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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