Diversity of indigenous arbuscular mycorrhizal fungi in rhizosphere of upland rice (*Oryza sativa* L.) varieties in Southwest Nigeria

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Arbuscular mycorrhizal fungi (AMF) have the potential to increase crop productivity and play a key role in the functioning and sustainability of most agroecosystems. However, limited information is available on the divervisity of AMF associated with upland rice varieties in Southwest Nigeria. Field survey was conducted to investigate colonization and diversity of AMF in 13 upland rice varieties commonly grown in Southwest Nigeria. Root and soil samples were collected from rice fields in 2012. The results showed natural root colonization of all the rice varieties by AMF with highest root colonization in ITA 157and Ofada. The spore densities retrieved from the different rhizospheres were relatively high, varying from 13 spores in UORW 111 to 174 spores in Ofada with a mean of 67.6 spores per 20 g dry soil. *Glomus* was observed to be the most abundant AMF genus. *Funneliformis mosseae* was the most frequently occurring AMF species (96.2%) with relative density (*RD*) of 32.2%, followed by *Glomus intraradices*, *Claroideoglomus etunicatum*, and *Glomus clareium*. This study showed that AMF naturally colonized the roots of these rice varieties and diversity of different AMF genera in rice rhizosphere. This study will help draw attention to natural colonization of AMF in rice producing areas of Nigeria that can influence future possibility of using inocula of the dominant AMF species in upland rice cultivation.

Keywords: Arbuscular mycorrhizal fungi, community structure, diversity, upland rice, spore density

1   Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops. In Nigeria, rice is a major staple food consume in many households. It is the only crop that is grown nation-wide in all agro-ecological zones, from the savannah to the coastal mangrove swamps of Nigeria. One of the limiting factors in sustainable utilization of agricultural land for rice production in Nigeria is the declining soil fertility. Arbuscular mycorrhizal fungi (AMF) are important and widespread components of soil microbial communities and agricultural ecosystems forming symbiotic relationships with roots of over 80% terrestrial plants, including many agricultural crops such

as rice, soybean etc. (Brundrett and Tedersoo, 2018; de Andrade Júnior et al., 2018). They are generally essential for many important ecosystem functions and processes, including nutrient cycling, plant productivity and sustainability (Van Der Heijden et al., 2015; Souza et al., 2016).

In many agricultural plants, AMF have shown the potential to increase crop productivity, thereby playing a key role in the functioning and sustainability of agroecosystems (Brundrett and Tedersoo, 2018; Silva-Flores et al., 2019). The most reported function of these symbiotic associations involves the transfer of nutrients such as organic carbon (C), in the form of sugars and

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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; Van Der Heijden et al., 2015). AMF-mediated improvement in nutrient uptake may lead to increased growth and development of plants, and may confer resistance to abiotic and biotic stress (Ventura et al., 2018; de Moura et al., 2018). They enhance host growth and survival by improving tolerance to drought, salinity effects and to some root pathogens and nematodes (Chen et al., 2018). AMF may improve soil structure, ameliorate drought and salinity stress, and affect the diversity of plant communities (de Moura et al., 2018). Thus, the widespread 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 (Oehl et al., 2017). A number of factors have been shown to act as environmental filters, structuring AMF communities, such as host plants, land use, fertilization and soil pH (Lin et al., 2012; Peyret-Guzzon et al., 2016; Oehl et al., 2017). 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 (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. Host preferences have also been demonstrated to exist to a certain extent in AMF (Pivato et al., 2007), but strict host specificity seems to be rare. However, it is well established that individual AM fungal species can differ in their associations with different plants (Adeyemi et al., 2019). Most studies on AMF community structure have been conducted at a small scale, with only a few authors reporting AMF diversity at the regional scale or larger (Hazard et al., 2013). Some AMF taxa have been reported to be surprisingly widespread (Davison et al., 2015), however, many cannot yet be directly linked to a certain set of agricultural practices or environmental conditions.

Colonization by indigenous or native AMF species in cereal crops in general and rice in particular has been reported earlier (Campos-Soriano et al., 2010). Despite this, in Nigeria, little attention has been paid to AMF associations in rice fields. Thus, it will be necessary to evaluate the natural association of AMF with rice plants, particularly in cultivar s that are commonly cultivated

by farmers, 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 southwest Nigeria, the aim of this study was to survey fields of different rice cultivars grown across the southwest Nigeria to determine the extent of AMF colonization and community structure in the rhizosphere. The study tested the hypotheses that AMF establish natural association with rice roots, and that the AMF colonization would differ among rice cultivars. This study was carried out on most of the rice cultivars grown in southwest Nigeria and demonstrated natural colonization of AMF in rice fields, which may have practical implications for increasing rice production and sustainability.

2 Material and methods

Study sites

The study was conducted across different rice fields in Southwest Nigeria in 2013. The region has a tropical climate with two main seasons, namely rainy (March – November) and dry season (December – February).

Field sampling

Root and soil samples were collected from rhizosphere of thirteen upland rice varieties grown in different locations in Southwest Nigeria (Table 1). Approximately 2 kg of soil and roots were collected from a depth of 0–30 cm using soil auger and stored in the refrigerator (10 ^{o}C) until processing. There were four sampling points in each field. At each sampling point, four subsamples (250 g) were collected, mixed and pooled to produce composite soil

Table 1 Upland rice varieties and coordinates of samples collection in 2013

No	Varieties	Location	Soil origin
1	Igbomo	Ekiti	Lat. 7° 79′N Long. 5° 51′E
2	ITA 157	Osun	Lat. 7° 62′N Long. 4° 73′E
3	ITA 150	Osun	Lat. 7° 62′N Long. 4° 73′E
4	NERICA 1	Ogun	Lat. 6° 95′N Long. 3° 50′E
5	NERICA 2	Ekiti	Lat. 7° 79′N Long. 5° 51′E
6	NERICA 8	Ogun	Lat. 6° 95′N Long. 5° 51′E
7	Ofada	Ogun	Lat. 6° 95′N Long. 3° 50′E
8	UDM 4A11	Ogun	Lat. 6° 95′N Long. 5° 51′E
9	UDRW 111	Ogun	Lat. 6° 95′N Long. 3° 50′E
10	UN 111	Ogun	Lat. 6° 95′N Long. 3° 50′E
11	UORG 311	Ekiti	Lat. 7° 79′N Long. 5° 51′E
12	UORW 111	Ekiti	Lat. 7° 79′N Long. 5° 51′E
13	WITA 4	Osun	Lat. 7° 62′N Long. 4° 73′E

samples. A total of 52 soil samples were thus collected. From these samples, the roots of the respective crop species were carefully freed from adhering soil and immediately fixed in 50% ethanol.

Soil analysis

Soil chemical and physical parameters were determined and analyzed using the following procedures; the soil pH was determined by the glass electrode in a 1 : 1 soil: water and in 1 : 1 KCl suspensions, particle size by the hydrometer method (Bouyoucos, 1951), organic carbon by the chromic acid oxidation procedure (Walkey and Black, 1934). Exchangeable bases were determined using the neutral ammonium acetate saturation, Na and K in solution were measured by flame photometer while Ca and Mg by Atomic Absorption Spectrophotometer, Exchangeable acidity was determined using the 1 M KCl extraction followed by 0.01 M NaOH titration. Total nitrogen was determined using the regular macro-Kjeldahl method, and available P by Bray 1 extraction method.

Staining and estimation of AMF root colonization

Roots in ethanol were rinsed thoroughly in tap water, cut into approximately 1 cm segments and cleared in hot KOH solution (10% w/v, at 90 °C) for 1 hour. The bleached roots were rinsed to remove excess KOH and stained in acidic glycerol containing methyl blue lacto-glycerol (1 : 1 : 1 :0.5 g) at 90 °C for 30 minutes (Phillips and Hayman, 1970). The stained root segments were mounted on microscopic slides and examined for AMF structures (hyphae, vesicles and arbuscules) under light microscope to determine percentage root colonization (Adeyemi et al., 2017, 2020):

$$Percentage\ root\ colonisation =$$
$$= \frac{number\ of\ root\ infected}{total\ number\ of\ roots} \times 100$$

AMF spore isolation and identification

AMF spore extraction was done in triplicates for individual soil sample. The spores were extracted by the modified wet sieving method (Błaszkowski, 2012). A sample of 25 g of air-dried field soil was mixed with distilled water. The resulting mixture was passed through 250, 150 and 40 μm sieves. The fraction retained in the 500 μm sieve was checked for large spores, spore clusters, sporocarps and organic matter debris. Soil materials retained by the 150 and 40 μm sieves were washed into centrifuge tubes using a small stream of distilled water. Tubes were centrifuged at 4,000 rpm for 2 minutes. The supernatants were decanted and subjected to sucrose centrifugation (70% (w/v)) gradient and centrifuged at 4,000 rpm for 2 minutes. The supernatant was passed through the

40 μm sieve, washed with distilled water and transferred to new Petri dishes. Spores, spore clusters and sporocarps were recovered and counted at 40× magnification. For identification, spores were picked under the dissecting microscope with a glass micropipette and subsequently mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1 : 1 (v/v); Brundrett 2002) to get permanent slides for spore observation and identification under a compound microscope at up to 400× magnification. The spores were identified at the genus level on the basis of size, spore-wall structure, Melzer's reaction, colour and presence or absence of subtending hyphae and compared with descriptions of fungal genera according to taxonomic criteria (Oehl et al., 2011) and information available on the international network for vesicular arbuscular mycorrhizal (INVAM, 2018).The relative abundance, which is the ratio of the number of spores from a particular genus with respect to the total number of spores recovered was calculated based on percentage.

Ecological AMF diversity indices

To determine differences in the structure of the AMF communities on different crops, the following parameters were calculated: The isolation frequency (*IF*) of occurrence was calculated as the percentage of samples in which a genus or species occurred among all samples, and it reflects the distribution status. Relative spore density (*RD*) was defined as the ratio between the spore densities of a particular genus or species to the total AMF spore densities and it shows the degree of sporulation ability of different AMF in a given soil. The importance value (*IV*) was used to evaluate the dominance of AMF species based on *IF* and *RD* and was calculated as *IV* = (*IF* + *RD*)/2. An *IV* ≥50% indicates that a genus or species is dominant; 10% < *IV* <50% applies to common genera or species; an *IV* ≤10% indicates that a genus or species is rare (Chen et al., 2012). Species richness (*SR*) is the number of AM fungal species recovered from each site per sample collection. Simpson's Index of Diversity (*D*) = 1 − $\sum(P)2$ where *P* = *n*/*N*, *n* is the relative abundance of the species calculated as the proportion of individuals of a given species to the total number of individuals in a community N. Shannon diversity index (*H*) by Shannon and Wiener is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present:

$$H = -\sum \left(P \ln(P) \right)^2$$

Statistical analysis

Spore density and root colonization (%) were subjected to log *e* (*x* + 1) and square root transformation respectively

for normalization of the data. Analysis of Variance was conducted to determine significant differences among the means at 5% probability level. Significant means were separated using Least Significant Difference (LSD). Pearson correlation analysis was used to detect the relationship between spore densities, percent root colonization and AMF relative abundance using the statistical package Genstat 12th edition

3   Results and discussion

Chemical and physical soil characteristics of the surveyed fields

The physico-chemical soil analyses are summarized in Table 2. The pH ranged from 5.1 to 6.8, and cation exchange capacity (CEC) values varied from 0.16 to 10.71 cmol kg^{-1}, while total amount of nitrogen (N) and available P contents were low. Organic carbon present in the soil was adequate.

AMF root colonization and spore density in the soil

The result showed that all the rice varieties root samples surveyed in this study were colonized by AMF. The mean percentage of AMF colonization was 50.1%, ranging from 33.6% to 76.2% (Figure 1). Percent AMF root colonization was highest in roots collected from ITA 150 (50%) and lowest in UORW 111 (33.6%). The spore densities (expressed as per 20 g dry soil) retrieved from different rhizosphere crops were relatively high, varying from 13 in UORW 111 to 174 in Ofada with a mean of 67.6 (Figure 2).
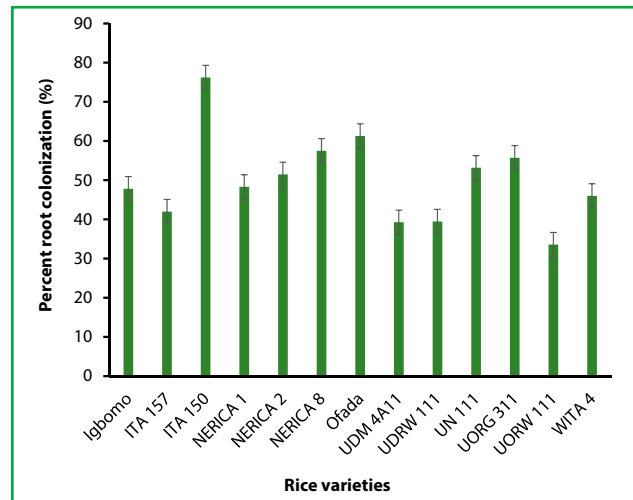

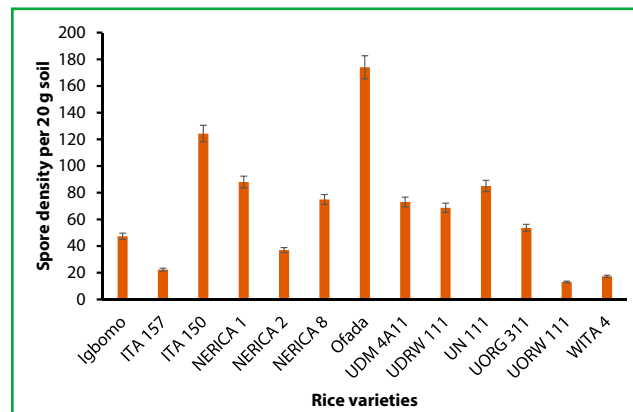**Figure 1**   Percent AMF root colonization of 13 rice varieties

Figure 2   AMF spore density in rhizosphere of 13 rice varieties

Table 2   Chemical and physical soil characteristics of the surveyed fields

| Varieties	pH	Sand (%)	Silt (%)	Clay (%)	OC (%)	N (%)	P (mg kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	K (cmol kg^{-1})	Na (cmol kg^{-1})	CEC (cmol kg^{-1})	Exch. acidity (cmol kg^{-1})
Igbomo	6.16	76.4	12.8	10.8	1.05	0.11	1.02	2.20	1.22	0.30	0.35	4.17	0.10
ITA 157	6.48	86.4	8.80	4.80	1.73	0.17	8.88	4.01	1.83	0.40	0.37	6.70	0.09
ITA 150	6.37	85.2	8.80	6.0	1.95	0.20	1.34	2.61	0.81	0.32	0.29	4.12	0.09
NERICA 1	7.10	56.4	28.8	14.8	0.96	0.10	2.41	3.41	2.44	0.39	0.38	6.68	0.06
NERICA 2	6.17	76.4	17.6	6.0	1.15	0.12	2.22	1.20	0.41	0.82	0.43	2.96	0.10
NERICA 8	6.80	88.4	4.80	6.80	1.50	0.15	1.94	3.21	2.20	0.16	0.30	5.94	0.07
Ofada	5.96	86.4	8.80	4.80	1.62	0.16	1.16	2.61	0.81	0.23	0.32	4.08	0.11
UDM 4A11	7.13	86.8	6.80	6.40	2.19	0.22	2.45	4.01	1.62	0.19	0.29	0.16	0.05
UDRW 111	5.1	60.4	24.8	14.8	1.06	0.11	1.11	1.60	0.61	0.57	0.43	3.33	0.12
UN 111	6.16	76.4	12.8	10.8	1.05	0.11	1.02	2.2	1.22	0.30	0.35	4.17	0.1
UORG 311	7.73	86.0	7.20	6.80	2.99	0.30	5.56	8.22	1.62	0.53	0.32	10.71	0.02
UORW 111	6.66	86.4	6.80	6.80	1.06	0.11	1.19	1.80	0.61	0.19	0.29	2.97	0.08
WITA 4	6.14	86.8	8.80	4.40	0.78	0.08	0.51	2.0	0.41	0.41	0.39	3.33	0.10
Mean	6.46	79.88	12.12	8.00	1.47	0.15	2.37	3.01	1.22	0.37	0.35	4.56	0.08

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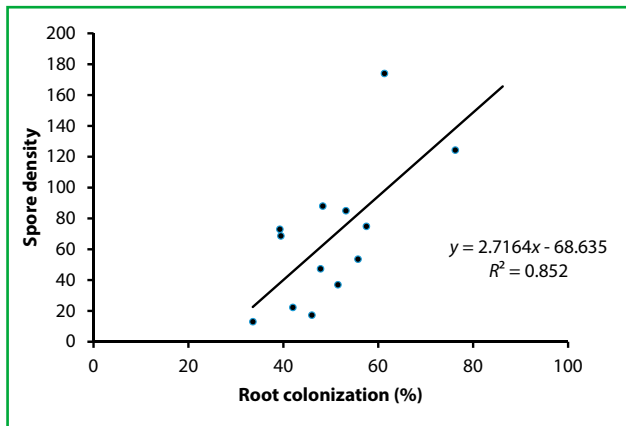
Figure 3 Relationship between AMF spore density and root colonization
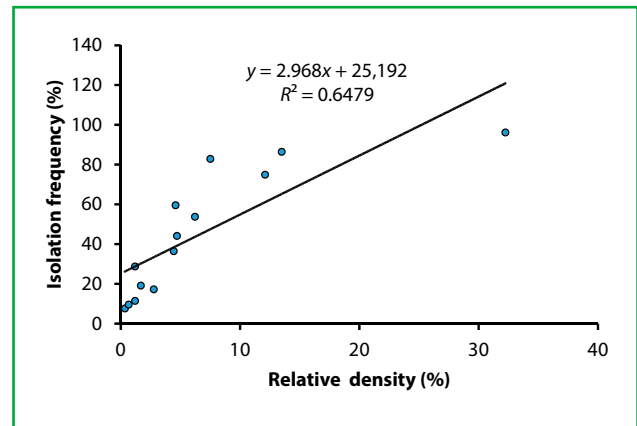

Figure 4 Relationship between AMF isolation frequency and relative abundance

There was a positive correlation between spore densities in the soil and the root colonization rates ($r^2 = 0.45$) (Figure 3).

AMF species richness and diversity

A total of 6810 AMF spores were identified and classified from the 52 rhizosphere soil samples. A total of 14 morphologically classifiable species of AMF were detected from this study. The most abundant genus was *Glomus* (Table 3). The relative density of AMF ranged from 0.34% to 32.2%, the isolation frequency from 7.9% to 96.2%, and the importance values ranged from 4.02 to 64.21 (Table 3). Relative density was positively correlated with the isolation frequency ($r^2 = 0.65$, $P <0.05$) (Figure 4). In addition, *Funneliformis mosseae* was the most

frequently occurring AMF species (96.2%) with an *RD* of 32.2%, followed by *Glomus intraradices* (*IF* = 86.5%, *RD* = 13.5%), *Claroideoglomus etunicatum* (*IF* 82.9%, *RD* 7.5%), and *Glomus clareium* (*IF* 75%, *RD* 12.1) (Table 3). Maximum AMF diversity was recorded in Ofada and ITA 150 (Figure 5).

The widespread importance of AMF in rice production have received some attention in recent times (Vallino et al., 2014; Wang et al., 2015; Zhang et al., 2015), but no study has investigated how native AMF species naturally colonize rice and to what extent the natural colonization by AMF differs among commonly grown rice varieties in the southwest of Nigeria. In the present study, the results indicate natural AMF colonization in all the sampling

Table 3 Spore count per species, relative spore density, isolation frequency and importance value of arbuscular mycorrhizal fungi (AMF) identified from 52 soil samples from 13 upland rice varieties in South-west Nigeria.

AMF species	S	RD (%)	IF (%)	IV (%)
Acaulospora scorobiculata	81	1.19	28.8	14.9
Acaulospora sp	23	0.34	7.69	4.02
Claroideoglomus claroideum	312	4.58	59.6	32.1
Claroideoglomus etunicatum	511	7.50	82.9	45.2
Funneliformis mosseae	2,195	32.23	96.2	64.21
Glomus intraradices	918	13.48	86.5	49.9
Glomus clareium	823	12.09	75.0	43.5
Glomus aggregatum	423	6.21	53.8	30.0
Glomus fasciculatum	320	4.70	44.2	24.4
Glomus sp	302	4.43	36.5	20.5
Glomus geosporus	114	1.67	19.2	10.4
Gigaspora gigantea	188	2.76	17.3	10.0
Scutellospora pellucida	81	1.19	11.5	6.34
Scutellospora sp	45	0.66	9.61	5.14
Total AMF species richness: 14 species	6,810	100		

S = absolute number of spores identified per species; *RD* = relative spore density; *F* = isolation frequency; *IV* = importance value
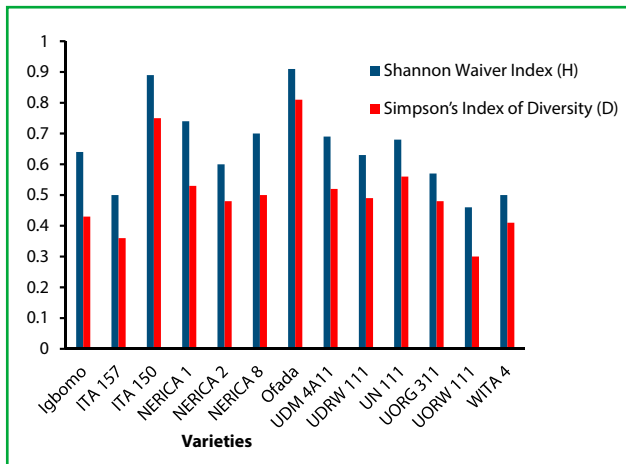
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Figure 5   AMF diversity in rhizosphere of rice varieties

rice fields, confirming our hypothesis that natural AMF colonization is widespread in most soils.

In this study, variation in the spore density and root colonization of AM fungi naturally associated with the rice cultivars varieties was observed with ITA 157 and Ofada having highest root colonization and spore density respectively. Host preferences have been demonstrated to exist to a certain extent in AMF, but strict host specificity seems to be rare (Pivato et al., 2007). This could possibly be attributed to the differences in rooting habits and nutrients demands of the crops (Oehl et al., 2017) or amount of carbon transfer from the hosts to AMF. It has also been reported that AMF community composition depends on host plant species and, therefore, plant species may have varying degrees of selectivity on AMF species that range from selective specialists to non-selective generalists (Davison et al., 2015; Oehl et al., 2017).

Environmental factors and agricultural management practices in rice fields such as fertilizer input and water management have been reported to influence the root colonization and diversity of AMF communities (Lumini et al., 2011; Barber et al., 2013). Soil P has been known to have a negative impact on AMF symbiosis and at lower soil P concentrations; plants tend to allocate a higher fraction of available carbon to AMF, thus stimulating AMF colonization (Johnson, 2010). In general, AMF spore density was found to be positively correlated with root colonization in this study. This could be due to the fact that some AMF species rely more on extensive formation of hyphal networks in roots and survival through spore formation as primary infective propagules in soils.

There was a prominent distribution of AMF species among the rice varieties, with higher isolation frequency of *Funneliformis mosseae*, *Glomus intraradices* and *Claroideoglomus etunicatum*. High diversity may

be important for buffering an ecosystem against disturbances. Occurrence of maximum number of species results in higher index of diversity. Maximum diversity observed in the Ofada and ITA 150 indicated shared dominance of many AM fungal genera.The genus *Glomus* was the most dominant and widely distributed. It has been previously reported that *Glomus* species are the most abundant among the glomeromycotan genera in tropical areas (Snoeck et al., 2010; Oehl et al., 2017), regardless of the type of hosts and intensity of disturbance in the different ecosystems. Furthermore, *Glomus* species have the abiltiy to produce a relatively high number of spores within a very short period of time (Oehl et al., 2017). The significant reduction in relative spore abundance of *Gigaspora* and *Scutellospora* may be due to soil disturbances due to agricultural practices such as tillage. Furthermore, Gigasporaceae have been reported to rely on spores as their primary infective propagules. Moreover, soil fertility and physical properties also play a key role for their occurrence in tropical soils (Lekberg et al., 2007).

4   Conclusions

All the rice varieties are managed with each variety having its own cultivation practice. The difference in cultural practices can cause changes in the suitability for growth of AM fungi. In the present study, dominance of *Glomus* genus was recorded in the rhizosphere of all the varieties. The information gathered from this study suggests the suitability of some of the AMF species to be used as inocula, and can be used to further investigate the impact of the symbiotic relationship between AMF and rice, which is becoming increasingly relevant for sustainable agriculture, where soil organisms may be useful for crop production.

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