization reduces arsenate uptake via downregulation of transporters in the direct epidermal phosphate uptake pathway [35]. Thus as a result of the two combined forces, As(V) uptake can be reduced by AMF in short-term low-affinity uptake system.

In this study, the mycorrhizal inoculation of both Guangyinzhan and Handao 502 tended to reduce As(III) (Table 1, Figs. 1 and 3) and MMA (Table 1, Figs. 2 and 4) uptake in both high- and low-affinity uptake systems compared with no AMF. This is different from the result of Yu et al. [16] who indicated that mycorrhizal inoculation (*G. mosseae*) did not significantly influence the As(III) uptake by maize roots. This may be due to the different species of host plants on AMF response. Meharg and Jardine [10] showed that glycerol competes with As(III) for uptake into rice roots. Later, Ma et al. [36] found that As(III) transport belonged to the NIP subfamily of aquaporins in rice roots and shared the same highly efficient pathway as silicon, and that mutation in Lsi1, a silicon influx transporter significantly decreased As(III) uptake. Hence, the rice roots inoculated with AM fungi *G. intraradices* are able to release a substance that can either combine with Lsi1 as a substrate or can down-regulate Lsi1, causing inhibition of As(III) uptake in rice roots. Li et al. [37] also demonstrated that the NIP aquaporin Lsi1 mediates the influx of MMA into rice roots. Lsi1 is regarded as much more highly expressed in rice roots than other NIP genes [36,38], and would be the main aquaporin involved in As transport in rice roots. Thus, the reason for mycorrhizal inoculation reducing MMA uptake by rice may be the same as As(III) uptake. Both As(III) and MMA uptake can be controlled by Lsi1, therefore we speculate that the rice inoculated with *G. intraradices* is able to affect the NIP aquaporin Lsi1 in short-term. Furthermore, in As(III) and MMA treatments, the total As concentration in roots also decreased significantly ($P < 0.05$), because of the presence of AMF. It is consistent with the previous result that mycorrhizal inoculation can reduce As(III) and MMA influx notably, regardless of rice varieties. Thus, it can be explained that mycorrhizal roots may release some signaling molecule to down-regulate the expression of Lsi1, or mycorrhizal roots could produce some substances which can combine with Lsi1 as a substrate and bring a competitive relationship with As(III) and MMA.

In the low-affinity uptake system, DMA uptake rate was much less than As(V), As(III) and MMA significantly in both Guangyinzhan and Handao, regardless of AMF inoculation (Table 2, Figs. 3 and 4). This is in accordance with the result of Marin et al. [39] and Abbas and Meharg [15]. In the present study, DMA uptake by rice roots did not affect by mycorrhizal inoculation in the short-term (Table 1). Abbas and Meharg [15] suggested that DMA may be taken up by the phosphate transporters. However, Li et al. [37] demonstrated that the NIP aquaporin Lsi1 mediates a reduced amount of DMA influx into rice roots. Therefore, DMA uptake by rice roots is not yet clear. The factors which mediate the DMA uptake by rice deserve further investigation.

In the arsenate treatment, the ratio of As(V)/As(III) in shoots reduced because of the presence of AMF, while the ratio of As(V)/As(III) in roots increased due to the existence of AMF (Fig. 5). Yu et al. [16] also found more As(V) and less As(III) were accumulated in mycorrhizal roots, compared to those in nonmycorrhizal maize roots. This is due to lower arsenate reductase (AR) activities in mycorrhizal roots leading to less As(V) being reduced to As(III), compared with nonmycorrhizal roots [16]. The reduction in the ratio of As(V)/As(III) in shoots may be due to the fact after As(V) is transported into the shoots, more As(V) is reduced to As(III) by arsenate reductase in mycorrhizal rice, and then more As(III) is pumped into vacuole or out of cell to detoxify As [20,40]. Our study suggested that As(V) is the predominate As speciation in shoots in the 1 mM arsenite treatment (for 20 min). This is in accordance with the results obtained by Abedin et al. [27], who reported that the proportion of As(V) was 72–84% in shoots of rice grown under flooded conditions. However, it is different from the results of Su et al. [41], who indicated that As(III) was found to be the predominant form of As transported in the xylem sap of rice in the 5 μM arsenite treatment (for 24 h), and Meng et al. [42], who showed that As(III) took up 90% in the shoots whether in wild type or transgenetic rice exposed to 10 μM arsenite (for 4 weeks). The opposing results may be due to the rice genotypes and the time and As concentrations of treatments, but further research is needed before

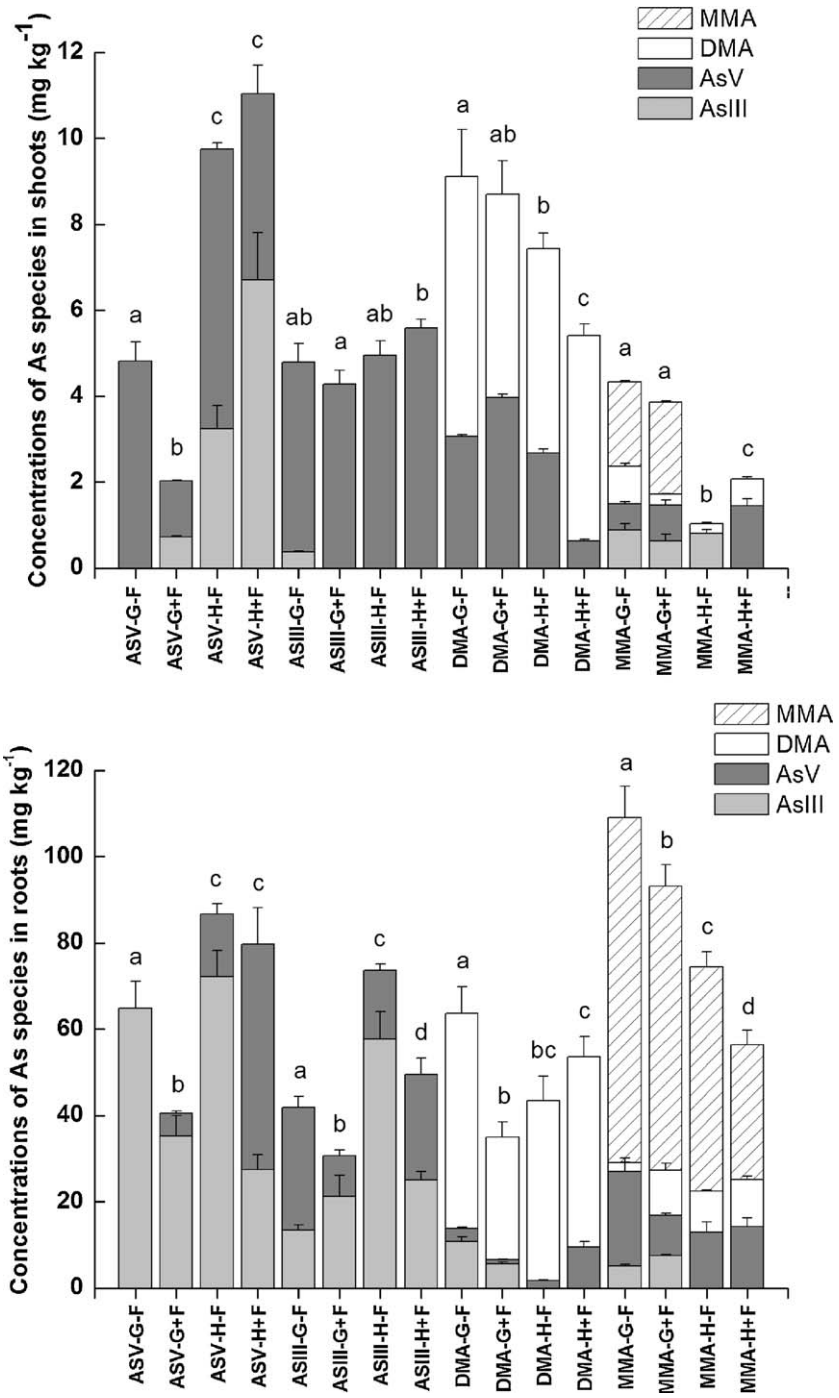


Fig. 5. Concentrations of As species [arsenate (As(V)), arsenite (As(III)), DMA, MMA] in shoots and roots of Guangyinzhan (G) and Handao 502 (H) inoculated with *Glomus intraradices* (+F) or uninoculated (−F) in solution added with 1 mM As(V), As(III), DMA or MMA. Different letters above the bars in the same As species treatment indicate a significant difference at $P < 0.05$ (Duncan test), $n = 4$.

obtaining a more concrete conclusion. No organic As species was found in As(V) or As(III) treatment, which suggests that a long-time is needed for inorganic As to be transformed into organic species.

5. Conclusion

This study is the first to report the effects of mycorrhizal inoculation on As species uptake by rice in short-term. It reveals that mycorrhizal inoculation reduced the arsenate uptake by Guangyinzhan and Handao 502 significantly ($P < 0.001$) in low-affinity uptake system, which suggested that phosphorus (P) transporters may be influenced by AMF in high arsenate concentrations. Moreover, mycorrhizal inoculation also decreases the uptake of arsenite and MMA by Guangyinzhan and Handao 502 markedly ($P < 0.05$) in both low- and high-affinity uptake systems, which indicates that mycorrhizal roots may release some signaling molecules to down-regulate the expression of Lsi1 or combine with Lsi1 as a substrate. The findings of this research will contribute to our understanding of the role of AM fungi in As resistance to rice. Long-term As species uptake kinetics needs further investigation.

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