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Full Length Research Paper

Molecular diversity and distribution of arbuscular mycorrhizal fungi in karst ecosystem, Southwest China

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Nested polymerase chain reaction and denaturing gradient gel electrophoresis (DGGE) was used to study the molecular diverstiy and distribution of arbuscular mycorrhizal fungi (AMF) from microhabitats of three different vegetation types in karst ecosystem, Southwest China. We found molecular diversity in all three microhabitat types was high. The highest values of biodiversity index (4.06) and species abundance (68) were found in the shrubland soil surface, and the lowest values (3.16 and 29, respectively) were found in the secondary forest rocky crevice. The average values of biodiversity index and species abundance of the nine microhabitats studied were 3.67 and 48, respectively. The index value was higher than those in other areas of China; the possible reason might be the intricate karst ecological system structure, diverse plant species and a long-term mutual adaptation process. The AMF molecular diversity of different microhabitat types showed significant differences. The highest similarity index was only 0.45, which means the spatial heterogeneity of microhabitat types had significant influence on AMF molecular diversity. *Glomus* was the dominant genus in the karst area and should be a candidate for ecological restoration in karst areas. All together, our research provided an empirical, scientific basis for rational exploitation of AMF in karst ecosytem and rocky desertification restoration.

Key words: Arbuscular mycorrhizal fungi, molecular diversity, karst, microhabitat.

INTRODUCTION

Karst areas in Southwest China, located in the center of the East-Asia karst area (one of the three largest karst areas in the world), are one of two ecological fragile regions in China (Wang et al., 2004a; Zhou et al., 2010). Karst topography is a geologic formation shaped by rocky desertification of soluble bedrock, usually carbonate rocks, resulting in the fragility and vulnerability of the ecosystem in karst region (Parise and Pascali, 2003). The ecologically fragile karst system is characterized by droughts, phosphorus limited, calcium-rich, slightly alkaline, low primary productivity, soil erosion, surface collapse, land degradation and poverty of human life, which makes the recovery of vegetation even more difficult (Li et al., 2006; Wang et al., 2004a). It is widely accepted that karst rocky desertification is a serious ecological problem for ecological civilization construction and comprehensive sustainable development.

Arbuscular mycorrhizal fungi colonize roots of almost all land important plants (Smith and Read, 2008) and help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients (Sally, 2003) from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonisation of land by plants (O'Connor et al., 2002) and drought tolerance (Augé, 2001), ecological balance (Simard et al., 1997). The use of arbuscular mycorrhizal

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Abbreviations: AMF, Arbuscular mycorrhizal fungi; **DGGE,** denaturing gradient gel electrophoresis; **PCR,** polymerase chain reaction.

Table 1. Primers of Nested-PCR used in the present study.

Primer	Sequence	Length (kb)	Source
GeoA2	5′-CCAGTAGTCATATGCTTGTCTC - 3′	1.80	Schwarzott and Schüβlre (2001)
Geo11	5′-ACCTTGTTACGACTTTTACTTCC - 3′		
AM1	5′-GTTTCCCGTAAGGCGCCGAA - 3′	0.55	Helgason et al. (1998)
NS31 GC†	5′-TTGGAGGGCAAGTCTGGTGCC - 3′		Simon et al. (1992)
Glo1	5′-GCCTGCTTTAAACACTCTA - 3′	0.23	CORNEJO et al. (2004)

†The sequence of 5′- CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGG - 3′ was added at the 5′ end of NS31-GC.

fungi in ecological restoration (phytoremediation) and ecological rehabilitation projects has been shown to enable host plant establishment on degraded soil and improve soil quality and health (Straker et al., 2007; Noyd et al., 1996; Cuenca and Lovera., 1992). It should be noticed that function ecological of AMF is associated with rock desertation and limiting ecological factors of karst areas, which would have a promising potential in karst ecosystem protection and rocky desertification restoration.

Knowledge of AMF diversity and distribution characteristic may be crucial to the success of rational exploitation in plant protection projects on karst areas and restoration of rocky desertification. Karst regions display distinctive surface features with small furrows or karren, potholes, rocky, grike and stone pit, forming the microhabitat diversity of karst surface landforms (Zhou et al., 2003). As karst is a very distinctive type of topography, the co-existing plants have distinctive and regional AMF communities. However, so far there are few detailed studies of AMF diversity in karst areas, especially those using molecular biology techniques. This study investigated the AMF diversity from microhabitats of three different vegetation types (National Natural Reserve in MaoLan, GuiZhou Province, China) using nested polymerase chain reaction (PCR) and DGGE, providing an empirical, scientific basis for rational exploitation of AMF in karst ecosystem and rocky desertification restoration.

MATERIALS AND METHODS

Study site

The study sites were situated at South Libo County, MaoLan National Natural Preserve, GuiZhou province (Central-Asia humid and tropical monsoon climate zone), in the upper mountain forest zone, site is at 107°52´10″ to 108°05´40″ E/25°09´20″ to 25°20´50″ N and 758.8 m above sea level. The annual average precipitation is 1752 mm; average temperature, 18.6°C; average evaporation, 1343.6 mm; relative humidity, 83% and average 1272.8 h of sunshine. Karst topography and caves are composed mainly of limestone and dolomite in the chosen areas which forms an original, relatively stable and balanced karst ecosystem, limestone is a parent material of mollisols (Zhou, 1987).

Collection of soil samples

Samples were collected from three typical vegetation types respectively (karst original forest, secondary forest and shrubland). Three plots of 10 m × 10 m were randomly selected on each vegetation type. We surveyed all the microhabitats in each plot and three microhabitat types were classified in each plot. The microhabitat types are rocky gully (Depth/width ratio was less than 2 with varied thickness of soil at the bottom of karren), rocky crevice (a fracture or fissure in rock, Depth/width ratio was more than two with thin soil at the bottom of the grike) and soil surface [ratio of exposed rock was lower than 30%, soil covered uniformly, and the area was larger (length and width were more than 2 m)]. About 20 microhabitats of each microhabitat type were sampled from each plot. At each individual microhabitat, three to five points were collected from depth of 0 to 15 cm. Then the soils of the same microhabitat type of the same plot were pooled as the representative sample of this microhabitat type. Thus, we collected nine soil samples from each vegetation type (3 microhabitat types × 3 replicates = 9 soil samples). All together, 27 microhabitat soil samples were collected from three typical karst vegetation types. Soil samples were cut to pass a 2 mm mesh sieve using stainless steel shovels and were frozen in liquid nitrogen and stored at -20°C until use.

DNA extraction and nested polymerase chain reaction (PCR)

Community DNA was extracted from 0.5 g sample collected using Power Soil TM DNA isolation kit (MO BIO laboratories, USA). The extracted DNA was quantified and added at a concentration of 50 pg μL^{-1} for each PCR reaction (primers are listed in Table 1).

The extracted DNA was used as templates for the first PCR. PCR was performed by a nested procedure as described by Long et al. (2005). Oligonucleotide primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Company, Limited.

The first round of amplification was performed using the universal AMF 18S rDNA primers GeoA2 and Geo11. Nested PCR was performed in a total volume of 25 µL, containing 1 µL template solution, 10 mM Tris-HCl pH 8.3; 9.5 µL ddH_2O; 10 pmol of each primer (1 µl); 12.5 µl 2×Master mix (Promega, M712B). The cycling parameters were 4 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 54°C, and 7 min at 72°C, with a final extension phase of 10 min at 72°C then chilled to 4°C.

The PCR products were diluted 1:100 in Tris-EDTA buffer and used as templates in the second round using the primers NS31-GC and AM1. The cycling parameters were 2 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 65°C, and 45 s at 72°C, with a final extension phase of 7 min at 72°C then chilled to 4°C.

The PCR products were diluted 1:100 and used as templates in

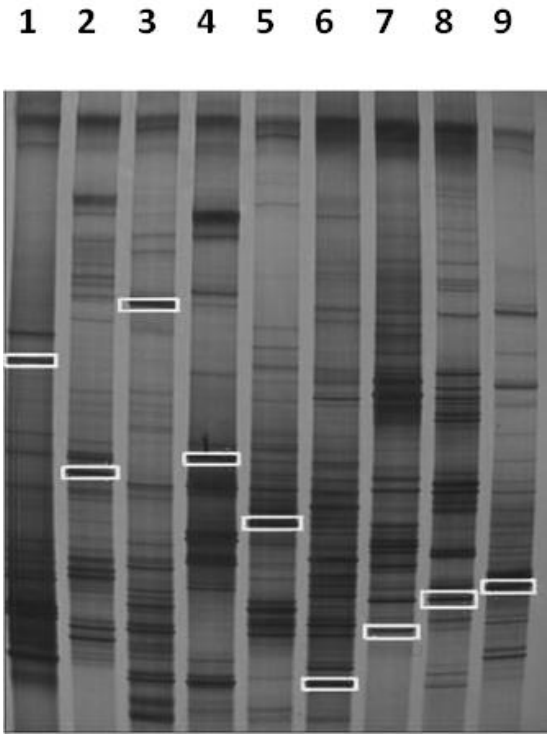

Figure 1. Comparison of DGGE profile of amplified AMF fragments from different samples and lanes. Remarked bands were selected for reamplification and gene sequencing. Lanes 1, Secondary forest Rocky crevice; 2, secondary forest rocky gully; 3, secondary forest soil surface; 4, shrubbery rocky crevice; 5, shrubbery rocky gully; 6, shrubbery soil surface; 7, original forest rocky crevice; 8, original forest rocky gully; 9, original forest soil surface.

the third round using the primers NS31-GC and GloI. The cycling parameters were 2 min at 94°C, followed by 30 cycles of 45 s at 94°C, 1 min at 55°C, and 45 s at 72°C, with a final extension phase of 7 min at 72°C then chilled to 4°C. Then 4 µl of each round amplified products were subjected to electrophoresis in 1.0% agarose gel.

Denaturing gradient gel electrophoresis (DGGE) analysis and sequencing

In order to compare all the samples in one polyacrylamide gels (As the number of injection hole is much less than our sample numbers), about 8 µl of the PCR reaction (the third round) of each replicate of each microhabitat type was pooled according to Renker et al. (2006) and DGGE was based on this bulk sample. About 24 µl of PCR product from each bulk sample was analyzed on 8% polyacrylamide gels containing gradients of 20 to 55% denaturants (7 M urea and 40% deionized formamide were considered to be 100% denaturant). Electrophoresis was run at a constant voltage of 120 V for 10 h at 60°C in 1× TAE (2 M Tris base, 1 M acetic acid, and 50 mM EDTA, for 50× stock solution) running buffer. The gels were then stained with silver nitrate and scanned with an laser image analyzer (Gel Doc Documentation System, Bio-Rad Company, USA).

Selected DGGE bands were excised from the gel of the horizontal distribution experiment (marked as red rectangular in Figure 1). The PCR products were purified as follows: the excised bands were dissolved in 12 µl ddH_2O overnight, 3000 rpm centrifuged for 1 min, 50°C bathed for 30 min, 20 µL ddH_2O was added, 3000 rpm centrifuged for 1 min, 50°C bathed for another 30 min, 12000 rpm centrifuged for 1min, the 10 µL purified products were used as template to be reamplified with primers NS31 and GloI. The amplified products were sequenced by Beijing Liuhe Huada Biological Company. Sequences were compared with known sequences by using the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST/, (Altschul et al., 1997), and the nearest neighbor fungi sequences were aligned with sample 18S rRNA sequences by using ClustalX 1.83. A phylogenetic tree was inferred by the neighbor-joining method with the Mega 4.1 package. The nucleotide accession numbers for the sequences reported in this paper are JN153040 to JN153048.

Statistical analysis

DGGE profile of amplified AMF fragments from different samples was analysed by Bio-Rad QUANTITY ONE 4.4.0 software. Comparisons of banding profiles were performed by calculating the dicer coefficient (Cs) using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. Cs=2j/(a + b). (j indicates bands in common, a indicates bands in sample A, b indicates band in sample B ; two bands were considered identical when their size were the same or differed less than 2% from their lightness.). Shannon's index (H) and richness(S) were also used to characterize AMF diversity (Luo et al., 2004), using the formula:

$$H = \sum_{i=1}^{S} \mathrm{Pilnpi}$$

H, Shannon's index; S, total bands number of each sample; Pi, relative abundance of i th band of each sample.

RESULTS

Denaturing gradient gel electrophoresis (DGGE) profile analysis

DGGE analysis yielded a high resolution band profile each sample contained many bands which suggested abundant AMF species and diversity (Figure 1). There were significant differences in its band number, intensity and migration rate, demonstrating the molecular diversity of different microhabitats.

AMF cluster analysis

Based on the similarity of AMF community composition in three studied microhabitat types, the results of the cluster analysis are shown by a dendrogram (Figure 2). The AMF community composition of different microhabitat types showed significant differences. The highest similarity index was only 0.45 (5#: shrubbery rocky gully; 6#: shrubbery Soil surface); the similarity index between original forest Rocky crevice and original forest Rocky gully was 0.41; the similarity index between secondary forest Rocky gully and shrubbery Rocky crevice was 0.34.

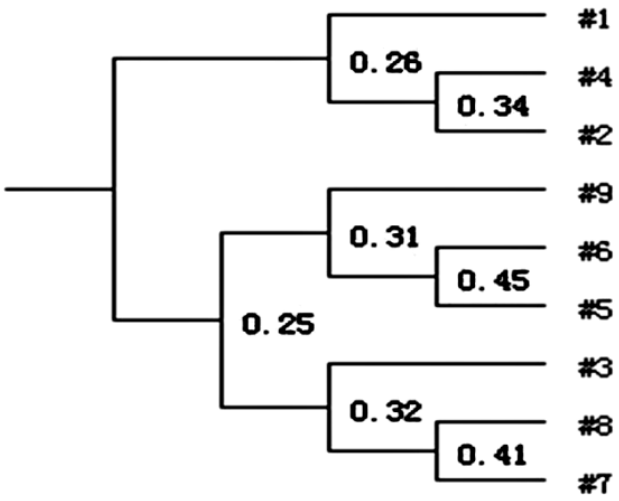

Figure 2. DGGE cluster analysis of 18S rDNA AMF communities in soil samples. Lanes 1, secondary forest Rocky crevice; 2, secondary forest rocky gully; 3, secondary forest soil surface; 4, shrubbery rocky crevice; 5, shrubbery rocky gully; 6, shrubbery soil surface; 7, original forest rocky crevice; 8, original forest rocky gully; 9, original forest soil surface.

Table 2. AMF Shannon index and richness of each sample.

Sample	Shannon's Diversity Index (*H*)	Richness
Secondary forest rocky crevice	3.16	29
Secondary forest rocky gully	3.65	48
Secondary forest soil surface	3.71	50
Shrubbery rocky crevice	3.68	50
Shrubbery rocky gully	3.77	48
Shrubbery soil surface	4.06	68
Original forest rocky crevice	3.89	57
Original forest rocky gully	3.74	48
Original forest soil surface	3.33	36

Diversity and Richness analysis of arbuscular mycorrhizal fungi (AMF)

The AMF molecular diversities in all three microhabitat types were high (Table 2). The highest values of biodiversity index were 4.06 (shrubbery Soil surface), the lowest biodiversity index of secondary forest rocky crevice was 3.16 and the mean biodiversity index of all three microhabitat types was 3.67. The biodiversity index of each microhabitat type varied as follows: The Shannon's index of original forest showed rocky crevice (3.89) > rocky gully (3.74) > soil surface (3.33); the Shannon's index of secondary forest from 3.71, 3.65 to 3.16 for soil surface, rocky gully and rocky crevice, respectively and the Shannon's index of shrubbery from 4.06, 3.77 to 3.68 for soil surface, rocky gully and rocky crevice, respectively. The highest species richness of the shrub land soil surface was 68, the lowest value was 29 (secondary forest rocky crevice) and the average values of species abundance was 48. The species richness of each microhabitat type also varied differently. The species richness of original forest was from 57, 48 to 36 for rocky crevice, rocky gully and soil surface, respectively; the species richness of secondary forest from 50, 48 to 29 for soil surface, rocky gully and rocky crevice, respectively while the species richness of shrubbery was from 68, 50 to 48 for soil surface, rocky gully and rocky crevice, respectively.

Phylogenetic analysis of arbuscular mycorrhizal fungi (AMF) sequences

PCR-DGGE of amplified 18S rRNA genes from the soils

Table 3. Alignment of DGGE sequenced clone to its most-similar GenBank sequence.

n	GenBank accession no.	Closest genera	Similarity (%)
1 (Secondary forest rocky crevice)	JN153043	Uncultured *Glomus* isolate DGGE gel band 22-2 18S ribosomal RNA gene (HQ874639)	95
2 (Secondary forest rocky gully)	JN153044	Uncultured *Glomus* clone Ap7 18S ribosomal RNA gene (EU350053)	95
3 (Secondary forest soil surface)	JN153040	Uncultured *Glomus* clone FEA6HBX02GK5NA 18S ribosomal RNA gene (GU198582)	93
4 (Shrubbery rocky crevice)	JN153045	Uncultured *Glomus* gene for 18S ribosomal RNA (AB556933)	97
5 (Shrubbery rocky gully)	JN153046	Uncultured *Glomus* partial 18S rRNA gene (AJ563889)	96
6 (Shrubbery soil surface)	JN153047	Uncultured *Glomus* isolate DGGE gel band 22-2 18S ribosomal RNA gene (HQ874639)	91
7 (Original forest rocky crevice)	JN153048	Uncultured *Glomus* isolate DGGE gel band 22-1 18S ribosomal RNA gene (HQ874638)	88
8 (Original forest rocky gully)	JN153041	Uncultured *Glomus* isolate DGGE gel band 11-2 18S ribosomal RNA gene (HQ874635)	86
9 (Original forest soil surface)	JN153042	Uncultured *Glomus* clone OEF89 18S ribosomal RNA gene (EU340303)	99

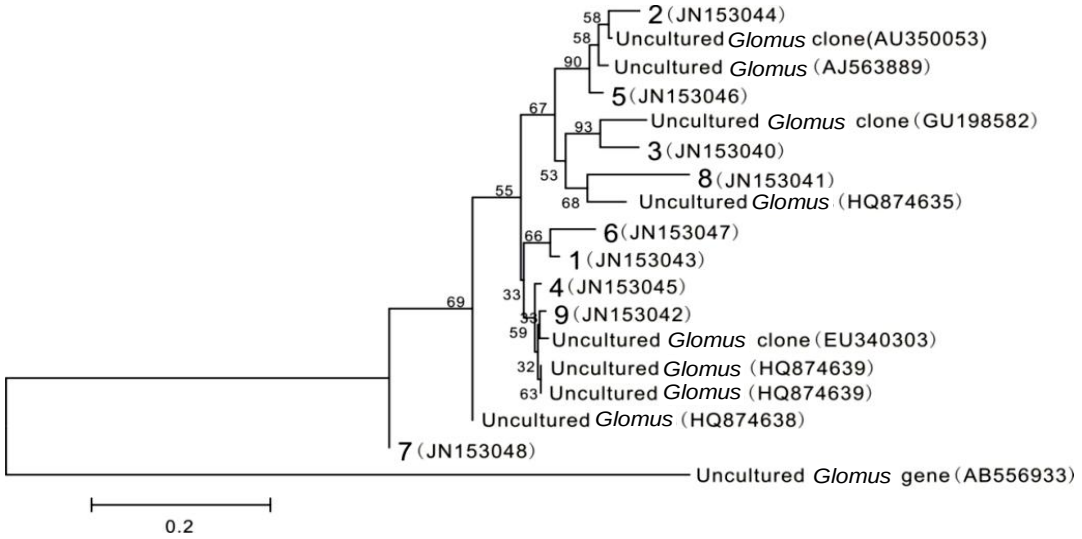

Figure 3. Phylogenetic tree of AMF partial 18S rRNA gene sequences in the soil of different microhabitat with the existing SSU rRNA gene sequences. Bootstrap support values with 1000 replicates are given along the branches.

(the clear and abundant band) were being excised for sequence analysis. Comparison of our amplified sequences with published sequences deposited in the NIH Genbank database resulted in similarity ranging from 86 to 99% (Table 3). The amplified products derived from three microhabitat types were most similar to organisms in the genus *Glomus*. The phylogenetic analysis revealed that all sequences clustered in at least nine discrete sequence groups, all belonging to the genus *Glomus* (Figure 3). This phenomenon suggested that *Glomus* was the dominant genus through the

karst area and should be a candidate for screening high ecological restoration strains for karst areas.

DISCUSSION

The DGGE is widely used in microbial community composition and dynamics under culture-independent condition, and is a powerful tool to investigate the AMF diversity (Solís-Domínguez et al. 2011; Oliveira et al., 2009). To our knowledge, this is the first molecular diversity study of AMF in the karst region by using DGGE and Nested PCR. Our results indicate that the AMF molecular diversity in karst region was high. The highest values of biodiversity index (4.06) and species abundance (68) were found in the shrubland soil surface, which is much higher than those of south-eastern coast, XiShuangBanna and Dujiangyan (Zhang et al., 1998, 2003; Fang et al., 2006). The higher molecular diversity and species richness of AMF in the investigated karst area in the present study could be due to several reasons: (1) The investigated karst area is situated at Middle Asia (tropical and monsoon climate), the structure, composition and function of ecosystems are extremely complex which formed by morphologically diversified plant species (Tu, 1989; Zhu, 1993), the diversity of plant species is higher than that of evergreen broad leaved forest (at the same latitude) (Wang et al.,2003), it's found that plant species diversity exert a reciprocal influence on associated AMF (Kernaghan, 2005; Pagan et al., 2011). (2) Compared with non-karst area, spatial heterogeneity and biodiversity of microhabitat in karst area may affect the diversity of AMF (Gilbert and Anderson, 1998), Zhang et al. (2004) showed microhabitat heterogeneity can not only enhance biodiversity but also benefit environmental stability. (3) AMF is an aerobic organism, aerobic conditions often occur in porous black limestone soils (soils with low clay content, high grit or sand soil) in karst area which favours the growth of aerobic microorganism (Xiao, 2009). It is found that spores density is higher in sandy and loamy soils than that in clay soil (Gai et al., 2000). (4) Among the mineral nutrients, phosphorus uptake is associated with AMF growth, low level soil available phosphorus could promote the growth of AMF, while the high level soil available phosphorus might inhibit the growth and development of AMF (Tawaraya et al*.,* 1994). The majority of the phosphorus in the calcareous soil is found as insoluble phosphates, which inhibit the phophorus uptake by plants. In this study, the soil available phosphorus of investigated area is 4.01 $mg \cdot kg^{-1}$ (Xiao, 2009), lower than 5 $mg \cdot kg^{-1}$ (standard phosphorus deficiency threshold), which is beneficial for AMF growth and development. In conclusion, the higher AMF diversity of karst areas is associated with biodiversity of plant species and ecosystem peculiarities in these areas and due to a long-term mutual adaptation process.

According to the DGGE cluster analysis, the highest similarity index of the AMF community composition was only 0.45 (Figure 2), the biodiversity index and species richness varied inconsistently among three microhabitat types. This phenomenon suggested that the topographic features of microhabitat can significantly affect the AMF molecular diversity. The reasons about this phenomenon might be: (1) illumination conditions, hydrothermal conditions, rainfall amount and evaporation varied among microhabitat types. Rocky crevice is more intensely illuminated by sun and favors the growth of xerophilous plants and heliophiles; rocky gully is relatively moist and favors the growth of ombrophyte and sciophiles; while soil surface favors growth of many plant species. Sykorová et al. (2007) found that the host plant species can have a major influence on the AMF communities within the roots. So, a range of different outcomes of plant growth is a main reason of molecular variability in an AMF population of different microhabitat. (2) The spatial distribution of soils is affected by micromorphology and microtopography of microhabitat. Liu et al. (2008) found soil distribution and soil properties (clay content, aggregate content, effective nutrients) varied sharply within the microhabitats in our studied area, and the soil texture, soil organic matter and nutrient elements significantly affected the diversity of AMF (Lekberg et al., 2007; Wang et al., 2009). In conclusion, the diverse microhabitats in karst areas induce spatial heterogeneity of soil properties and water distribution, resulting in the diverse distribution pattern of plant species (Liu et al., 2008) and high diversity of AMF. The biodiversity index and species abundance of microhabitat in each microhabitat type were varied and inconsistent. For microhabitat type of original forest, rocky crevice had the highest level of biodiversity index and species abundance, whereas for shrubbery and secondary forest, the soil surface had the highest level of biodiversity index and species abundance. This phenomenon indicated that the molecular diversity of AMF in each microhabitat was affected by multiple environmental factors, and the reciprocal influence mechanism was complex and random.

Sequencing results showed that, all the sequence types in each sample belonged to unnamed or uncultured microorganisms, suggesting there are many AMF species that have not been reported or discovered before. Since named or unnamed AMF positively affect soil quality and fertility, the formation of symbiotic structures. Knowledge of AMF distribution, diversity and association with different plant types may be crucial to the success of rational exploitation in plant protection projects on karst areas and restoration of degraded ecosystems

This study investigates the AMF diversity of three representative vegetation types, all together, a total of nine different bands were chosen randomly in nine selected samples from three microhabitat types (marked in red rectangular in Figure 1), sequence results showed

all of which belonged to *Glomus*, suggesting the *Glomus* is the dominant genus (population bigger than 1%) in this karst ecosystem (Muyzer et al., 1993). Genus *Glomus* was also found to be more dominant in all locations (Shi et al., 2007; Li et al., 2010), as *Glomus* was the dominant genus in this karst area and should be a candidate strain for ecological restoration in karst areas.

The abundant AMF molecular diversity in karst area means there may be abundant AMF functional diversity (Koch et al., 2006). Kuhn et al. (2001) and Corradi et al. (2007), Corradi and Sanders (2006) showed that mycorrhizal fungi have evolved through accumulation of mutations to be multi-genomic, thus forming mutualistic symbioses with the roots of approximately 80% of all plant species and improving plant nutrition and promoting plant diversity. Requena et al. (2001) found in a desertified Mediterranean ecosystem, inoculation with indigenous AMF was more efficient than non-indigenous AMF in enhancing the establishment of key plant species and increasing soil fertility and quality. Our results show that AMF were involved in a complex array of mutualisms which help maintain the microhabitat, the AMF have coevolved with the plants that produce the substrate in which they live, benefiting plant species by enhancing plant nutrient acquisition, improving soil quality, and increasing resistance to environmental stresses (drought, desertification, calcium-rich and low-phosphorus). The diversity and species composition of plant communities also exert a reciprocal influence on associated AMF communities which may promote and maintain both plant and AMF diversity. The conclusion of this study is in accordance to Navarro-Fernández et al. (2011).

It should be noticed that plants develop extremely complex root systems in karst areas, it is difficult to specify the individual plant which AMF colonize, thus lacking host specify verb. AMF genetic diversity was investigated by molecular biotechniques in this study; it needs to be further corraborated by microscopic analysis of the morphology of their spores to identify the symbiotic community currently colonizing the roots of an individual plant. All together, our research provided an empirical, scientific basis for rational exploitation of AMF in karst ecosystem and rocky desertification restoration.

REFERENCES

Augé RM (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3-42.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

CORNEJO P, Azcón-Aguilar C, Barea JM, Ferrel N (2004). Temporal temperature gradient gel electrophoresis (TTGE) as a tool for the characterization of arbuscular mycorrhizal fungi. FEMS Microbiol. Lett. 241:265-270.

Corradi N, Croll D, Colard A (2007). Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. Appl. Environ. Microbiol. 73:366-369.

Corradi N, Sanders IR (2006). Evolution of the P-type II ATPase gene family in the fungi and presence of structural genomic changes among isolates of *Glomus intraradices*. BMC Evolutionary Biology. pp. 6-21.

Cuenca G, Lovera M (1992). Vesicular arbuscular mycorrhizae in disturbed and revegetated sites from La Gran Sabana, Venezuela. Canadian J. Bot. 70:73-79.

Fang H, Damodaran PN, Cao M (2006). Arbuscular mycorrhizal status of Glomus plants in tropical secondary forest of Xishuangbanna, southwest china. Acta Ecologica Sinica. 26:4179-4185. (in Chinese with English abstract)

Solís-Domínguez FA, Valentín-Vargas A, Chorover Jon, Maier RM (2011). Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings. Science of the Total Environ. 409:1009-1016.

Gai JP, Liu RJ, Li XL (2000). Ecological distribution of arbuscular mycorrhizal fungi on wild plants in different vegetation regions of Shandong. Chinese J. Ecol. 19:18-22.

Gilbert OL, Anderson P (1998). Habitat Creation and Repair. Oxford: Oxford University Press. p: 3-6

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998). Ploughing up the wood-wide web? Nature. pp. 394-431.

Kernaghan G (2005). Mycorrhizal diversity: Cause and effect? Pedobiologia 49:511-520.

Koch AM, Croll D, Sanders IR (2006). Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. Ecological Lett. 9:103–110.

Kuhn G, Hijri M, Sanders IR (2001). Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. Nature 414:745-748.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB (2007). Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol. 95:95-105.

Li YB, Wang SJ, Wei CF, Long J (2006). An analysis on the frangibility of Karst Ecosystem. Tropical Geography 26:303-307. (in Chinese with English abstract)

Li T, Li LF, Sha T, Zhang HB, Zhao ZW (2010). Molecular diversity of arbuscular mycorrhizal fungi associated with two dominant xerophytes in a valley-type savanna, southwest China. Appl Soil Ecol. 44:61-66

Liu F, Wang SJ, Luo HB, Liu YS, Liu HY (2008). Micro-habitats in katst forest ecosystem and variability of soils. Acta Pedologica Sinica. 45:1055-1062. (in Chinese with English abstract)

Long LK, Yang SZ, Yao Q, Zhu HH (2005). DNA extraction from arbuscular mycorrhizal fungi and analysis by Pcr-Denaturing Gradient Gel Electrophoresis. Mycosystema 24(4):564-569. (in Chinese with English abstract)

Luo HF, Qi HY, Zhang HX (2004). Assessment of the bacterial diversity in fenvalerate-treated soil. World J. Microbiol. Biotechnol. 20:509-515.

Muyzer G, De Waal EC, Uitterlinden AG (1993). Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes encoding for 16SrRNA. Appl. Environ. Microbiol. 59:695-700.

Navarro-Fernández CM, Aroca R, Barea JM (2011). Influence of arbuscular mycorrhizal fungi and water regime on the development of endemic Thymus species in dolomitic soils. Appl. Soil Ecol. 48:31-37.

Noyd RK, Pfleger FL, Norland MR (1996). Field response to added organic matter, arbuscular mycorrhizal fungi, and fertilizer in reclamation of taconite iron ore tailing. Plant Soil. 179:89-97.

O'Connor PJ, Smith SE, Smith FA (2002). Arbuscular mycorrhizal influence plant diversity and community structure in a semiarid herbland. New Phytol. 154:209-218.

Oliveira CA, Sá NMH, Gomes EA, Marriel IE, Scotti MR, Guimarães CT, Schaffert RE, Alves VMC (2009). Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). Appl. Soil Ecol. 41:249-258.

Pagan MC, Utida MK, Gomes EA, Marriel IE, Cabello MN, Scotti MR (2011). Plant-type dependent changes in arbuscular mycorrhizal communities as soil quality indicator in semi-arid Brazil. Ecolo

Indicators.11: 643-650.
Parise M, Pascali V (2003). Surface and subsurface environmental degradation in the karst of Apulia (southern Italy). Environ. Geolo. 44:247-256.
Renker C, Weißhuhn K, Kellner H, Buscot F (2006). Rationalizing molecular analysis of field- collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, that is the question. Mycorrhiza 16:525-531.
Requena N, Perez SE, Azcon AC (2001). Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiolo. 67:495-498.
Sally E (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol. 133:16-20.
Schwarzott D, Schüßler A (2001). A simple and reliable method for SSU rRNA gene DNA extraction, amplification and cloning from single AM fungal spores. Mycorrhiza. 10:203–207.
Shi ZY, Zhang LY, Li XL, Feng G, Tian CY, Christie P (2007). Diversity of arbuscular mycorrhizal fungi associated with desert ephemerals in plant communities of Junggar Basin, northwest China .Appl. Soil Ecol. 35:10-20.
Simard SW, Perry DA, Jones MD (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. Nature. 388:579-582.
Simon L, Lalonde M, Bruns TD (1992). Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Appl. Environ. Microbiol. 58:291–295.
Smith SE, Read DJ (2008). Mycorrhizal symbiosis. 3rd Ed. London, UK: Academic Press.
Straker CJ,Weiersbye IM, Witkowski ETF (2007). Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines: In. Root colonization and spore levels. South Afr. J. Bot. 73:218-225.
Sykorová Z, Wiemken A, Redecker D (2007). Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss supper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. Appl. Environ. Microbiol. 73:5426-5434.
Tawaraya K, Saito M, Morioka M (1994). Effect of phosphate application to arbuscular mycorrhizal onion on the development and succinat dehydrogenase activity of internal hyphae. Soil Sci. Plant Nutr. 40:667-673.
Tu YL (1989). Preliminary study on karst forests in Guizhou. Carsologica Sinica. 8:282–290. (in Chinese with English abstract).
Wang MY, Hu LB, Wang WH, Liu ST, Li M, Liu RJ (2009). Influence of long-term fixed fertilization on diversity of arbuscular mycorrhizal fungi. Pedosphere 19:663-672.
Wang SJ, Liu QM, Zhang DF (2004a). Karst rocky desertification in southwestern China: Geomorphology, landuse, impact and rehabilitation. Land Degrad. Dev. 15:115–121.
Wang ZP, Li XG, Shi YS (2003). A comparison study on the species diversity between the gap and non-gap in Jinyun Mountain. Chinese J. Appl. Ecol. 14:7-10.
Xiao DA (2009). The geochemical characteristics of soil water and epikarst springs and their response to vegetation-soil degradation (PhD dissertation). Beijing: Graduate university of Chinese academy of science.
Zhang MQ, Wang YS, Xing LJ (1998). The ecological distribution of AM fungi community in south and east coast of China. Mycosystema. 17:274-277. (in Chinese with English abstract).
Zhang WH, Lu T, Ma KM (2004). Analysis on the environmental and spatial factors for plant community distribution in the arid valley in the upperreach of Min jiang River. Acta Ecologica Sinica 24:552-559.
Zhang Y, Guo LD, Liu RJ (2003). Diversity and ecology of arbuscular mycorrhizal fungi in Dujiangyan. Acta Phytoecologica Sinica. 27:537–544. (in Chinese with English abstract).
Zhou YC, Wang SJ, Lu HM, Xie LP, Xiao DA (2010). Forest soil heterogeneity and soil sampling protocols on limestone outcrops: Example from Sw China. Acta Carsologica 39(1):115–122.
Zhou YY, Li SS, Huang TF (2003). Features of karst forest ecosystem in China and its conservation in utilization-a case study on Maolan, Mulun, Nonggang typical karst forests in Southwest China. J. Guangxi Teachers College (Natural Science Edition). 20:67-72. (in Chinese with English abstract).
Zhou ZX (1987). Scientific investigation report of Maolan Karst forest. Guizhou People's Press, Guiyang. pp. 1-23. (in Chinese).
Zhu SQ (2003). Ecological research on Karst forest (Ⅲ). Guizhou Science and Technology Press, Guiyang. pp. 52-62. (in Chinese).