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RESEARCH ARTICLE

Mycorrhizal colonization status of lowland rice (*Oryza sativa* L.) in the southeastern region of China

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Abstract The accumulation, distribution, and speciation of contaminants, such as arsenic, in rice can be affected by soil microorganisms such as arbuscular mycorrhizal fungi (AMF). As a potential measure to control contaminant acquisition in rice, the status and performance of AMF in the field need to be investigated. Root samples of rice plants were collected in seven different cities in Guangdong, Jiangxi, Hubei, and Jiangsu Provinces in China in order to investigate the colonization rate of AMF. The total DNA of the roots was extracted, followed by PCR and sequencing, and further confirmed the existence of AMF. The highest colonization rates (19.5 ± 7.2%) were observed in samples from Huizhou City, Guangdong Province. Sequences of ribosomal DNA derived from Pingtan (PT) and Shuikou (SK) in Huizhou shared a similarity of 73 and 86% to *Glomus* cf. *clarum* Att894-7 (FM865542) and "uncultured fungus" (EF434122.1), respectively. The moisture tolerance of the AMF from different sources was tested by subjecting to different levels of water content in the soil. Only AMF from PT, SK, and LJ colonized rice under a condition of 100% of the soil water holding capacity (WHC), but not those isolated from upland plants. The AM colonization rate could be governed by the lighting conditions and temperature. AMF isolated in paddy fields has been shown to have more tolerance to moisture than other

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upland species. Radial oxygen loss (species and stress dependent) could be an essential factor influencing the colonization rate and requires more investigation.

Keywords Waterlogged · Mycorrhizae · Lowland rice · Fungal identification · PCR · Arsenic

Introduction

Contamination (e.g., arsenic pollution) in paddy rice (Meharg and Rahman 2003; Meharg 2004) has given rise to a significant amount of research, focusing on the mechanism of the contaminants uptake (Abedin et al. 2002; Ma et al. 2008; Li et al. 2009a; Zhao et al. 2009) and the development of mitigation measures using water management (Takahashi et al. 2004; Arao et al. 2009), silicon addition (Guo et al. 2005; Guo et al. 2007; Bogdan and Schenk 2008; Li et al. 2009b; Wu et al. 2016), and mycorrhizal inoculation (González-Chávez et al. 2011; Li et al. 2011; Chen et al. 2012; Chen et al. 2013).

It has been shown that the potential role of arbuscular mycorrhizal fungi (AMF) in rice production system should not be neglected (Smith et al. 2010; Smith and Read 2008). Mycorrhizal symbiosis not only alters the root architecture (Smith and Read 2008), but also the way of plants in taking up nutrients (Barrett et al. 2011; Liu et al. 2012) and in regulating pollutant transporters (Chen et al. 2007; Chen et al. 2012; Paszkowski et al. 2002). AMF also interact with other soil microbes which can improve nutrient cycling and the soil structure (Barea et al. 2005; Bonfante and Anca 2009). In addition, paddy fields are considered as one of the major contributors to global warming, emitting methane (Neue 1993), while mycorrhizal symbiosis plays a critical role in the global carbon cycle (Cheng et al. 2012; Kaschuk et al. 2009; Treseder and Allen 2000). The abovementioned studies have drawn attention to investigation on the status and performance of existing mycorrhizae in rice paddy fields, which is important to soil ecology, soil carbon cycling, rice nutrients, and contaminant acquisition.

It has been pointed out that AMF are not suitable for growing under anaerobic/flooded conditions (Bohrer et al. 2004; Carvalho et al. 2001; Oliveira et al. 2001; Smith and Read 2008; Yan et al. 2008). However, mycorrhizal symbiosis associated with *Armeria maritima*, *Salicornia europaea*, *Carex*, and *Scirpus* grown in wetlands has been observed, and such plants do not harbor AMF in aerobic conditions (Bauer et al. 2003; Hildebrandt et al. 2001). A greater number of plant species has been eventually found to be mycorrhizal, including plants found in wetlands, salt marshes, transition zones, lakes, and streams (Carvalho et al. 2004; Cornwell et al. 2001; Miller 2000; Miller and Sharitz 2000; Sudova et al. 2011; Sumorok and Kiedrzynska 2007; Wang and Zhao 2006).

Lowland rice grown in agriculture paddy fields can be potentially colonized by AMF. Li (2011) reported that the colonization rates of AMF in rice, collected from nine different sites in suburban areas in Chenzhou City, Hunan Province, China, were approximately 5% (Li 2011). A larger survey scale is worthwhile to gain a better picture on the AMF colonization in lowland rice from different areas of the Southeastern China: a region with high density of population with rice serving as the staple food. Thus, the objectives of this study are to (1) assess the colonization status of AMF associated with rice growing in the fields of Southeastern China and (2) investigate soil moisture tolerance of the AMF cultured from the field.

Materials and methods

Sample collection

The paddy rice plants (including roots, shoots, and rhizospheric soils) were collected by digging soil samples of dimensions $30 \times 30 \times 30$ cm^3. Ten villages from seven cities were covered (Table 1). In each village, there were four sampling sites covered. Four bunches of rice plants were collected in each sampling site, and the distance between the bunches was about 20 m. The samples were transported to the laboratory within 3 days. Roots, shoots, and soils were separated and the roots were preserved in > 80% of ethanol and stored at −20 °C prior to the colonization rate measurement and DNA extraction. The shoots were oven-dried at 60 °C for 72 h (Cornelissen et al. 2003) and the soils were air-dried before further analyses.

Soil properties analyses

Due to the higher AMF colonization rates (~19.5%) (Fig. 1a) in rice plants from Huizhou, the soils collected in Huizhou were analyzed for pH, extractable phosphorus (ExP), potassium (K), magnesium (Mg), iron (Fe), lead (Pb), zinc (Zn), manganese (Mn), copper (Cu), and arsenic (As). For pH, the sieved soils (2 mm) were weighed (~5 g) and put in a flask mixed with 100 ml of deionized water. The flask was shaken for 2 h and measured by a pH meter (420A, Orion, USA). The ExP content was extracted using 0.5 M of $NaHCO_3$ solution followed by the molybdenum blue method using a spectrophotometer (UV-1601, Shimadzu, Japan). For K, Mg, Fe, Cu, and Mn, the soil (~0.5 g) was digested according to EPA method 3051 (Environmental Protection Agency, USA) and measured using flame atomic absorption spectrometry (FAAS) (SpectrAA-20, Varian, USA). For Pb, Zn, and As determination, inductively-coupled plasma mass spectrometry (ICP-MS) (Elan9000, PerkinElmer, USA) was used. Standard reference material (SRM 2709, San Joaquin Soil, NIST, USA) and digestion blanks were used for quality control and assurance. The recovery rates of metals were within 85 ± 10%.

Table 1 Description of the paddy field sampling sites

Province	City	Village	Geographic parameters	Rice cultivars
Guangdong	Huizhou	Shuikou	114° 27′ 51.8″ E/23° 09′ 30.8″ N	Boyou 998
		Pingtan	114° 35′ 65.6″ E/23° 05′ 19.7″ N	Qiuyou 998
		Liangjing	114° 31′ 36.8″ E/22° 58′ 14.1″ N	Huayou 665
		Lianghua	114° 39′ 44.4″ E/23° 06′ 21.8″ N	Boyou 998
	Heyuan	Guzhu	114° 42′ 48.4″ E/23° 30′ 48.1″ N	Huayou 625
	Meizhou	Sanjiao	116° 07′ 06.9″ E/24° 15′ 51.8″ N	Quanyou 2689
	Shaoguan	Lechang	113° 20′ 12.5″ E/25° 08′ 19.8″ N	Tianyou 116
Hubei	Jingzhou	Gong'an	112° 17′ 29.9″ E/29° 59′ 05.4″ N	Liangyou 6326
Jiangxi	Ji'an	Shuangcun	115° 13′ 15.7″ E/27° 23′ 28.1″ N	Quanyou 463
Jiangsu	Xuzhou	Longhe	118° 21′ 37.1″ E/34° 15′ 53.1″ N	Xuyou 631

AMF colonization investigation

The slide length method (Giovannetti and Mosse 1980) was used to assess the AMF colonization rates. AMF photos were taken using a microscope (Axioskop2, Carl Zeiss, Germany).

Polymerase chain reaction for amplification of rDNA fragments of AMF in rice root

Total DNA of the rice roots was extracted using a DNA extraction kit (DNeasy Plant Mini Kit, Qiagen, Germany). AMF specific primers (Krüger et al. 2009), targeting on a range containing small subunit (SSU) ribosomal DNA (rDNA), internal transcribed spacer (ITS) region, and large subunit (LSU) rDNA were used for the PCR (including first and nested PCR) using a Phusion High-Fidelity PCR Kit (Finnzymes, Finland). PCR products were loaded on 1% agarose gel with 1 × sodium borate buffer (Brody and Kern 2004) at 150–200 V and imaged using a gel imaging system (Gel Doc, Bio-Rad Laboratories, USA) after ethidium bromide staining (1 μg ml^{-1}) (Krüger et al. 2009). The PCR products were sequenced using primer NDL22 (van Tuinen et al. 1998). The obtained sequence data were used to align with the known AMF sequences in the database of National Center for Biotechnology Information (NCBI).

Trap culture of AMF and spores extraction

The corresponding rhizospheric soils (containing pieces of rice roots) from Huizhou (sub-sites: Shuikou, Pingtan, Liangjing, and Lianghua) were air-dried at 25 °C for 1 week. The soils were mixed with sterilized sand (soil:sand = 3:1). Three kilograms of the mixture was put into a pot (diameter 16.8 cm, height 15 cm). Approximately 50 seeds of Sudan grass (*Sorghum bicolor*) (provided by Guangdong Academy of Agricultural Sciences, Guangzhou, China) were sown. Deionized water was added every 2 days. The grasses were allowed to grow for 4 months in a greenhouse (28/22 °C, day/

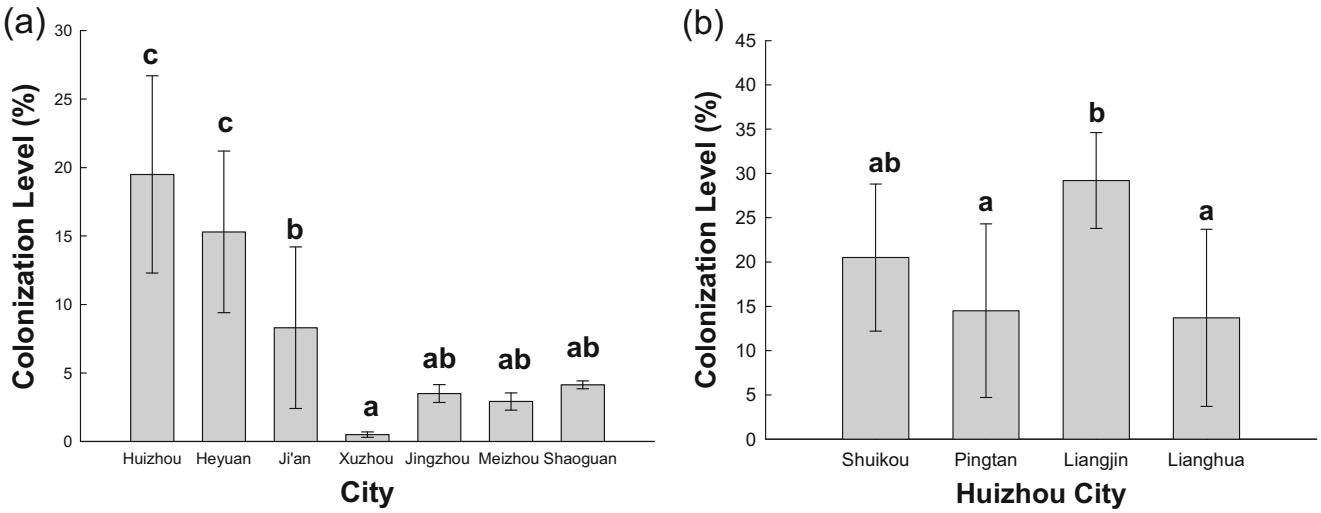

Fig. 1 Comparison of the AMF colonization rates among different cities (**a**), and AMF colonization rates of rice collected from four different sites in Huizhou City (**b**). Different *letters* (*a*, *b*, and *c*) indicate significant difference between different treatments at the level of $P < 0.05$ (Duncan's multiple range test). Data are mean ± S.D. ($n = 4$)

night, 85% of relative humidity), under a random block design. In addition to natural sunlight, 12-h photon flux density of 300 mmol m^{-2} s^{-1} was provided via an assembly of cool-white fluorescent lamps.

The spores in the soils were extracted using the method provided by the International Culture collection of AMF (INVAM 2016) using 100 g of soil for each pot. Extracted spores were observed using a stereomicroscope (SZXZ-ILLT, Olympus, Japan).

AMF moisture tolerance test

AMF medium cultured using the soils from Shuikou (SK), Liangjing (LJ), Lianghua (LH), and Pingtan (PT) in Huizhou City were used for the tolerance test due to their higher colonization rates (Fig. 1). The AMF medium were mixed with sterilized sand (medium:sand = 1:3) and inoculated to a lowland rice (Boyou 998, a commercial cultivar in Guangdong Province). Different soil moisture contents were achieved by adjusting to 25, 50, 75, and 100% of the soil water holding capacity (WHC). There were three plants in each pot and three replicates for each treatment.

The other three AMF inocula from Beijing Academy of Agriculture and Forestry Sciences, including *Rhizophagus intraradices* (BJ09), *Funneliformis mosseae* (GZ01A), and *F. mosseae* (NM01A), isolated from *Solanum lycopersicum*, *Styphnolobium japonicum*, and *Allium tenuissimum*, respectively, were also used for comparison. Plants without AM inoculation served as a control. Hoagland solution (Hoagland and Arnon 1950) was applied when plants showed signs of phosphorus deficiency (purpling of leaf sheaths) or nitrogen deficiency (chlorosis of young leaves) (INVAM 2016). After 2 months, the rice roots were collected to investigate the levels of AMF colonization.

Statistical analyses

One-way ANOVA was used to compare the mean values of the soil properties and colonization rates of AMF. Duncan's multiple range test at a probability level of 5% was used for post hoc comparison to separate the differences. The Pearson correlation was used to explore the correlation coefficients among the parameters. All statistical analyses were conducted using SPSS v16.

Results

Soil properties from Huizhou City

The highest concentration of extractable P was derived from LH (37.9 mg kg^{-1}). The arsenic concentration (29.9 mg kg^{-1}) from PT was significantly higher ($P < 0.05$) than at other sites (Table 2). The concentrations of Mn (136 mg kg^{-1}), Zn (45.6 mg kg^{-1}), Pb (34.1 mg kg^{-1}), and Mg (652 mg kg^{-1}) were significantly ($P < 0.05$) higher in soil from SK than those of other sites, while the pH value (4.63) was significantly lower ($P < 0.05$). The highest concentration of Cu (13.4 mg kg^{-1}) was derived from LH.

AMF colonization status

The colonization rates of AMF in roots collected from different cities varied (Fig.1a). The highest colonization rates (19.5 ± 7.2%) were observed in the samples collected in Huizhou City, followed by Heyuan City (Fig. 1a). The highest colonization rate in all samples was found in the LJ site in Huizhou City (29.2%) (Fig. 1b). Structures of vesicles and hyphae were mostly observed, while arbuscules could only be found in some roots. Both vesicles and hyphae were the most abundant structures in the samples from Huizhou City (Fig. S1).

Identification of AMF using PCR

Target rDNA products derived from Huizhou City, including first and nested PCR products (1500–1800 bp), were amplified (Fig. 2). Nested PCR products (~1500 bp) derived from PT are shown in lanes N1-N3 of Fig. 2a. First PCR products (~1800 bp) and nested PCR products (~1500 bp) of SK are shown in lane F2 of Fig. 2b and in lane N3 of Fig. 2c, respectively. For lane N3 of Fig. 2c, the concentration of the target fragment (~1500 bp) was low, with only a trace amount observed. The first PCR products of PT and SK (Fig. 2d) were used for sequencing.

The basic local alignment search tool (BLAST) from the NCBI was used to align the sequence data of PT and SK. For the PT site, the sequence of the PCR product showed a similarity of 73% to the arbuscular mycorrhizal fungus *Glomus* cf. *clarum* Att894-7 (accession no.: FM865542). For the SK site, the results showed that the sequence shared a similarity of 86% to the "uncultured fungus" (accession no.: EF434122.1).

Cultured spores

The AMF spores, extracted in the soils from LJ, SK, PT, and LH, are shown in Fig. S2. The spore density was the highest in PT which was 93 ± 6 in 100 g soil (air-dried), followed by LJ, LH, and SK which were 49 ± 4, 32 ± 2, and 30 ± 5, respectively (data refer to mean values ± S. D., with $n = 3$).

Moisture tolerance test

Although the colonization rates were lower than 4% for all plants, AMF was present in the root grown under the condition of 100% of soil WHC (Table 3). All AM species could

Table 2 Soil properties of the four sites from Huizhou City

Element (mg kg^{-1})	PT	SK	LJ	LH
Mn	73.0 ± 18.4 b	136 ± 6.3 c	39.9 ± 4.5 a	27.8 ± 0.7 a
Zn	37.4 ± 2.5 b	45.6 ± 2.5 c	18.7 ± 1.4 a	40.3 ± 2.4 bc
Pb	24.4 ± 6.2 a	34.1 ± 4.1 a	28.8 ± 3.6 a	30.5 ± 3.9 a
Fe	13,830 ± 419 d	11,660 ± 1100 c	5484 ± 88 a	7593 ± 193 b
Cu	6.0 ± 0.2 b	10.9 ± 0.6 c	4.2 ± 0.2 a	13.4 ± 0.8 d
Mg	222 ± 23.0 a	652 ± 75.4 b	97.8 ± 8.7 a	181 ± 11.8 a
K	906 ± 154 b	± 39.8 b	322 ± 34.8 a	351 ± 45.5 a
As	29.9 ± 0.5 d	4.7 ± 0.2 b	3.0 ± 0.1 a	12.7 ± 0.6 c
Extractable P	10.8 ± 1.2 a	16.8 ± 2.5 ab	19.6 ± 0.5 b	37.9 ± 4.0 c
pH	5.5 ± 0.2 b	4.6 ± 0.1 a	5.5 ± 0.1 b	5.3 ± 0.01 b

Different letters within the same row indicate significant difference among sites at the level of $P < 0.05$ (Duncan's multiple range test). Data are mean ± S.D. ($n = 3$). The unit mg kg^{-1} is not applicable to pH

PT Pingtan site, *SK* Shuikou site, *LJ* Liangjing site, *LH* Lianghua site

colonize the rice under conditions of 50 and 75% of soil WHC, except for the species from LH which formed symbiosis with rice under 25% (2.9%) and 100% (3.6%) of soil WHC. No colonization was observed in rice inoculated with the purchased AMF (i.e., BJ09, GZ01A, and NM01A) under 100% of soil WHC. However, SK, AMF colonization was observed in the plants inoculated with inocula cultured using the soils from PT (1.7%), LH (3.6%), and LJ (1.8%) (Table 3). Both vesicles and hyphae were observed.

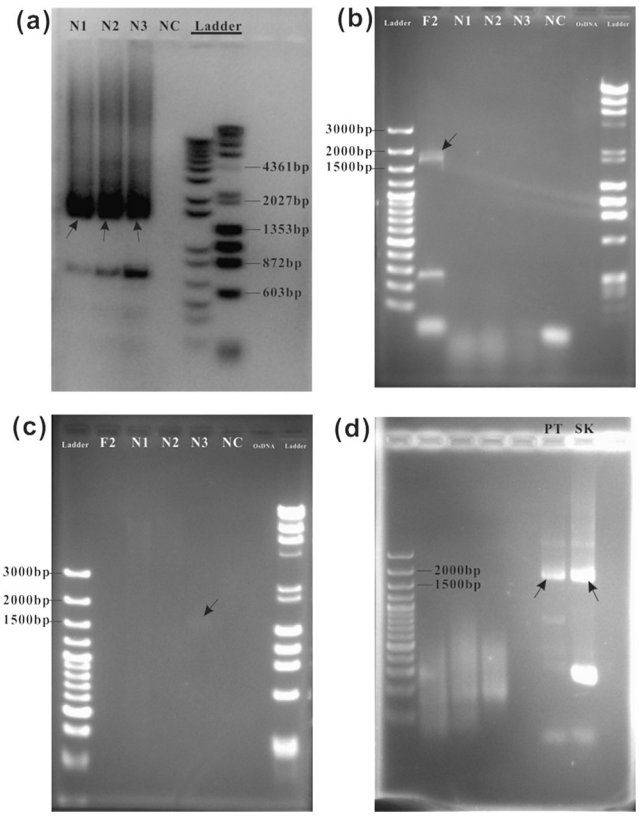

Fig. 2 Images of PCR products amplified by first and nested PCR. *N1-3* nested PCR product, *F2* first PCR product, *NC* negative control, *OsDNA* rice DNA template, *PT* and *SK* PCR product derived from PT and SK, respectively. Nested PCR product (~1500 bp) amplified using the samples collected in PT, Huizhou City (**a**). First PCR product (~1800 bp) of SK is presented in lane F2. There was no presence in N1, N2, and N3 for nested PCR products (**b**). Nested PCR product (~1500 bp) of SK is presented in lane N3 (**c**). First PCR products derived from PT and SK (**d**)

The biomass of rice plants grown under the conditions of 75% (2.22–2.48 g pot^{-1}) and 100% (2.40–2.51 g pot^{-1}) of soil WHC was significantly higher ($P < 0.05$) than those under 25% (0.62–0.81 g pot^{-1}) or 50% (1.30–1.58 g pot^{-1}) (Table S1).

Discussion

AMF colonization observed in lowland rice

The AMF colonization rates in lowland rice ranged from 0.5 to 19.5% (Fig. 1). Similarly, Miller (2000) showed that the plants in flooded areas form mycorrhizal association and the colonization rates were lower than 20%. AMF colonization rates in *Isoëtes lacustris* and *I. echinospora* from submerged fields were 4.0 and 13.3%, respectively (Sudova et al. 2011). These ranges of AMF colonization rates are similar to the results of the present study, showing the relatively lower AMF colonization rates in wetland plants, whereas in upland plants, the colonization rates could be up to 80–90% (Smith and Read 2008).

The colonization rates of AMF in the rice fluctuated even under controlled conditions. Previous greenhouse studies showed that AMF colonization rates in rice, under non-flooded conditions, were 25.9–40.4% (cultivar: Gulmont) (Dhillion and Ampornpan 1992) and 22–43% (cultivar: Shafagh) (Hajiboland et al. 2009a), while under flooded conditions, the rates were 7–27% (cultivar: Shafagh), 8–27% (cultivar: Fajr) (Hajiboland et al. 2009b), and 12–27% (cultivar:

Table 3 The results of AMF colonization rate of the moisture tolerance test

% of WHC	SK	PT	LH	LJ	BJ09 (Ri)	GZ01A (Fm)	NM01A (Fm)	Control
25	0	0	2.9 ± 0.7	0	0	0	0	0
50	0.1 ± 0.04	1.5 ± 1.0	0	0.1 ± 0.03	0	3.5 ± 1.4	0.1 ± 0.03	0
75	0	0	0	0	0.2 ± 0.15	0	0	0
100	0	1.7 ± 0.7	3.6 ± 1.0	1.8 ± 0.7	0	0	0	0

SK, PT, LH, and LJ are inocula cultured from rhizospheric soils collected in Shuikou, Pingtan, Lianghua, and Liangjing sites in Huizhou City, respectively. BJ09, GZ01A, and NM01A are the purchased inocula which are *Rhizophagus intraradices* (Ri), *F. mosseae* (Fm), and *F. mosseae* (Fm), respectively. Data are mean ± S.D. ($n = 3$)

WHC water holding capacity, "*0*" not detected

Shafagh) (Hajiboland et al. 2009a). A field study also showed that AMF colonization rates in rice could be up to 40% (cultivar: Vandana) under non-flooded conditions (Maiti et al. 2011). Together with the present study, it indicates that the percentages of AMF colonization in rice are relatively lower under flooded conditions than non-flooded conditions. This implies that it is important to find out whether a low colonization rate of mycorrhizae (< 20%) can affect the plant performance significantly (e.g., contaminant uptake).

The present results also showed that the AMF colonization rates were negatively correlated with the latitudes of the sampling sites ($R = -0.68$, $P = 0.046$). This could be caused by the effects of different light conditions and temperatures at different sites. It has been shown that higher light density results in higher levels of AMF colonization in onions (*Allium cepa* L.) (Son and Smith 1988). The AMF colonization rate observed in Shaoguan was 4.13% (Fig. 1a) which was similar to that of < 5% reported by Li (2011) in lowland rice collected from nine different sites in paddy fields in Chenzhou, Hunan, China (Li 2011), which has a similar latitude as Shaoguan.

Mycorrhizal symbiosis is also AM species and host dependent. The colonization rates varied significantly between different host plants in a single site while the colonization rates of one particular host plant species in different sites showed minor differences (Carvalho et al. 2003; Wang et al. 2004). Rice samples of the present study included several rice cultivars such as Boyou 998, Qiuyou 998, and Huayou (Table 1). The cultivar Boyou 998 was grown in SK and LH and the colonization rates of AMF on rice from these sampling sites were 20.5 and 13.7%, respectively (Fig. 1b). This was similar to a study in which minor differences in colonization rates (9.2, 8.0, 7.8, and 9.0%) were observed in the same host (*Cirsium setosum*) grown in different sites (Changyi, Wudi, Shouguang, and Dongying, respectively) in the Yellow River Delta, China (Wang et al. 2004). This was also the case for other plant species, *Tamarix chinensis* (colonization rate 2.5–3.1%), *Phragmites communis* (0.3–0.6%), *Suaeda glauca* (0.8–1.0%), and *Aeluropus littoralis var. sinensis* (2.8–3.5%), which were collected in those four sites (Wang et al. 2004). Since rice cultivars varied from province to province in the present study, it is therefore not appropriate to compare the colonization rates among different sites.

The most common AM structures observed within the roots were vesicles and hyphae in the present study, which is similar to the studies focusing on field samples of floating mats (*Typha angustifolia* L., *T.* × *glauca* Godr., and *Typha latifolia* L.) (Stenlund and Charvat 1994). Hyphae were the most common structures observed in plants *I. lacustris*, *I. echinospora* (Sudova et al. 2011), and lowland rice (Li 2011), rather than vesicles and arbuscules. Although arbuscules were not found, the abundance of hyphae, vesicles, and spores indicated the presumed symbioses occurred between AMF and their hosts under anaerobic conditions (Stenlund and Charvat 1994). This raises the question as to whether resources exchange within these limited numbers of arbuscule-containing cells can significantly affect the plant performance.

PCR and sequencing results further confirmed the AMF colonization

Our results showed that the lengths of the base pair of the amplified rDNA fragments were in line with the results obtained by Krüger et al. (2009), with ~1800 bp for first PCR and ~1500 bp for nested PCR (Fig. 2). These findings, together with the AMF morphological results (Fig. S1), confirmed the existence of the AMF in the samples. The DNA sequence of this AMF species *Glomus* cf. *clarum* Att894-7 was first deposited in the NCBI database by Stockinger et al. (2009). For samples from SK, the obtained rDNA sequence has 86% similarity with the "uncultured fungus" (accession no.: EF434122.1). This sequenced fragment of DNA was derived from soil samples and first deposited in the NCBI database by Taylor et al. (2007). Further identification, as well as on more other samples, is needed.

Relationship between soil properties and AMF colonization rates in Huizhou

AMF colonization rates in rice from Huizhou City was not significantly correlated with extractable P concentrations

(R^2 = −0.224, P = 0.242). In contrast, Son and Smith (1988) (studied onions, *Allium cepa* L.), Dhillion and Ampornpan (1992) (studied rice), and Paszkowski et al. (2002) (studied lowland rice) showed that lower P content led to significantly higher levels of AMF colonization. This contradiction may be caused by (1) a waterlogged condition offers mobilized nutrients, especially P and N; thus, the initiation of symbiosis may become unnecessary (Bowden 1987; Jensen et al. 1998; Lucassen et al. 2004; Young and Ross 2001) and (2) the initial nutrient concentrations in soils (before cultivation) were different from site to site which made it difficult to compare after cultivation.

The lowland rice, especially that obtained in Guangdong Province, possessed the colonization rates up to 19.5% (Fig. 1a). This may be due to the lowland rice roots being able to secrete oxygen through the aerenchymatous roots (radial oxygen loss) to the rhizosphere (Xu et al. 2007) via radial diffusion, therefore providing oxygen to the AMF, which are considered to be oxygen dependent (Smith and Read 2008; Wang et al. 2004). Evaluation of the radial oxygen loss for different cultivars could be essential in investigating why the AM colonization rate depends on species but not locations (Wang et al. 2004).

Moisture tolerance test

Although the AMF colonization rates were low (< 4%), AMF could be observed in the roots of the same cultivar inoculated with AMF isolated from PT, LH, and LJ under the condition of 100% of soil WHC. In contrast, AMF were absent in rice inoculated with BJ09 (*R. intraradices*), GZ01A (*F. mosseae*), and NM01A (*F. mosseae*), which are considered as upland species (Table 3). Both the host and AMF species have important effects in the colonization rate. By studying more than 20 plant species collected from salt marshes, it has been shown that the colonization rate varied from 0% (*Puccinellia distans*, *Triglochin maritimum*) to 96% (*Aster tripolium*) (Hildebrandt et al. 2001). Another study also showed that the colonization rates varied from 3 to 90%, even in one individual plant species (*Phalaris arundinacea*) of the 21 wetland species investigated (Bauer et al. 2003). However, the radial oxygen loss of the wetland plants, which can be considered as one of the important factors governing the colonization rate, was not investigated. Under field conditions, the radial oxygen loss can be different even in the same species. This could be one of the key factors leading to the distinct AMF colonization rates in the same species. Li et al. (2011) also showed that the colonization rate of AMF was 35.8% in lowland rice (cv. Guangyingzhan) inoculated with *R. intraradices* and was 5.15% inoculated with *Glomus geosporum*. Most AMF investigated in the present study could colonize the rice grown under the condition of 50% of soil WHC (Table 3). Li (2011) also showed that the AMF colonization rates in rice were the highest under the condition of 50% of soil WHC.

Suitable combinations of rice and AMF species under waterlogged conditions should be further investigated. The results of moisture tests in the present study indicated AMF collected in paddy fields may have evolved an adaptation to the higher level of moisture content when compared with other AMF isolated from upland plants.

Conclusions

Among the seven different sampling sites (Huizhou, Heyuan, Shaoguan, Meizhou, Ji'an, Xuzhou, and Jingzhou), the highest AMF colonization rate was observed in the samples from Huizhou City (19.5 ± 7.2%). The colonization rates of all samples were not significantly correlated with metal concentrations (Mn, Zn, Pb, Fe, Cu, Mg, and As), macro nutrients (P and K), and pH but were significantly affected by the latitudes of the sampling sites (R = −0.68, P = 0.046), which may be caused by light intensity and temperature differences.

Hyphae were mostly observed followed by vesicles and arbuscules within the roots. The sequence of amplified rDNA fragments showed that *Glomus* cf. *clarum* was the potential AMF species colonized rice plant in the PT site, Huizhou City. Further studies are needed to identity AMF species in other sampling sites using PCR and sequencing techniques.

It has been shown that lowland rice was colonized by most AMF species, which were collected from SK, PT, and LJ, as well as the upland species *R. intraradices* and *F. mosseae*, under 50% of soil WHC. For 100% of soil WHC, AMF isolated from Huizhou were able to colonize the rice plants, but not those AM species isolated from aerobic conditions. These results indicate that AMF collected from paddy fields may have adapted to a higher level of moisture content.

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