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## Natural Colonization of Rice by Arbuscular Mycorrhizal Fungi in Different Production Areas

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**Abstract:** Interactions between plants and soil microorganisms can influence the other interactions in which plants participate, including interactions with herbivores. Many fungi, including arbuscular mycorrhizal fungi (AMF), form symbiotic relationships with the roots they inhabit, and potentially alter defense against pests. The objective of this study was to document the extent of root colonization by AMF on non-flooded rice plants grown under conditions typical of commercial fields. We hypothesized that AMF naturally colonized rice plants in different rice producing field locations. Rice plant samples were collected from areas across the southern United States, including Texas, Mississippi, Arkansas and two research stations in Louisiana. We quantified the amount of AMF colonization in insecticide-free rice plants over three consecutive years (2014–2016). The results revealed natural colonization of AMF in all rice producing areas. In all the three years of survey, rice-AMF associations were the greatest in Arkansas followed by Mississippi and Texas. This research will help draw attention to natural colonization of AMF in rice producing areas that can impact future rice research and production by facilitating agricultural exploitation of the symbiosis.

Key words: arbuscular mycorrhizal fungus; rice; root colonization; soil quality; agriculture

Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are important components of soil microbial communities. AMF form mutualistic associations with roots of most terrestrial plants, including many agricultural crops. In many agricultural plants, these mutualistic associations have shown the potential to increase crop productivity, thereby playing a key role in the functioning and sustainability of agroecosystems (Gianinazzi et al, 2010). The most important function of these symbiotic associations involves the transfer of nutrients such as organic carbon (C), in the form of sugars and lipids (Jiang et al, 2017; Luginbuehl et al, 2017), to the fungi by the plants, and the transfer of phosphorus (P) and nitrogen (N) to the plants by the fungi (Smith and Read, 2008). AMF-mediated improvement in mineral uptake may lead to increased growth and development of plants, and may confer resistance to abiotic and biotic stress (Liu et al, 2007; Smith and Read, 2008; Gianinazzi et al, 2010). In addition to these benefits to plants, AMF may improve soil structure, ameliorate drought and salinity stress, and affect the diversity of plant communities (van der Heijden et al, 1998, 2010; Rillig and Mummey, 2006; Smith et al, 2010). The benefits of AMF may be critical to increasing agricultural yields and productivity in a low-input manner.

AMF share a long history of coevolution with plants in various ecosystems, resulting in their adaptation to specific areas (Gosling et al, 2006). The majority of research on AMF associations involves laboratory or greenhouse experiments, in which plants are cultivated in sterilized soil, with particular AMF species. They ignore indigenous AMF species that could alter plant responses or compete with the AMF inoculant (Munkvold et al, 2004). In addition, these studies ignore the complexity of soil biological communities that could influence the establishment of the AMF symbiosis and its impact on plant

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fitness (Lekberg and Koide, 2005).

During the last two decades, different aspects of the association of crop plants with AMF have been studied extensively in different geographical regions and under different agricultural conditions (Srivastava et al, 1996; Gianinazzi et al, 2010). Those studies have shown variable effects of AMF on crop plants, ranging from mutualistic to parasitic. The effects of AMF can depend on soil moisture, the inorganic nutrient available in the soil, pH, species of AMF and the host plant species. Along with these factors, a number of agricultural management practices affect the soil environment, and therefore, mycorrhizal abundance and activity.

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops. In the United States of America, it is cultivated in two distinct regions, California and several southern states, including Arkansas, Louisiana, Mississippi, Missouri and Texas. In the southern region, the majority of rice acreage is grown under a delayed-flood cultural system in which rice is drill-seeded and surface-irrigated as necessary to establish a stand (Hamm et al, 2010). Timing of the permanent flood in this system varies, but flooding is generally delayed until rice begins to tiller, four to five weeks after planting. The period from seeding to flooding favors the colonization of root systems by AMF (Dhillion, 1992; Secilia and Bagyaraj, 1994).

Colonization by indigenous or native AMF species in cereal crops in general and rice in particular has been reported earlier (Maiti et al, 1995; Sawers et al, 2008; Campos-Soriano et al, 2010; Cosme et al, 2011). Despite this, in USA, almost no attention has been paid to AMF associations in rice. In another study, we showed that the performance of insects and a pathogen on rice is enhanced when plants are colonized by AMF, and AMF colonization can be manipulated by inoculating plots with a commercial AMF product (unpublished data). It will be necessary to evaluate the natural association of AMF with rice plants, particularly in regions where rice is produced, to facilitate agricultural exploitation of the symbiosis.

Given the paucity of information on the natural association of AMF with rice in different production areas of the United States, our goal was to survey rice fields from several locations in the southern United States to determine the extent of AMF colonization associated with commercial varieties before flooding. We tested the hypotheses that AMF establish natural association with rice roots, and that the AMF colonization would differ among locations. Unlike previous studies of natural AMF colonization in rice (Dhillion, 1992; Dhillion and Ampornpan, 1992), this study was carried out in most of the rice-producing areas of the southern United States and demonstrated natural colonization of AMF in rice fields, which may have practical implications for increasing rice production and sustainability.

## MATERIALS AND METHODS

## Sampling sites

Sampling to determine the extent of natural AMF colonization was conducted over three production seasons from 2014 to 2016. Four (2014 and 2015) or five (2016) collection sites were included in each year to represent a range of production environments in the southern United States (Table 1). The climate in the rice-producing regions of the southern United States belongs to the humid subtropical type, with average annual rainfall of 1 000 to 1 600 mm. In these areas, the summers are warm and humid, and the daily maximum temperatures usually range from 32 °C to 37 °C during the growing season. Average temperatures in late spring are about 23 °C, while 28 °C in summer and about 25 °C in early fall (US Climate Data, 2018).

Environmental conditions and cultural practices varied from year to year and site to site, but in all cases were typical of rice fields in the southern USA. In all these environments, rice was grown as a single crop per year, drill-seeded and irrigated. Plot sizes at all the sites were at least  $1.5 \text{ m} \times 6.0 \text{ m}$ . At the Winnsboro (WB) site, rice was grown in experimental plots in fields that had been under a continuous rice cultivation system for several years; for the Crowley (CR) and Beaumont (BM)

Rice field	County, State	Coordinate	Soil type	Variety -	AMF colonization percentage (%)		
					2014	2015	2016
H. Rouse Caffey Rice Research	Acadia, Louisiana	30°14′23.4″ N	Silt loam	Cocodrie	$3.8\pm0.4$	$19.0 \pm 2.1$	$59.3\pm4.1$
Station, Crowley (CR)		92°20′46.1″ W		Jupiter	$3.8 \pm 0.9$	NS	NS
				Lemont	$1.8 \pm 0.4$	NS	NS
				Mermentau	NS	NS	$22.0\pm4.1$
				Cheniere	NS	NS	$58.0\pm2.2$
Macon Ridge Research Station,	Franklin, Louisiana	32°08′33.0″ N	Sharkey clay	CL151	NS	NS	$16.0\pm0.6$
Winnsboro (WB)		91°42′23.6″ W					
Delta Research & Extension	Washington, Mississippi	33°25′24.1″ N	Sharkey clay	Cocodrie	$16.7 \pm 2.6$	NS	NS
Center, Stoneville (SV)		90°54′39.1″ W		Wells	NS	$29.8\pm2.8$	$27.0\pm0.8$
Texas A&M AgriLife Research &	Jefferson, Texas	30°04'19.8" N	League clay	Antonio	$5.8 \pm 0.8$	$25.8\pm1.3$	$18.0\pm1.8$
Extension Center, Beaumont (BM)		94°17′58.1″ W					
Rice Research & Extension Center,	Stuttgart, Arkansas	34°28′31.9″ N	Dewitt silt loam	Wells	$11.8 \pm 1.1$	$61.4 \pm 6.3$	$61.2\pm4.6$
Stuttgart (ST)		91°25′05.6″ W					

Table 1. Arbuscular mycorrhizal fungi (AMF) colonization percentage (presence of hyphae, arbuscular and vesicles) in fields during 2014–2016.

NS, Not sampled. Values are Mean  $\pm$  SE (n = 7 to 10).

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