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Arbuscular mycorrhizal fungi in the *Jatropha curcas* rhizosphere

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***Jatropha curcas* L. is a Euphorbiaceae characterized as drought tolerant, with low nutrient exigency and resistant to pests and diseases; furthermore, its seeds have a high content of good quality oil, which makes it a potential plant species for biodiesel production. The association of *J. curcas* with arbuscular mycorrhizal fungi (AMF) may enhance some of these promising features. The aim of this work was to assess AMF community associated with different genotypes of *J. curcas* (different accessions of a germoplasm bank) grown in the same area and in plants of the same genotype grown in different regions to identify promising fungi in this association. The AMF community was assessed by morphological analysis and by polymerase chain reaction-denaturing gradient gel electrophoresis fingerprinting approach (PCR-DGGE) and sequencing of 18S rDNA. Twenty-seven species of AMF were identified morphologically, in addition to five additional ones identified by sequencing of DGGE bands. In both analyses, some genera and species were found in common, including *Glomus* and *Acaulospora*. In the same accession or in samples obtained from the same genetic material, but cultivated in neighboring regions, the AMF community had a greater similarity, showing a possible influence of the genetic material and of climatic conditions on the AMF community. Regardless of the AMF community, these plants present a high percentage of mycorrhizal colonization and a relatively high number of AMF spores, suggesting an important relationship with mycorrhizal association.**

Key words: Nested PCR-DGGE, *Jatropha curcas,* arbuscular mycorrhizal fungi (AMF) community, Glomus, spore morphology.

INTRODUCTION

The necessity to reduce the use of fossil fuels has intensified research to develop technologies for renewable energy (Sharma and Singh, 2009). As an alternative, the production of biofuels has been increased

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worldwide. As a substitute of petrodiesel, biodiesel must be technically feasible, economically competitive and environmentally sustainable (Demirbas, 2007).

Within this context, *Jatropha curcas* L. is an important plant due to seed quality (Behera et al., 2010). *J. curcas* (Euphorbiaceae), known as the physic nut, is native of tropical America and has been broadly dispersed through the tropical and subtropical areas of Africa and Asia (Schmook and Serralta-Peraza, 1997; Openshaw, 2000). It is a perennial shrub, with 5 to 7 m height (Achten et al., 2008; Drumond et al., 2009), and an average lifespan of 50 years (Achten et al., 2008). Besides having a high content of good quality oil in their seeds, the plant is considered drought tolerant and able to grow in soils with low nutrient contents, requires little manual labor for cultivation, does not compete with other cash crops and tolerates well pests and diseases (Openshaw, 2000; Jongschaap et al., 2007; Achten et al., 2008; Behera et al., 2010).

The beneficial association between *J. curcas* and arbuscular mycorrhizal fungi (AMF) has been demonstrated (Openshaw, 2000; Achten et al., 2008; Charoenpakdee et al., 2010). This association occurs between some soil fungi and most terrestrial plants, is present in nature more than 400 million years and is found in approximately 80% of plants, including most of agricultural, horticultural and forestry species (Pozo and Azcón-Aguilar, 2007). Plants that participate in mycorrhizal symbioses have an increasing nutrient uptake (Smith et al., 2010), a higher tolerance to drought and salt stresses than non-mycorrhizal plants (Augé, 2001), and a greater resistance to the effects of heavy metals (Rozpadek et al., 2014). Besides increase, the resistance to pathogens and act as plant growth promoters (Pozo et al., 2002; Hernández-Montiel et al., 2013). Considering that environmental factors, such as soil moisture (Helgason and Fitter, 2005; Silva et al., 2014), pH, rainfall and soil type (Hazard et al., 2013) can affect AMF community and there are only a few studies emphasizing the diversity of AMF associated with physic nut under distinct edaphoclimatic conditions, identification of the common species in the rhizosphere of *J. curcas* is important, which information can be used in crop management in the field, or even in the production of mycorrhizal seedlings, in order to fully exploit the potential of this association taking into account the characteristics of each locality.

Identification of AMF species has relied mainly on the analysis of spore morphological characteristics such as color, shape and size as well as spore-wall properties (Morton, 1988; Schenck and Perez, 1990). However, changes in the spore wall, resulting from interactions with the environment and differential sporulation patterns between species of AMF, make it difficult to identify field-collected spores (Rodríguez-Echeverría and Freitas, 2006), especially in cases where microbial activity is high. In addition, an evaluation solely based in the morphology of spores provides an incomplete interpretation of the community structure of these fungi in the environment (Ma et al., 2005; Hempel et al., 2007).

The use of molecular tools to assess AMF species diversity under field conditions has allowed to detect species with low sporulation rates in soil which would have a greater difficulty to be detected by morphological analysis. In addition, molecular approaches do not usually require steps associated with the cultivation and production of fungi spores in trap cultures (Kowalchuk et al., 2002). In this context, denaturing gradient gel electrophoresis fingerprinting approach (DGGE) has been used to analyze the AMF community, allowing access to these fungi in the root systems of plants, in soil samples or even through a spore bank (Kowalchuk et al., 2002).

The aims of this study were to analyze the richness of the AMF species associated with distinct genotypes of *J. curcas* and to compare the diversity of these fungi in different soil and climatic conditions using the classical method of identification (morphology), complemented with molecular assessments.

MATERIALS AND METHODS

Sampling

Samples of soil and root system of *J. curcas* were collected at the germplasm bank of experimental station of Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Unidade Regional Norte de Minas (URENM) in Nova Porteirinha, and on commercial plantations in Viçosa and Canaã, in the state of Minas Gerais, Brazil, between the months of February and April 2010. In all areas, there were only *J. curcas* plants as monocrop, so the AMF community was not influenced by any other plant species. The soils at EPAMIG are sandy loam in texture with average rainfall annual of 876 mm, whereas those at the commercial plantation are sandy clay with average rainfall annual of 1,221.4 mm (Table 1).

At the EPAMIG experimental station, 44 accessions of *J. curcas* were originally obtained from five different regions (MA-Matinha; PA-Paciência; BA-Banavit; BR-Barbosa and SE-Sub-estação Janaúba), and three plants per accession were sampled. Due to the limited number of plants in Canaã and Viçosa, six and nine plants, respectively, were selected at random.

The soil samples were collected using a cylindrical ring of 5-cm diameter and 20-cm depth to obtain a standardized volume of the samples. Two samples of the soil and root system per plant under the canopy were collected to obtain a composite sample.

Samples of the root system were collected manually at the same points of soil sampling for evaluating the percentage of roots with mycorrhizal colonization.

Morphological characterization of AMF

AMF spores were extracted from a 100 cm^3 aliquot of each soil sample using the wet-sieving technique (Gerdemann and Nicholson, 1963), followed by centrifugation in water and then in a 45% sucrose solution. Subsequently, the quantification and separation of spores were performed under a dissecting microscope using morphological characteristics (shape, color and

Table 1. Physical and chemical characteristics of soils collected from the areas of Viçosa, Canaã e Nova porteitinha.

Area	pH	P	K	Ca^{+2}	Mg^{+2}	Al^{+3}	H+Al	SB	$CEC_{(t)}$
	H_2O	----mg/dm³----		------------------------cmolc/dm³---------------------					
Canaã/MG	4.40	45.70	65.00	3.00	0.40	0.40	5.78	3.57	3.97
Nova Porteirinha/MG	6.65	30.00	290.00	3.10	1.80	0	1.49	5.64	5.64
Viçosa/MG	5.20	19.60	158.00	4.00	1.20	0.10	2.81	5.60	5.70
	$CEC_{(T)}$		V	m	OM	P-rem	Clay	Silt	Sand
	cmolc/dm³		---------%---------		dag/Kg	mg/L	------------%------------		
Canaã/MG	9.35		38.00	10.00	3.70	33.20	36	10	54
Nova Porteirinha/MG	7.13		79.00	0	0.80	50.9	12	22	66
Viçosa/MG	8.41		67.00	2.00	2.90	29.60	33	17	50

SB, sum of bases; $CEC_{(t)}$, effective cationic exchange capacity; $CEC_{(T)}$, cationic exchange capacity in pH 7,0; V(%), base saturation; m(%), Al saturation; OM, organic matter; P-rem, remaining phosphorus.

size).

For AMF species identification, the spores were separated according to their morphotypes and mounted on slides with pure polyvinyl-lacto-glycerol (PVLG) and in PVLG mixed with Melzer (1:1 v:v). Identification was made using the descriptions of reference cultures from the International Culture Collection of Vesicular-arbuscular and Arbuscular Mycorrhizal Fungi (INVAM, 2010) (at http://invam.caf.wvu.edu) and by consulting the protocols available at the AMF-phylogeny website (www.lrz.de/~schuessler/amphylo). The genera and families presented in this paper follow the consensus classification of Redecker et al. (2013).

Root colonization

For evaluation of mycorrhizal colonization, roots were kept in FAA (formalin: Alcohol-ethanol: acetic acid, 0.5:9:0.5) and stored for later analysis. The roots were subjected to bleaching in a solution of KOH 10 % (w:v) for 12 h, washed in water and subsequently immersed in HCl 1 % (v:v) for 5 min, followed by staining in 0.05 % trypan blue in lactoglycerol (w:v) at 70°C for 40-60 min (Phillips and Hayman, 1970). Root colonization was quantified by using the gridline-intersect method (Giovannetti and Mosse, 1980).

Soil DNA extraction and reference AMF species

Analysis by denaturing gradient gel electrophoresis (DGGE) was performed using DNA fragments corresponding to the 18S rDNA genes from AMF, as described by Liang et al. (2008), with modifications.

Approximately 10 g of soil sample for each treatment was crushed with the aid of the mortar and pestle to break the aggregates. From these samples, only 1 g was used for the extraction of the total DNA using an *UltraClean™ Soil DNA Isolation* kit (*MO BIO Laboratories, Solana Beach, CA, USA*), according to the manufacturer's recommendations.

The total DNA of reference AMF species was extracted using the same kit, but the AMF were concentrated from 50 cm^3 using a wet-sieving technique (Gerdemann and Nicholson, 1963) or using all the spores present in a Petri dish of an *in vitro* culture of the fungus *Rhizophagus clarus* (= *Glomus clarum*) to concentrate the spores and to ensure sufficient amounts of DNA for use as parameters in subsequent studies.

The reference markers were: a strain of *R. clarus* from *in vitro* collection (Laboratory of Mycorrhizal Associations, Universidade Federal de Viçosa -Viçosa, Brazil), *Acaulospora koskei* SCT406A, *Acaulospora tuberculata* SCT250B, *Gigaspora albida* PRN201A, *Gigaspora decipiens* SCT304A and *Dentiscutata heterogama* (= *Scutellospora heterogama*) PNB102A. Fungal isolates were obtained from the International Culture Collection of Glomeromycota (CICG - www.furb.br/cicg, at Universidade Regional de Blumenau, Blumenau, Brazil).

These reference markers were used to verify the pattern of bands in the DGGE gel of isolated AMF species, to verify the correlation of these species by morphological and molecular characterization, and besides being used as a reference in the DGGE gels.

Nested-PCR strategy for amplification of 18S rDNA fragments

The primers used in the first round for amplification of the 18S rDNA were AM1 (5'-GTTTCCCGTAAGGCGCCGAA-3') (Helgason et al., 1998) in combination with the universal primer for eukaryotes, NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') (Simon et al., 1992).

Polymerase chain reactions (PCR) were performed in thin-walled PCR tubes, 0.5 mL, using the enzyme Go Taq DNA Polymerase Flex® (Promega, Madison, USA) in a volume of 50 µL according to the manufacturer's recommendations. Negative controls consisted of Milli-Q water and replacing the DNA sample to confirm the results. All material used in the preparation of the reactions was previously sterilized and nuclease free.

The DNA template used for amplification of the desired region consisted of 5 µL of the DNA extracted from the AMF spores, which were used as a reference, and DNA extracted from the soil. The reaction mixture for performing the PCR was composed of 200 µmol L^{-1} each deoxynucleoside triphosphates, 1.5 µmol L^{-1}, $MgCl_2$, 0.2 µmol L^{-1} of each primer and 1.25 U GoTaq DNA polymerase Flex®. Acetylated bovine serum albumin (BSA, Promega) was also added to each reaction to potentiate the action of polymerase (0.8 µg µL^{-1}). The PCR amplifications were performed in a thermocycler (Mastercycler epgradient, Eppendorf) using the following steps: a first cycle of 1 min at 94 °C, 1 min at 66 °C and 1 min 30 s at 72 °C, followed by an additional 30 cycles of 30 s at 94 °C, 1 min at 66 °C and 1 min 30 s at 72 °C and finally a 10 min final extension at 72 °C. To confirm the presence of the amplified product, aliquots of of 5 µL of the products of PCR reactions were submitted to electro-phoresis on agarose gel 0.8 % (w:v) stained with ethidium bromide (0.5 µg mL^{-1}) and visualized under UV light photodocumentation

imaging system (Loccus Biotecnologic L-Pix Chemi).

The amplicon corresponding to the first PCR reaction resulted in DNA fragments of approximately 560 bp. To obtain a smaller DNA fragment for carrying out the DGGE technique a second round of PCR reactions was performed (Nested-PCR).

The product of the first PCR reaction was diluted 10 times in sterile Milli-Q water and 1 µL containing about 25 ng μL^{-1} of the DNA used as template. We used the primers NS31-GC (5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGT TGGAGGGCAAGTCTGGTGCC-3') (Kowalchuk et al., 2002) and Glo1 (5'-GCCTGCTTTAAACACTCTA-3') (Cornejo et al., 2004), employing the same reaction mixture as in the first round of PCR. An initial denaturation of 5 min at 94°C was performed, followed by 35 cycles of 45 s at 94°C, 45 s at 52°C, 1 min at 72°C and the extension end of fragments at 72°C for 30 min. To confirm the presence of the product, an aliquot of 5 µL of PCR was verified by electrophoresis on agarose gel 1.5 % (w:v) stained with ethidium bromide (0.5 µg mL^{-1}) and visualized under UV light photodocumentation imaging system (Loccus Biotecnologic L-Pix Chemi).

Analysis of the PCR products by DGGE

From the products obtained by the nested-PCR technique using the primers, Glo1 and NS31-GC, approximately 250 ng of DNA from each samples were analyzed by DGGE (Modelo *Dcode™ System* – BIO-Rad California, USA).

The references were performed as described for the field samples and approximately 300 ng of the DNA mixture of these species were used as marker for the analysis of DGGE.

The polyacrylamide gel used contained 8% (w:v) acrylamide:bisacrylamide (37.5:1) in Tris-acetate-EDTA (TAE) 1X (Tris/acetic acid/EDTA, pH 8.0). A linear denaturing gradient was formed with the aid of the trainer Hoefer gradient SG50 (Amersham Biosciences) and the mixture of two stock solutions of polyacrylamide, to obtain a final gradient ranging from 36 to 50% that was used for all analysis, where the condition of 100% of the denaturing agents consisted of urea 7 mol L^{-1} (Sigma, Cat # U5378) and 40 % formamide (v:v) (Sigma, Cat # F9037) and another solution was created without these compounds.

All the DGGE analysis were performed in 1X TAE buffer at a constant temperature of 60°C at 80 V for a period of 10 min, followed by 60 V for 20 h. The gels had a thickness of 0.75 mm and dimensions of 16 x 16 cm and were stained, after completion of electrophoresis, for 30-40 min in solution of 1X SYBR Gold® (Sigma-Aldrich) according to the manufacturer's recommendations. The images of the gels were observed under UV light and were then captured and digitized using a photodocumentation imaging system (Loccus Biotecnologic L-Pix Chemi).

Selection and DNA fragment sequencing

Based on the different profiles obtained by DGGE, the bands showing greater intensity in each area were selected (Figure 3). The relative intensities of the bands were considered to be the frequency, which these species occur and their DNA fragments were collected with the aid of sterile tips, and were transferred to 0.5 mL microtubes containing 30 µL of sterile Milli-Q water for reamplification using PCR.

The new PCR reaction was performed in an identical manner to that used in the nested-PCR, using of the primers NS31 and Glo1. The selected fragments were sequenced by Macrogen, Inc. (Korea). Subsequently, the sequences obtained were analyzed using the BLASTn tool -NCBI (Altschul et al., 1997).

Statistical analysis

Each treatment consisted of grouping three plants collected in Viçosa and Canaã and by three replicates of each accession obtained in Nova Porteirinha. They were evaluated in relation to the number of spores and the percentage of colonization. The data were subjected to an analysis of variance (ANOVA) at α level of 5%. The means were compared using a Tukey test ($P \leq 0.10$). The data relating to the spore counts were previously via normalized log (x+1) and mycorrhizal colonization via an $\arcsin\sqrt{(x/100)}$ transformation for a subsequent ANOVA. Considering the large number of samples collected in Nova Porteirinha, the frequency (Freq) of each species found in the area was calculated according to the following formula: Freq = (number of accessions where the AMF species was found / total number of accessions)*100.

To analyze the profile of AMF in these soils and generate the dendrogram representing the distance and pattern of bands corresponding to the 18S rDNA gene of AMF, the images of the obtained gels were analyzed and aligned based on the external markers with the reference species by BioNumerics version 6.0 (Applied Maths, Inc., Austin, Texas, USA).

RESULTS

There were no differences ($P \leq 0.10$) in mycorrhizal colonization between samples of the *J. curcas* root collected in Viçosa and Canaã, as well as among the accessions collected in Nova Porteirinha. However, Canaã region presented a higher number of spores ($P \leq 0.10$) per 100 cm^3 of soil (Figure 1). All root samples analyzed showed typical structures of AMF colonization, with hyphae, arbuscules and/or vesicles; morphologically distinct AMF spores were observed in all soils.

Diversity of AMF by morphological characteristics

A total of 27 morphospecies of AMF were detected in all areas, belonging to nine genera and seven families in the Glomeromycota. Twelve species were identified only at the genus level and most of them formed glomoid spores and assigned conservatively to the genus *Glomus*. The largest number of species was recovered from Nova Porteirinha (21) where the largest number of samples was obtained, followed by Viçosa (14) and Canaã (7).

Glomus was the most common genera recovered (12 species) followed by *Acaulospora* (8 species). Other genera were represented by one species each (Table 2). In Nova Porteirinha, *Acaulospora morrowiae* was the most frequent species (93 % frequency), followed by *A. mellea* (74 %), *Glomus* sp. (72 %), *Pacispora* sp. (72 %) and *R. diaphanum* (= *Glomus diaphanum)* (69%). The remaining species were found in less than 50% of the samples (Table 2).

Identification of AMF by molecular tools

DNA extraction from soils and referencing the AMF spores as markers was performed successfully. After diluting

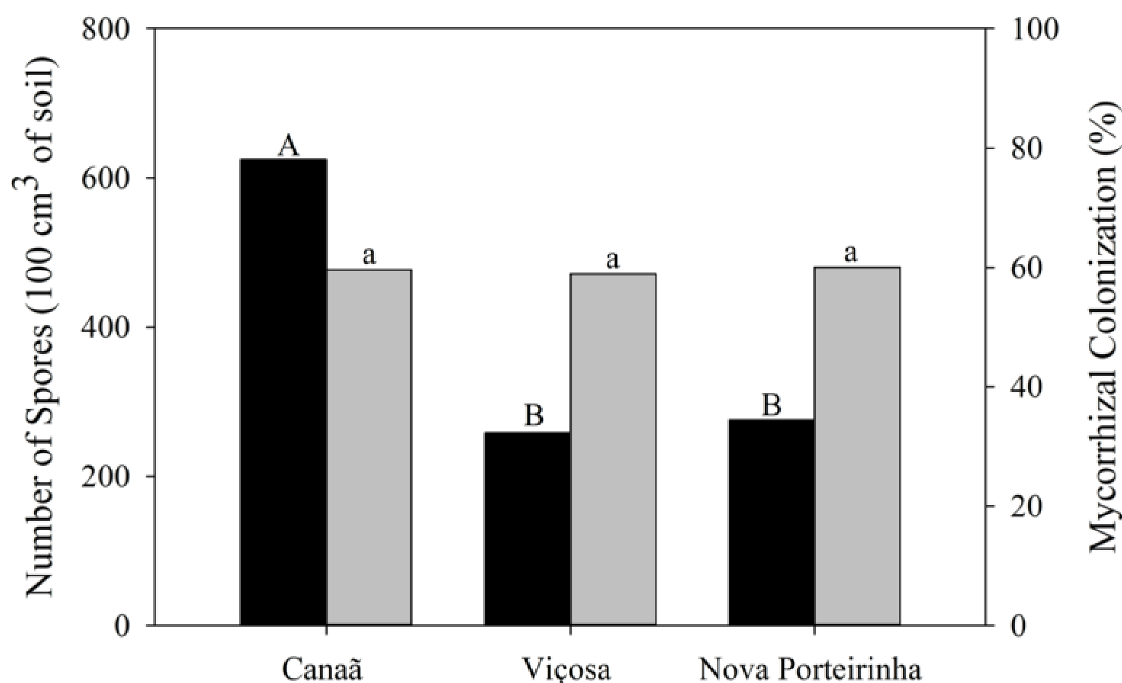

Figure 1. Number of spores of Arbuscular Mycorrhizal Fungi (■) (per 100 cm^3) and mycorrhizal colonization (■) in the rhizosphere of *J. curcas* in Canaã, Viçosa and Nova Porteirinha. Means followed by the same uppercase letter do not differ from each other Tukey test (P≤ 0.10) for number of spores and means followed by the same lowercase letter do not differ from each other by Tukey test (P≤ 0.10) for mycorrhizal colonization (%).

the products of this first round of amplification and its subsequent amplification with primers Glo1/NS31-GC (nested-PCR), we obtained amplicons of the expected size (approximately 230 bp) in all samples, as also observed by Cornejo et al. (2004) and Liang et al. (2008).

The amplicons obtained by nested-PCR generated a profile of several bands in the DGGE gel, characteristic for each reference species analyzed (Figure 2).

Some predominant bands in the profiles of the species used as references were eluted and sequenced. The obtained sequences were analyzed using the BLASTn tool (NCBI) that showed the same species identified by morphological techniques or at least as belonging to the same family (Table 3). All species used as reference markers species that were identified by morphological characteristics were confirmed by molecular analysis.

The nested-PCR, using the primer pair Glo1/NS31-GC, resulted in DNA fragments corresponding to the partial 18S rDNA sequence in all analyzed samples. The profiles of separation of fragments in these bands of DGGE gels are shown in Figure 3.

Difference on distribution patterns of bands were observed in samples between regions. Band positions from Viçosa and Canaã samples showed a more pronounced difference between replicates. Although some variables interfere with the molecular analysis of soil microbial communities, it is possible to make a comparison between the compositions of communities of microorganisms in the areas under study using BioNumerics software (Figure 3).

Similarity between AMF communities was larger between accessions of the same region. Likewise, the samples from Canaã 01 and 02 and Viçosa 01 and 03 formed a cluster, indicating that genetic material of the same origin occurring in fairly remote regions, with similar climatic conditions, results in similar AMF communities in rhizosphere of *J. curcas*.

Sequencing of selected DGGE bands and identification of AMF

From the sequence analysis performed by the BLASTn tool (NCBI) identity values ranged from 81-100% (Table S1).

Five species of four genera, beyond those already identified by morphological characteristics were identified after sequencing of the 18S rDNA gene fragments: *Gigaspora decipiens* Hall and Abbott, *Gigaspora gigantea* (Nicol. and Gerd.) Gerd. and Trappe, *R. clarus* Nicol. and Schenck, *Scutellospora dipapillosa* (Koske and Walker) Walker and Sanders and *Dentiscutata heterogama*

Table 2. Arbuscular Mycorrhizal Fungi (AMF) occurring in rhizosphere of *J. curcas* in Viçosa (V), Canaã (C) and Nova Porteirinha (NP) and the frequency of AMF occurrence in Nova Porteirinha.

AMF species	V	C	NP	Freq NP* (%)
Family Acaulosporaceae				
Acaulospora delicata Walker, Pfeiffer and Bloss	+	-	+	18.6
Acaulospora excavata Ingleby and Walker	-	-	+	9.3
Acaulospora mellea Spain and Schenck	-	+	+	74.4
Acaulospora morrowiae Spain and Schenck	-	-	+	93.0
Acaulospora paulinae Blaszkowski	+	-	-	-
Acaulospora scrobiculata Trappe	+	-	+	25.6
Acaulospora walkeri Kramadibrata and Hedger	-	-	+	9.3
Acaulospora sp.	-	-	+	4.6
Family Archaeosporaceae				
Archaeospora trappei (Ames and Linderman) Morton and Redecker	-	-	+	2.3
Family Claroideoglomeraceae				
Claroideoglomus etunicatus (Becker and Gerdemann)	+	-	+	2.3
Family Glomeraceae				
Rhizophagus diaphanum (Morton and Walker) Schussler and Walker	+	+	+	69.8
Funneliformis mosseae (Nicol. and Gerd.) Schussler and Walker	+	-	+	4.6
Glomus viscosum (Nicol.)	+	-	-	-
Glomus sp	-	-	+	2.3
Glomus sp 1	+	-	-	-
Glomus sp 2	+	+	+	72.1
Glomus sp 3	+	+	-	-
Glomus sp 4	+	-	-	-
Glomus sp 5	+	+	-	-
Glomus sp 6	-	+	+	7
Glomus sp 7	-	+	+	2.3

(+) presence or (-) absence of the species in the area. * Freq = (number of accessions where the AMF species were found / total number of accessions)*100.

(Nicol. and Gerdemann) Sieverding, Souza and Oehl.

These species were not recovered previously as spores and therefore increased AMF diversity associated with *J. curcas* to 32 species.The bands eluted in the same position in the gel (Figure 3), collected from the different accessions of *J. curcas* in Nova Porteirinha, generally indicated that the AMF species were phylogenetically close to each other, especially at the genus level (Table S1).

DISCUSSION

Assessment of AMF diversity based on field-collected spores indicated the dominance of the family Glomeraceae, represented in this study by *Glomus, Rhizophagus* and *Funneliformis,* while molecular analysis revealed the prevalence of members of the Gigasporaceae (*Gigaspora, Scutellospora* and *Dentiscutata*) (Table S1). Similar results were found by Alguacil et al. (2012) who found species of *Glomus* being predominantely associated with *J. curcas*.

The predominance of small size *Glomus* spores may be linked to survival and propagation strategies found in this genus (Liang et al., 2008). The largest number of *Glomus* species in all three areas of study may be related to the high adaptability of this genus to the variations of temperature and soils, besides its ability to survive in a pH ranging from acidic to alkaline (Ho, 1987) and adapting to the disturbances in the soil (Oehl et al., 2010).

We also detected members of *Acaulospora*, *Archaeospora*, *Pacispora* and *Paraglomus* in rhizosphere of this plant. *Acaulospora* and *Glomus* have been reported as the most frequently found genera associated with *J. curca,* with 16 and 10 species, respectively (Charoenpakdee et al., 2010). Furthermore, these authors also found *Entrophospora* (1 species), *Gigaspora* (2 species) and *Scutellospora* (5 species). In the study by Charoenpakdee et al. (2010) as our, the species

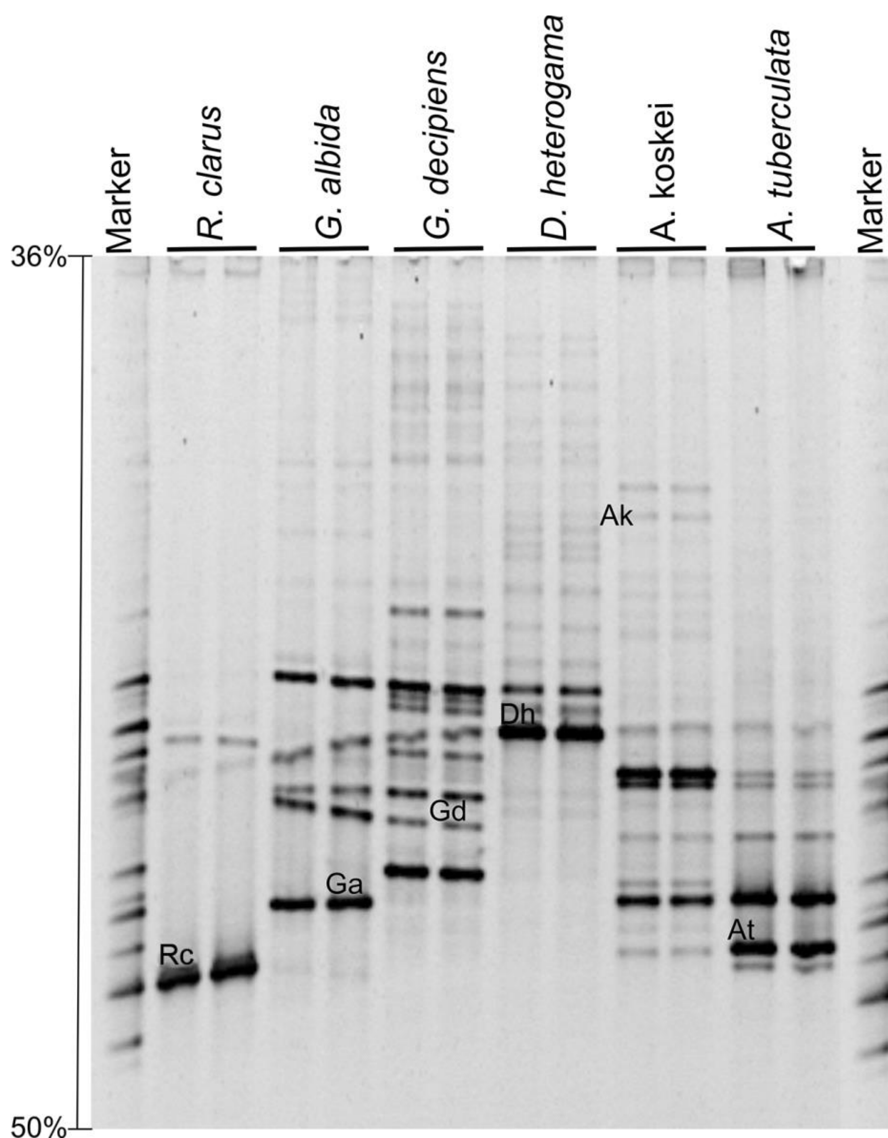

Figure 2. Profile of the bands corresponding to the 18S rDNA fragments of AMF species used as markers obtained by DGGE.

Table 3. Identity of the bands selected and eluted from the DGGE gel of Arbuscular Mycorrhizal Fungi used as reference markers.

Position of the gel band	Closest match from GenBank (% sequence similarity by BLASTn)	Genbank accession no.
Rc	*Rhizophagus clarus* (99%)	AJ852597.1
Ga	*Gigaspora* sp (98%)	EF447242.1
Gd	*Gigaspora decipiens* (100%)	AY641812.1
Dh	*Dentiscutata heterogama* (100%)	NG_017177.1
Ak	*Acaulospora* sp (96%)	AY919854.1
At	*Acaulosporaceae* (98%)	GU198548.1

The codes Rc, Ga, Gd, Dh, Ak, At, indicate the bands eluted and sequenced in DGGE gel shown in Figure 2. Only a few sequenced bands are shown in this table."

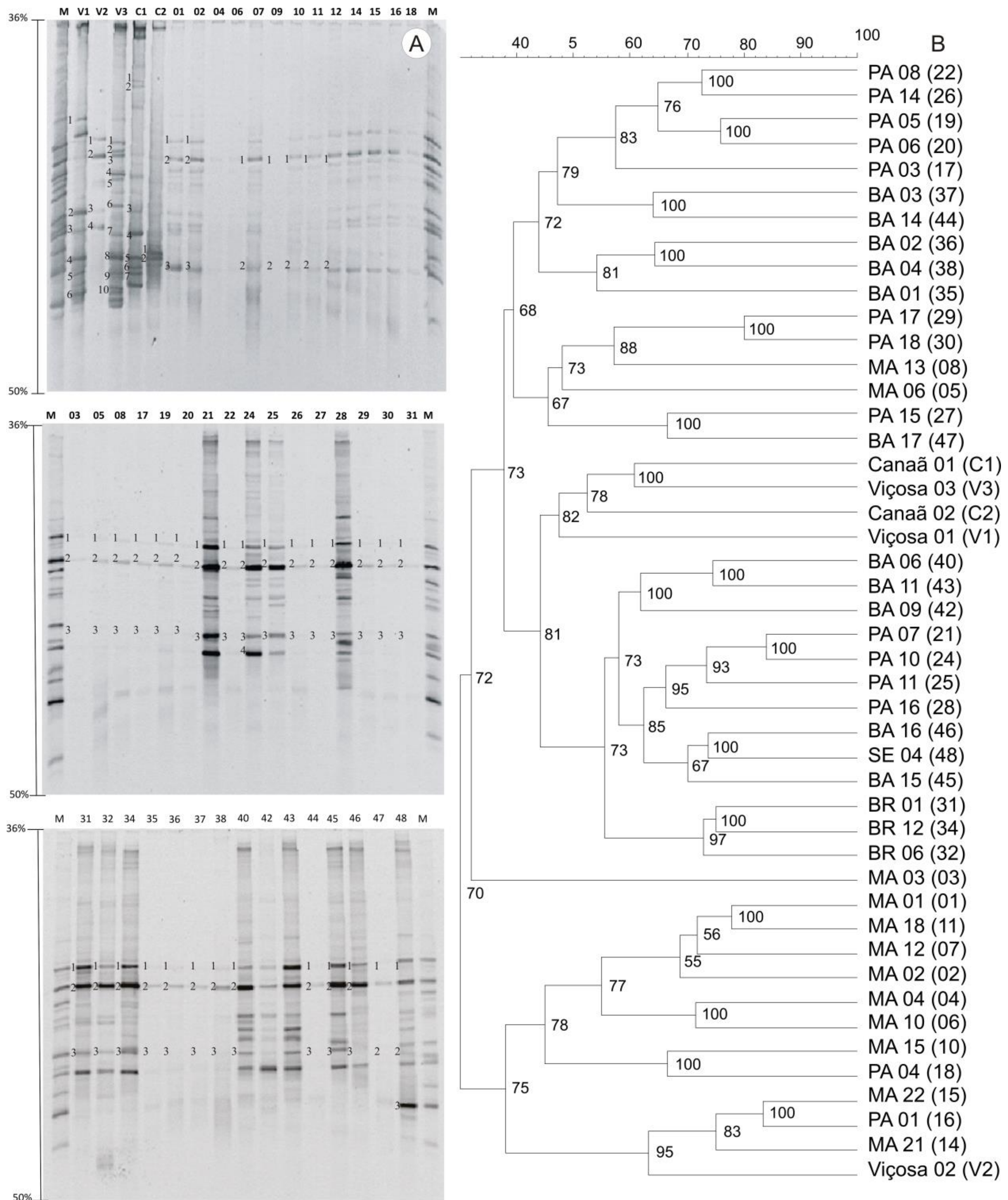

Figure 3. DGGE profile of AMF 18S rDNA fragments from Viçosa, Canaã and Nova Porteirinha. **(A)** The denaturant gradient increases from 36% on the top to 50%. M, Markers. Samples were collected in (V) Viçosa, (C) Canaã and (01 to 48) identification of each accession was collected in Nova Porteirinha/MG. The bands numbered indicate the ones which were eluted, amplified in PCR, sequenced and analyzed by BLASTn. **(B)** The UPGMA tree inferred from AMF 18S rDNA fragments from DGGE gels. The accessions of *J. curcas* deposited in the bank germplasm originally obtained from five different regions are identified by the abbreviations: MA, Matinha; PA, Paciência; BA, Banavit; BR, Barbosa and SE, Sub-estação Janaúba. The numbers indicate cophenetic correlations, which are estimates of the faithfulness of each subcluster of the dendrogram.

Table S1. Identity of the bands selected and elutes from the DGGE gel of Arbuscular Mycorrhizal Fungi samples obtained in Viçosa (V), Canaã (C) and for each accession in Nova Porteirinha (Ac).

Position the gel band	Closest match from GenBank (% sequence similarity by BLASTn)	Genbank accession no.
V1-1	Uncultured *Glomus* clone NES17#G16 18S ribosomal RNA gene, partial sequence (94 %)	GU353935.1
V1-2	Uncultured *Glomus* partial 18S rRNA gene, clone 30_14.S-NT (95 %)	AM412085.1
V1-3	Uncultured *Glomus* clone HDALG14 18S ribosomal RNA gene, partial sequence (98 %)	GQ336527.1
V1-4	Uncultured *Glomus* clone HDALG14 18S ribosomal RNA gene, partial sequence (97 %)	GQ336527.1
V1-5	*Glomus* sp. CH3263078 partial 18S rRNA gene, isolate CH3263078 (94 %)	FR690122.1
V1-6	*Glomus* sp. CH3263078 partial 18S rRNA gene, isolate CH3263078 (96 %)	FR690122.1
V2-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
V2-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (99 %)	AJ852609.1
V2-3	*Gigaspora decipiens* isolate DGGE band AU102-5 18S ribosomal RNA gene, partial sequence (100 %)	AY641812.1
V2-4	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (100 %)	EF447242.1
V3-1	Uncultured *Glomus* isolate DGGE band 123 14.c2.1.1.14c2 18S ribosomal RNA gene, partial sequence (97 %)	HQ323622.1
V3-2	Uncultured *Glomus* clone T22L1SP 18S small subunit ribosomal RNA gene, partial sequence (97 %)	EF177648.1
V3-3	Uncultured *Glomus* clone T22L1SP 18S small subunit ribosomal RNA gene, partial sequence (94 %)	EF177648.1
V3-4	Uncultured *Glomus* clone K179c6 18S ribosomal RNA gene, partial sequence (95 %)	DQ336464.1
V3-5	Uncultured *Gigasporaceae* clone FVDWSEP01EB9KY 18S ribosomal RNA gene, partial sequence (92 %)	GU198545.1
V3-6	Uncultured *Glomus* partial 18S rRNA gene, isolate PS41G (81 %)	FM955850.1
V3-7	Uncultured *Glomus* clone G1C4A1Z 18S small subunit ribosomal RNA gene, partial sequence (92 %)	EF177562.1
V3-8	Uncultured *Glomus* clone G10_2L2SP 18S small subunit ribosomal RNA gene, partial sequence (93 %)	EF177547.1
V3-9	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (94 %)	AJ852609.1
V3-10	Uncultured *Glomus* clone HDAMG10 18S ribosomal RNA gene, partial sequence (89 %)	GQ340787.1
C1-1	Uncultured *Glomus* clone DNA62_3 18S ribosomal RNA gene, partial sequence (96 %)	HM440265.1
C1-2	_	_
C1-3	Uncultured *Glomus* clone K230c5 18S ribosomal RNA gene, partial sequence (89 %)	DQ336521.1
C1-4	Uncultured *Glomus* partial 18S rRNA gene, isolate PS41G (94 %)	FM955850.1
C1-5	Uncultured *Gigasporaceae* clone FVDWSEP01CE9TJ 18S ribosomal RNA gene, partial sequence (87 %)	GU198546.1
C1-6	_	_
C1-7	Uncultured *Glomus* clone 14 group 5 18S small subunit ribosomal RNA gene, partial sequence (93 %)	EF109875.1
C2-1	Uncultured *Gigasporaceae* clone FVDWSEP01EB9KY 18S ribosomal RNA gene, partial sequence (91 %)	GU198545.1
C2-2	Uncultured *Glomus* small subunit ribosomal RNA gene, partial sequence (97 %)	DQ371697.1
Ac- 01-1	*Gigaspora gigantea* partial 18S rRNA gene, clone G-5 (90 %)	AM746154.1
Ac- 01-2	*Scutellospora heterogama* partial 18S rRNA gene, clone pWD163-2-6 (92 %)	AJ306434.1
Ac- 01-3	Uncultured *Glomus* clone NES01#D16 18S ribosomal RNA gene, partial sequence (90 %)	GU353768.1
Ac- 02-1	_	_
Ac- 02-2	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (98 %)	NG_017177.1

Table S1.Contd.

Position the gel band	Closest match from GenBank (% sequence similarity by BLASTn)	Genbank accession no.
Ac- 02-3	Uncultured *Glomus* partial 18S rRNA gene, isolate PS41G (82 %)	FM955850.1
Ac- 03-1	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 03-2	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (99 %)	NG_017177.1
Ac- 03-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 05-1	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (99 %)	NG_017177.1
Ac- 05-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 05-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 07-1	_	_
Ac- 07-2	_	_
Ac- 08-1	Uncultured *Gigasporaceae* clone LES13#I21 18S ribosomal RNA gene, partial sequence (96 %)	GU353712.1
Ac- 08-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 08-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (99 %)	EF447242.1
Ac- 09-1	*Scutellospora dipapillosa* rDNA for small subunit rRNA (87 %)	Z14013.1
Ac- 09-2	_	_
Ac- 10-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (94 %)	AJ852609.1
Ac- 10-2	Uncultured *Glomus* clone FVDWSEP01EPG8A 18S ribosomal RNA gene, partial sequence (85 %)	GU198598.1
Ac- 11-1	Uncultured *Glomus* clone NES34#D30 18S ribosomal RNA gene, partial sequence (96 %)	GU353956.1
Ac- 11-2	*Glomus clarum* 18S rRNA gene, isolate UFPE08 (93 %)	AJ852597.1
Ac- 12-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (99 %)	AJ852609.1
Ac- 12-2	*Glomus clarum* 18S rRNA gene, isolate UFPE08 (100 %)	AJ852597.1
Ac- 17-1	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (99 %)	NG_017177.1
Ac- 17-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 17-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 19-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 19-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 19-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 20-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 20-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 20-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 21-1	Uncultured *Gigasporaceae* clone LER04#P36 18S ribosomal RNA gene, partial sequence (93 %)	GU353463.1
Ac- 21-2	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (100 %)	NG_017177.1
Ac- 21-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 21-4	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (99 %)	EF447242.1
Ac- 22-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 22-2	_	_

Table S1. Contd.

Position the gel band	Closest match from GenBank (% sequence similarity by BLASTn)	Genbank accession no.
Ac- 22-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 24-1	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (99 %)	NG_017177.1
Ac- 24-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (95 %)	AJ852609.1
Ac- 24-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 24-4	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (99 %)	EF447242.1
Ac- 25-1	*Scutellospora heterogama* partial 18S rRNA gene, clone pWD163-2-6 (96 %)	AJ306434.1
Ac- 25-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (99 %)	AJ852609.1
Ac- 25-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 26-1	*Scutellospora heterogama* partial 18S rRNA gene, clone pWD163-2-6 (97 %)	AJ306434.1
Ac- 26-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (92 %)	AJ852609.1
Ac- 26-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 27-1	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (96 %)	NG_017177.1
Ac- 27-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (99 %)	AJ852609.1
Ac- 27-3	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (96 %)	NG_017177.1
Ac- 28-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 28-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 28-3	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (92 %)	NG_017177.1
Ac- 29-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 29-2	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 29-3	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (95 %)	NG_017177.1
Ac- 30-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 30-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 30-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 31-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (93 %)	AJ852609.1
Ac- 31-2	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (97 %)	NG_017177.1
Ac- 31-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 32-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 32-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 32-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 34-1	*Scutellospora heterogama* partial 18S rRNA gene, clone pWD163-2-6 (97 %)	AJ306434.1
Ac- 34-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (95 %)	AJ852609.1
Ac- 34-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 35-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 35-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (93 %)	AJ852609.1
Ac- 35-3	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (91 %)	NG_017177.1

Table S1. Contd.

Position the gel band	Closest match from GenBank (% sequence similarity by BLASTn)	Genbank accession no.
Ac- 36-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 36-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 36-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (99 %)	EF447242.1
Ac- 37-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 37-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 37-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 38-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 38-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (99 %)	AJ852609.1
Ac- 38-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (98 %)	EF447242.1
Ac- 40-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 40-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 40-3	Uncultured *Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 44-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 44-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 44-3	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 45-1	*Scutellospora heterogama* partial 18S rRNA gene, clone pWD163-2-6 (98 %)	AJ306434.1
Ac- 45-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (100 %)	AJ852609.1
Ac- 45-3	*Uncultured Gigasporaceae* clone FVDWSEP01EB9KY 18S ribosomal RNA gene, partial sequence (96%)	GU198545.1
Ac- 46-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (98 %)	AJ852609.1
Ac- 46-2	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (96 %)	AJ852609.1
Ac- 46-3	*Uncultured Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (94 %)	EF447242.1
Ac- 47-1	*Scutellospora heterogama* strain INVAM FL225 18S ribosomal RNA, partial sequence (98 %)	NG_017177.1
Ac- 47-2	*Uncultured Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (97 %)	EF447242.1
Ac- 48-1	*Scutellospora heterogama* 18S rRNA gene, isolate UFPE19 (97 %)	AJ852609.1
Ac- 48-2	*Uncultured Gigaspora* clone K15c2 18S ribosomal RNA gene, partial sequence (96 %)	EF447242.1
Ac- 48-3	*Glomus clarum* 18S rRNA gene, isolate UFPE08 (96 %)	AJ852597.1

The last number shows the identification of each eluted bands in each sample.

Acaulospora excavata, *Acaulospora morrowiae, Acaulospora scrobiculata, Claroideoglomus etunicatus, Cetraspora pellucida* and *Dentiscutata heterogama* were found. AMF diversity is a key factor for improving the sustainability of ecosystems, especially those with low fertility conditions (Ma et al., 2005). The high AMF species diversity found here suggest that this component of the soil biota plays a role to allow *J. curcas* grow in different habitats.

Glomeraceae is the most widespread and abundant family of AMF (Öpik et al., 2008; Öpik et al., 2010). The predominance of *Glomus* also occurs in various ecosystems, in association with various plant species as in recovered areas of Atlanti

forest in different stages of regeneration (Bonfim et al., 2013) in the rhizosphere of medicinal plants in the region of Goa, India (Radhika and Rodrigues, 2010), or associated with plants of *Agave potatorum* in semi-arid regions in Mexico (Carballar-Hernández et al., 2013), indicating that this genus seems to be more adapted to different soil conditions and ecosystems (Bonfim et al., 2013) and may become a good alternative for production of inoculum to be used even in the period of formation of *J. curcas* seedlings.

The percentages of colonization obtained in our study with averages close to 60% for all areas are close to the 54% found by Alguacil et al. (2012) and with less variation than those found by Charoenpakdee et al. (2010) (38-94%). According to these authors the presence of colonization in various conditions such as soil pH ranging from acidic to alkaline, low to moderate content of organic material, or even, high or low P availability demonstrate that this plant may present a high dependence on mycorrhizal colonization. The variation in the percentage colonization may be related to the diversity of AMF species present near the root system (Berbara et al., 2006) or compatible symbiotic plant-fungus relationship (Pouyu-Rojas et al., 2006; Porras-Soriano et al., 2009).

Although not all the bands have been sequenced, the gel profiles and the analysis of the sequences suggest that the AMF communities in the three regions analyzed, with different climatic conditions, present different characteristics and the grouping of DGGE band in the gel of samples obtained in the same regions (Figure 3) may indicate different genetic compatibility between the different accessions *J. curcas* and AMF.

The DGGE technique provides a good estimator of the community structure of these fungi in soil ecological studies (Öpik et al., 2003). The species identified by molecular analysis differed from the majority of species identified by morphological analysis except for species of *Glomus*, although some representatives have been identified at genus level, in both approaches. This difference may be attributed the reduced number of spores of some species found in the field or the dilution of spore in the sample preparation (Smith and Read, 1997).

As the band profile was generated by material collected directly from the soil rhizosphere, and there are spores of AMF forming multiple bands in the DGGE gel, the number of AMF found in the field, represented by the bands in the gel, can underestimate the community of these fungi in the area (Ma et al., 2005). Additionally, fragments of less abundant rDNA may be present in the same positions of large ones in the gel, which can occult the presence of the former and detection of possible species (Kowalchuk et al., 2002). However, the DGGE allows rapid comparisons between AMF communities from various regions and the analysis of multiple samples simultaneously, without the need for cultivation of fungi on host plants, making it a good tool for ecological studies of these microorganisms (Kowalchuk et al., 2002; De Souza et al., 2004).

Even samples obtained at each accession of *J. curcas* in Nova Porteirinha have shown differences in the presence or absence of some AMF species, previously identified by morphological characteristics, the distribution pattern of bands in the DGGE gels was very homogeneous, with presence of dominant bands occurring in the same position (Figure 3). However, it must be remarked that for morphological identification 100 cm^3 of soil was used, whereas for molecular analysis, only 1 g was used, which can be related to the lower abundance of AMF using this molecular tool.

The sequencing of bands found in different positions on the gel showed the presence of the same species, confirming the polymorphism of the 18S rDNA genes within the same AMF species (Öpik et al., 2003; Cornejo et al., 2004; Liang et al., 2008). Similar behavior was also observed by Liang et al. (2008), who worked with the following species markers: *Acaulospora scrobiculata*, *Gigaspora gigantea*, *Glomus intraradices, Funneliformis mosseae* (= *Glomus mosseae*) and *Dentiscutata heterogama*. This feature may be due the spores contain thousands of nuclei and eventually some may undergo some changes in the genes (Sanders and Croll, 2010).

One factor that may influence the analysis of the community of AMF by DGGE is the AM1 primer, which is specific to the orders Glomerales and Diversisporales and not specific to Archaeosporales and Paraglomerales (Ma et al., 2005). This contributes to the underestimation of the evaluation of diversity of AMF under field conditions. Our results partially corroborate that the members of *Paraglomus* and *Archaeospora* were detected from spores collected in the field (Table 2) but not from molecular analysis. Spores of *Acaulospora* were also found, although no molecular sequences were detected. This fact was also reported by Kowalchuk et al. (2002), which can be attributed to the selection of only some of the bands to be sequenced indicating that the use of both methods are important to obtain a more complete result of the diversity of AMF in areas of study.

Furthermore, it has been reported that this primer can amplify fragments of some ascomycetes and basidiomycetes (Helgason et al., 1998; Douhan et al., 2005). However, with the nested-PCR strategy, which combines the specificity of the partial AMF AM1 primer with the resolving power of the DGGE gel with the primer pair NS31-GC/Glo1 (Cornejo et al., 2004), it was possible to view a profile of the AMF species present in *J. curcas* rhizosphere.

Only the relative amount of AMF spores in the soil does not reflect their functional importance, that is it does not allow inferences about the intensity of mycorrhizal colonization or the importance of the distribution of hyphae in soil (Douds Jr. and Millner, 1999). However, this information allows us to carry out studies to understand

the composition of these communities of fungi and track changes due to environmental or anthropogenic disturbances.

So far, it has been reported, that some AMF species colonize *J. curcas* in some regions of Thailand, identified by the characterization of morphospecies in the rhizosphere of this plant (Charoenpakdee et al., 2010) and in Guantánamo, Cuba, by molecular analysis (Alguacil et al., 2012). This is a fundamental step towards understanding the dynamics and influence of these fungi on this plant at the field level.

Our studies revealthat in the region of Nova Porteirinha, characterized by a semi-arid climate, higher species diversity of these AMF are present in the rhizosphere of *J. curcas* compared with the regions of Viçosa and Canaã. This may be related to the greater diversity of plants present in regions with semi-arid climates with characteristics of greater reliance on mycorrhizal fungi (Tao and Zhiwei, 2005).

Conclusions

In *J. curcas*, *Glomus* seems to be the most abundant species of AMF and the genotype this plant may influence the AMF community. Regardless of the AMF community present in the area of cultivation, these plants present a high percentage of mycorrhizal colonization and high number of spores in their rhizosphere. The joint use of morphological and molecular methods for identification of AMF species provides more complete information about the diversity of AMF present in the rhizosphere of plants in the field.

Conflict of Interest

The author(s) did not declare any conflict of interest.

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