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Restriction Analyses of PCR Amplified Partial SSU Ribosomal DNA to Distinguish Arbuscular Mycorrhizal Fungi from Other Fungi Colonizing Plant Roots

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Roots of *Glycine max* and *Miscanthus sinensis* and soil samples were collected from various field sites at Goesan, Chungbuk in Korea. Microscopic observations of the roots indicated high colonization rates of both arbuscular mycorrhizal fungi (AMF) and other fungi. The partial small subunit of ribosomal DNA genes were amplified with the genomic DNA extracted from their roots by nested polymerase chain reaction (PCR) with universal primer NS1 and fungal specific primers AM1. Restriction fragment length polymorphism (RFLP) was analyzed using the combinations of three restriction enzymes, *HinfI*, *AluI* and *Asu*C21. Nucleotides sequence analysis revealed that ten sequences from *Miscanthus sinensis* and one sequence from *Glycine max* were close to those of arbuscular mycorrhizal fungi. Also, 33% of total clones amplified with NS31-AM1 primers from *M. sinensis* and 97% from *G. max* were close to *Fusarium oxysporum* or other pathogenic fungi, and they were successfully distinguished from AMF. Results suggested that these techniques could help to distinguish arbuscular mycorrhizal fungi from root pathogenic fungi in the plant roots. Especially, DNA amplified by these primers showed distinct polymorphisms between AMF and plant pathogenic species of *Fusarium* when digested with *Asu*C21.

KEYWORDS: Arbuscular mycorrhizal fungi, *AsuC21*, *Fusarium*, Glomales, *Glycine max*, *Miscanthus sinensis*

Arbuscular mycorrhizal (AM) fungi (order Glomales, Zygomycetes) are important for plants because they play a key role in plant nutrient uptake, especially phosphorus uptake (Smith and Read, 1996), and have protective role against various plant pathogenic fungi (Newsham *et al.*, 1995). Previous studies have shown that individual species of arbuscular mycorrhizal fungi differ in their ability to promote plant growth (van der Heijden *et al.*, 1998), therefore, identifying AM fungal species within plant roots is critical factor for agricultural and ecological studies. However, taxonomy of AM fungi have been relied on morphological features of spores collected from soil and it is not possible to identify AM fungal species within plant roots below the genus level using morphological features. In addition, it is difficult to discriminate active fungal symbionts of an individual plant from an AM fungal spore community in the soil around the roots due to the complex root system.

In recent years, molecular techniques have been used to study phylogenetic relationships of AM fungi, and attempts have been made to use ribosomal genes as a tool for the identification of AM fungal species (Redecker *et al.*, 1997). However, only few studies have been focused on the direct identification of mixed populations of AM fungi in actively colonized root using PCR based detection methods with specific primers (Vandenkoornhuyse *et al.*, 2002).

Helgason *et al.* (1998) designed a primer pair NS31-AM1 to detect AM fungal species composition in roots. However, one of the problems applying these primers to roots collected from field condition was amplification of other fungal DNAs associated with roots as well. The objective of this study was, therefore, to apply PCR based strategies to detect AM fungi in actively colonized roots of *Glycine max* and *Miscanthus sinensis*.

Materials and Methods

The soil and roots of *G. max* and *M. sinensis* were collected from field sites in Chungbuk, Korea in September of 2001. Soil chemical composition was analyzed in soil laboratory in Chungbuk National University. AM fungal spores were extracted from soil using wet-sieving and sucrose density gradient centrifuge methods (Daniels and Skipper, 1982) and observed under light microscopes. Root samples were stained with 0.05% trypan blue (Koske and Gemma, 1989) and colonization of AM fungi were observed.

Total DNA was extracted from the plant roots according to (Ausubel *et al.*, 1999). Nested PCR were conducted with genomic DNAs from plant roots. First amplification of a partial sequence of the SSU rRNA gene were performed with universal eukaryotic primers NS1 (GTA GTC ATA TGC TTG TCT C, 10 pmol) and NS4 (CTT CCG TCA ATT CCT TTA AG, 10 pmol) for 30 cycles (1 cycle at 94°C for 3 min, at 42°C for 1 min, at 72°C for 1 min 30 secs; 28 cycles at 94°C for 1 min 30 sec, at 42°C for 1 min, 72°C for 1 min 30 sec; 1 cycle

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at 94°C for 30 sec, at 42°C for 1 min, at 72°C for 10 min) using AccuPower PCR premix (Bioneer Co., 1 U Taq DNA polymerase, 250 uM dNTP, pH 9.0 10 mM Tris-HCl, 40 mM KCl, 1.5 mM $MgCl_2$). PCR product was diluted to 1 : 100 and used for template of the second PCR. The second amplification was performed using general fungal primer AM1 (GTT TCC CGT AAG GCG CCG AA, 10 pmol) designed to exclude plant DNA (Helgason *et al.*, 1999) and universal eukaryotic primer NS31 (TTG GAG GGC AAG TCT GGT GCC, 10 pmol; (Simon *et al.*, 1992). The PCR was performed for 30 cycles (10 cycles at 95°C for 1 min, 58°C for 1 min, 72°C for 2 min, 19 cycles at 95°C for 30 sec, 58°C for 1 min,

Table 1. Chemical properties of soil planted with *Miscanthus sinensis* and *Glycine max* at Chungbuk, Republic of Korea

	M. sinensis	*G. max*
pH	6.98	6.19
Organic matter (g kg^{-1})	19.99	16.03
NO_3-N (mg kg^{-1})	6.23	7.58
NH_4-N (mg kg^{-1})	6.64	12.68
Av. P_2O_5 (mg kg^{-1})	55.95	14.84
K ($cmol^+$ kg^{-1})	0.61	0.35
Ca ($cmol^+$ kg^{-1})	19.06	15.35
Mg ($cmol^+$ kg^{-1})	1.56	1.39
Cation Exchange Capacity ($cmol^+$ kg^{-1})	10.07	8.71

Table 2. Arbuscular mycorrhizal fungal spores collected from soils planted with *Miscanthus sinensis* and *Glycine max* at Chungbuk, Republic of Korea

Glomalean fungal species		Number of spores/10 g dry soil (%)			
		Miscanthus sinensis		*Glycine max*	
Acaulosporaceae					
	Acaulospora scrobiculata	133	(61.9)		
Gigasporaceae					
	Gigaspora margarita	34	(15.8)	4	(4.8)
	Scutellospora erythropa	17	(7.9)	13	(15.5)
	S. sp.1			1	(1.2)
Glomaceae					
	Glomus mosseae	12	(5.6)	40	(47.6)
	Gl. sp. 1			7	(8.3)
	Gl. sp. 2	11	(5.1)	17	(20.2)
	Gl. sp. 3	1	(0.5)		
	Gl. sp. 4	1	(0.5)		
	Gl. sp. 5	6	(2.8)	2	(2.4)
Total		215	(100)	84	(100)

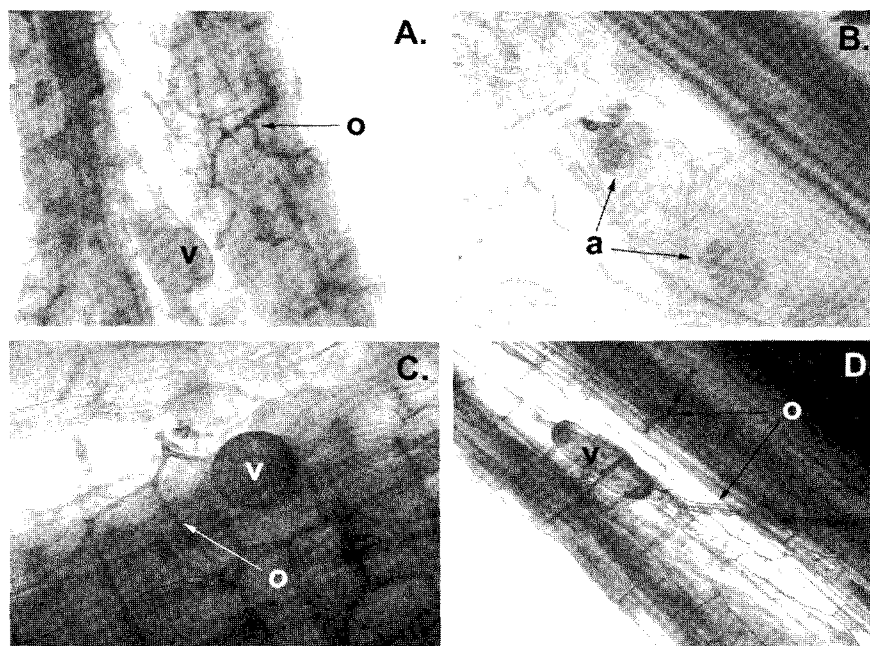

Fig. 1. Roots colonized by AM fungi (v: vesicle, a: arbuscules) and other fungal hyphae (o) stained with trypan blue. A and B: roots of *Glycine max*, B and C, roots of *Miscanthus sinensis*.

72°C for 3 min and 1 cycle at 95°C for 30 sec, 58°C for 1 min and 72°C for 10 min). PCR products were separated by electrophoresis on a 1% agarose gel and stained with EtBr. Expected fragments for cloning were removed from the gel and purified with gel purification Kit (Bioneer, Korea).

Purified DNAs were inserted to pGEM®-T Easy Vector and transformed to *Esherichia coli* strain JM109. Thirty putative positive *E. coli* transformants were selected from each plant sample. Recombinant plasmid DNAs were extracted from the bacterial transformants and used for PCR reaction using primers AM1 and NS31. The PCR products were digested with restriction enzymes, *Hinf*I and *Alu*I, and *Asu*C21. Restriction fragments were separated on a 3% agarose gel. Nucleotide sequence of one clone from each RFLP type was determined using automatic sequencer (ABIPRISM™, USA). DNA sequence analyses were performed with the BLAST software available through the National Center for Biotechnology Information (NCBI). Clustal X (ver. 1.81) was used for alignment of DNA sequence.

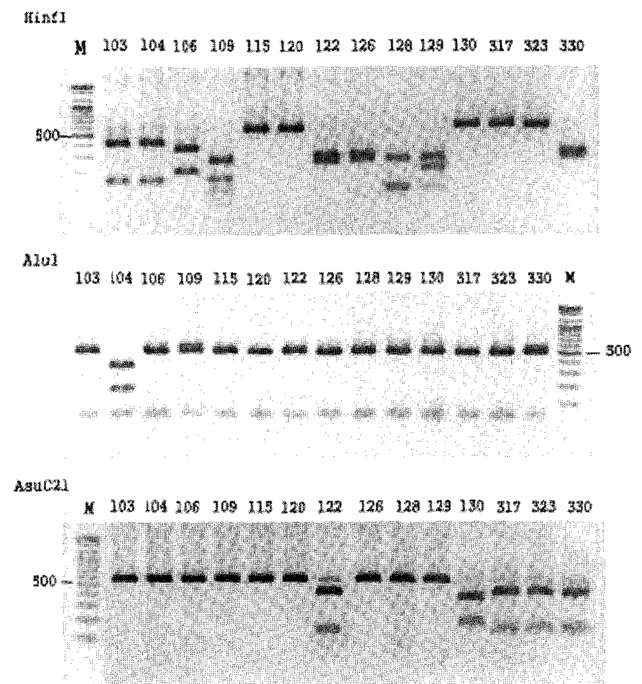

Fig. 2. The DNA fragments digested with restriction enzymes, *Hinf*I, *Alu*I and *Asu*C21 from the partial 18S rDNA bands; Lane M indicates molecular size marker (100 bp ladder) and lane numbers labled as 103 through 330 indicate isolates listed in Table 3.

Results and Discussion

Chemical properties, species richness of AM fungi and mycorrhizal root colonization rate showed significant difference in soil planted with *G. max* and higher phosphorus in soil planted with *M. sinensis* (Table 1). High nitrogen composition in soil planted with *G. max* should be due to presence of nitrogen fixing bacteria with *G. max*. As shown in Table 2, total 10 species of AM fungi were found in soils from both sites and five *Glomus* species could not be placed into described taxa. Eight species within 4 genus and 7 species within 3 genus were detected based on morphological features of spores from *M. sinensis* and *G. max* planted soils, respectively (Table 2). Total spore numbers and species composition in each soil were different between two sites. Total 215 spores of AM fungi were found from *M. sinensis* planted soil, compared to 84 spores from *G. max* planted soil. Among them, *Acaulospora scrobiculata* were dominated in soil

Table 3. Glomalean and other fungi identified by DNA sequences from roots of *Glycine max* and *Miscanthus sinensis*

Isolates	Fungal species identified by sequences from NCBI			Number of clones (%[a])			
	Fungal species	Accession number	Sequence similarity (%)	*G. max*		*M. sinensis*	
Glomalean fungi				**1**	**(3.3)**	**20**	**(66.7)**
C103	*Glomus fasciculatum*	GFA17640	540/550 (98%)	1	(3.3)	1	(3.3)
C104	*G. fasciculatum*	GFA17640	546/549 (99%)			5	(16.7)
C106	*Glomus* sp. Glo22	AF437698	499/508 (98%)			2	(6.7)
C109	*Glomus* sp. MO-G8	GSP418868	507/508 (99%)			1	(3.3)
C115	*G. sinuosum*	GSI133706	514/545 (94%)			2	(6.7)
C120	*Glomus* sp. WUM3	GVE301864	523/550 (95%)			2	(6.7)
C126	*G. mosseae* BEG69	GMU96141	522/545 (95%)			1	(3.3)
C128	*Glomus* sp. Glo13	AF437660	495/507 (97%)			2	(6.7)
C129	*Glomus* sp. Glo4	AF074353	496/507 (97%)			4	(13.3)
Pathogenic fungi and others				**29**	**(96.6)**	**10**	**(33.3)**
C130	*Chaetomium globosum*	AB048285	534/538 (99%)			1	(3.3)
C122	*Fusarium equisetium*	AF141949	523/545 (95%)			1	(3.3)
C317	*F. oxysporum*	AF219124	540/543 (99%)	24	(80)	8	(26.7)
C323	*Myrothecium* sp. BBA691	MSP301998	540/543 (99%)	4	(13.3)		
C330	*Cordyceps takaomontana*	AB044631	521/545 (95%)	1	(3.3)		

[a]Number of clones/total 30 clones.

planted with *M. sinensis* (62%), but *Glomus mosseae* was dominated in soil planted with *G. max* (79%). Host plant species has been suggested a possible factor for affecting AM fungal sporulation and communitiy composition (Bever *et al.*, 1996; Eom *et al.*, 2000). However, AM fungal composition in soils tested might be influenced by other factors as well as host plant species (Eom *et al.*, 1999). Mycorrhizal structures such as arbuscules and vesicles as well as other fungi with septate hypha were found in both roots (Fig. 1). Mycorrhizal root colonization rate was 70% in *M. sinensis* and 68 % in *G. max*, in spite of higher phosphorus content in soils of *M. sinensis* compared to soils of *G. max*. Significant differences in mycorhizal colonization rate and mycorrhizal structure between the sites were not detected under microscopes. However, there was much evidence that the spore counts or microscopic observations of roots do not reflect composition or abundance of AM fungi (Clapp *et al.*, 1995). Thus, molecular techniques were used to assess community composition of AM fungi colonizing on roots in this study.

Thirty of amplified PCR products from each plant were analyzed. Restriction analysis using enzymes *Hin*fI, *Alu*I and AscuC21 showed 14 distinct polymorphisms. Ten

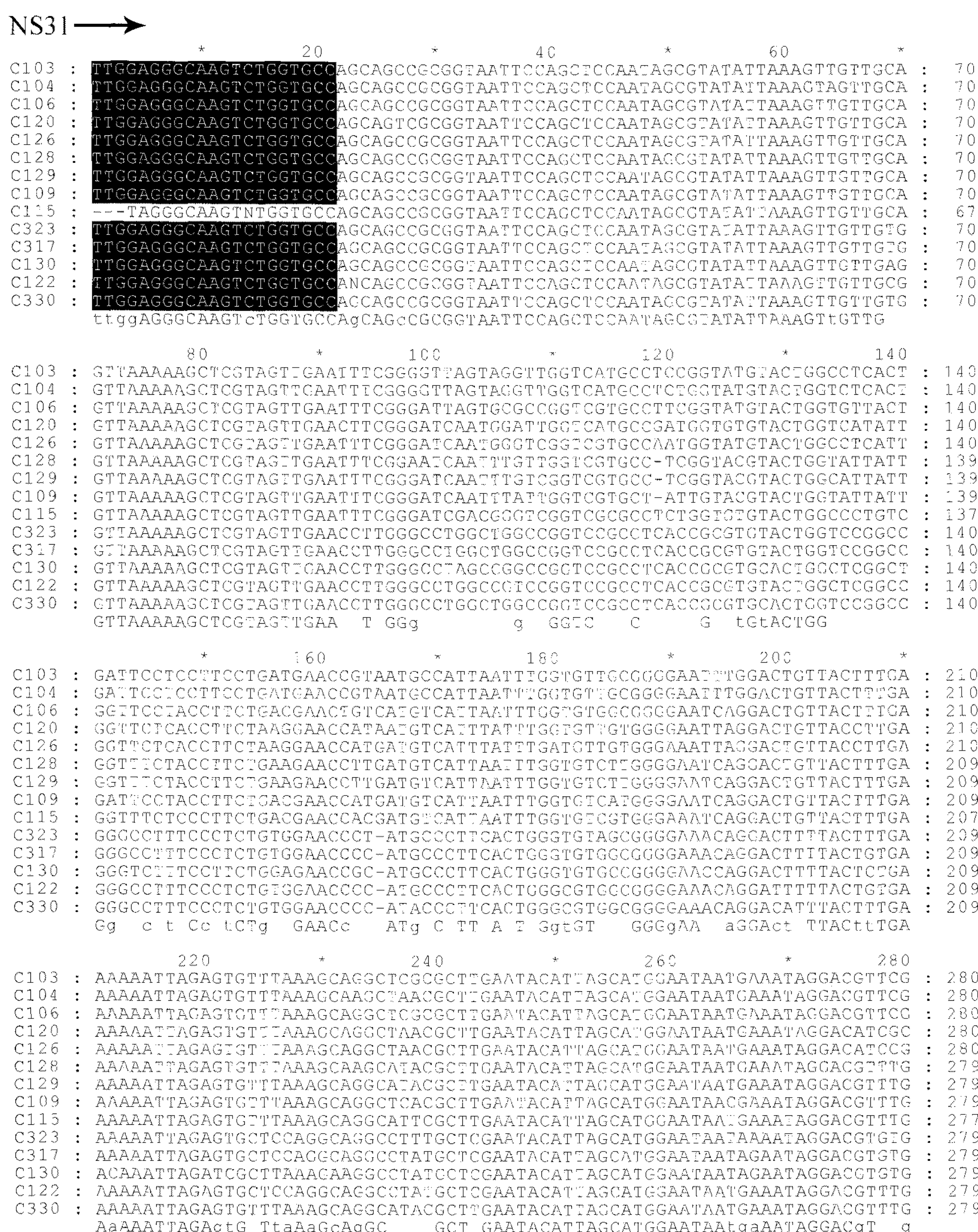

Fig. 3. The sequence alignment of partial 18s rDNA. Sequences were aligned using Clustal X. (Fig. continued)

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                  *      300        *       320        *       340        *
C103 : ATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAAGGGATAAGTTGGGGGCATTAGTATTCA : 350
C104 : ATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 348
C106 : ATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 348
C120 : ATTCTATTTTGTTGGTTTCTAGGATCGATGAAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 348
C126 : ATTCTATTTTGTTGGTTTCTAGGATCGATGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 348
C128 : ATTCTATTTTGTTGGTTTCTAGGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 347
C129 : ATTCTATTTTGTTGGTTTCTAGGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 347
C109 : ATTCTATTTTGTTGGTTTCTAGGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 347
C115 : ATCTTATTTTGTTGGTTTCTAAGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 345
C323 : GTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTAATA-GGGACA-GTCGGGGGCATCAGTATTCA : 347
C317 : GTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTAATA-GGGACA-GTCGGGGGCATCAGTATTCA : 347
C130 : GTTCTATTTTGTTGGTTTCTAGGACCGCCGTAATGATTAATA-GGGACA-GTCAGGGGCATCAGTATTCA : 347
C122 : ATTCTATTTTGTTGGTTTCTAGGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 347
C330 : ATTCTATTTTGTTGGTTTCTAGGATCGACGTAATGATTAATA-GGGATA-GTTGGGGGCATTAGTATTCA : 347
       aT cTATTTTGTTGGTTTCTAgGAtcGacGtAATGATTAATA GGGAtA GTtgGGGGCATtAGTATTCA

               360        *       380        *       400        *      420
C103 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 420
C104 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 418
C106 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 418
C120 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 418
C126 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 418
C128 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
C129 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTC : 417
C109 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
C115 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 415
C323 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
C317 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
C130 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
C122 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAGGCATTTGCCAAGGATGTTTT : 417
C330 : ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCATTTGCCAAGGATGTTTT : 417
       ATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAaGCATTTGcCAAGGATGTTTt

                  *      440        *       460        *       480        *
C103 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 490
C104 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 488
C106 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 488
C120 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 488
C126 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 488
C128 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C129 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C109 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C115 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 485
C323 : CATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C317 : CATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C130 : CATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C122 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
C330 : CATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT : 487
       CATTAATCAaGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT

               500        *       520        *       540        *
C103 : GCCGACTAGGGATCGGATGATGTTA--TTTTTAATGACTCATTCGGCGCCTTACGGGAAAA : 549
C104 : GCCGACTAGGGATCGGATGATGTTAATTTTTTAATGACTCATTCGGCGCCTTACGGGAAAC : 549
C106 : GCCGACTAGGGATCGGATGATGTTAA-TTTTTAATGACTCATTCGGCGCCTTACGGGAAAC : 548
C120 : GCCGACTANGGATCGGATGATGTTGATTTTTTAATGACTCATTCNGCGCCTTACNGGAAAC : 549
C126 : GCCGACTAGGGATCGGATGATGCTTATTTTTTAATGACTCATTCGGCGCCTTACGGAAAC- : 548
C128 : GCCNACTAGGGATCGGATGATGTTAATTTTTTAATGACTCATTCGGCGCCTTACGGGAAAC : 548
C129 : GCCGACTAGGGATCGGATGGTGTTAATTTTTTAATGACTCATTCGGCGCCTTACGCGAAAC : 548
C109 : GCCGACTAGGGATCGGATGATGTTAATTTTTTAATGACTCATTCGGCGCCTTACGGGAAAC : 548
C115 : GCCGACTAGGGATCGGATGATGTTAATTTTTTAATGACTCATTCGGCGCCTTACGCGAAAC : 546
C323 : GCCGACTAGGGATCGGACGATGTTA--GAAAT--TGACTCGTTCGGCGCCTTACGGGAAAC : 544
C317 : GCCGACTAGGGATCGGACGGTGTTA--TATTT--TGACCCGTTCGGCGCCTTACGGGAAAC : 544
C130 : GCCGACTAGGGATCGGACGGTGTTA--TTTTT--TGACCCGTTCGGCGCCTTACGGGAAAC : 544
C122 : GCCGACTAGGGATCGGATGATGTTA--TTTTTAATGACTCATTCGGCGCCTTACGGGAAAC : 546
C330 : GCCGACTAGGGATCGGATGATGTTA--TTTTTAATGACTCATTCGGCGCCTTACGGGAAA- : 545
       GCCgACTAgGGATCGGAtGaTGtTa  ttttTaaTGACtCaTTCgGCGCCTTACgGgAAac

                                               <---- AM1
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groups of restriction pattern were from roots of *M. sinensis* and 4 groups from roots of *G. max*. A PCR product for each polymorphism was sequenced (Fig. 2). BLAST searches revealed that 9 sequences amplified using NS31-AM1 belong to the Glomales and all of the sequences were within genus Glomus. No sequences were detected within the Acaulosporaceae and Gigasporaceae. Also, Archaeosporaceae or Paraglomaceae were not detected but this was expected because the AM1 primer does not match the highly divergent sequences of these taxa (Redecker, 2000). Sequence analysis showed that 8 species of AM fungi were colonized in roots of *M. sinensis* (67% of the total number of clones). However, only 1 sequence of total 4 clones from roots of *G. max* was related to AM fungi (Table 3).

NS31-AM 1 primers used in this study would be one of the useful primers to study AM fungal community in roots because these primers were designed to exclude plant DNA from DNA extracts from plant roots {Helgason, 1998 #4529}. However, these primer pairs do not exclude other root inhabiting fungi such as *Fusarium,* and plant DNA (Redecker, 2000; Tae *et al.*, 2002). It was difficult to distinguish those non Glomalean sequences using enzymes, *Alu*I and *Hinf*I. In this study, several other restriction enzymes were screened for efficient detection of non-Glomalean clones (data not shown) and *Asu*C21

were selected. RFLP with *Asu*C21 (CC!(C/G)GG) revealed three more groups in *M. sinensis*, but these group showed same restriction pattern with *Hinf*I and *Alu*I (Fig. 2). However, non-Glomalean species such as *Fusarium oxysporum* and *Joinvillea ascendens* (102 and 124 in Fig. 2, respectively) had restriction sites with *Asu*C21, but Glomalean species such as *Glomus sinuosum* and *G. mosseae* (115 and126 in Fig. 2, respecitively) had no restriction site (Fig. 2). Using this enzyme, 33% of amplified product with NS31-AM1 primers from *M. sinensis* was close to *F. oxisporum* and other pathogenic fungi; they were 97% from *G. max*.

One of problems of AM1-NS31 primers was that they could not amplify a certain group of AM fungi including genus Archaeospora and Paraglomus and it should be solved for further ecological and physiological studies of AM fungi. Although species in Archaeosporaceae and Paraglomaceae have restriction sites with *Asu*C21, it is useful as long as amplified with AM1-NS31 primers because they could not amplify DNA of these species.

Analyses revealed that soil nutrient composition and mycorrhizal root colonization rate were similar in both plant growing sites. However, fungal species composition in roots significantly differed in two plant roots. In roots of *M. sinensis*, 67% of clones were Glomalean fungi, compared to 3% of clones in *G. max*. This was consistent with higher spore numbers in *M. sinensis* than that in *G. max* (Table 2). Only one species, *G. mosseae*, was found in both root and soil and the most dominant species, *A. scrobiculata* in *M. sienesis* and *G. mosseae* in *G. max* was not found in their roots. These discrepancies of AM fungal species composition between roots and soil confirmed that spore communities in soil would not necessarily reflect active AM fungal community in host plant root.

Most of sequences amplified from roots of *G. max* were *Fusarium* species related to root pathogen. A possible explanation is that *G. max* was a crop species and more vulnerable to pathogens than wild species, *M. sinensis*. Alternatively, soil nitrogen contents or geographical difference would be other factors. However, there was not enough samples in this study to explain this result and further experiments are needed.

Results in this study suggest that a PCR-based technique could be useful to distinguish arbuscular mycorrhizal fungi from other root pathogenic fungi. Especially, DNA amplified by these primers showed distinct polymorphisms between AMF and plant pathogenic species of *Fusarium* when digested with *Asu*C21.

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References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. 1999. Short protocols in molecular biology. 4th ed. John Wiley and Sons, Inc, New York.

Bever, J. D., Morton, J. B., Antonovics, J. and Schultz, P. A. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology* **84**: 71-82.

Clapp, J. P., Young, J. P. W., Merryweather, J. W. and Fitter, A. H. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* **130**: 259-265.

Daniels, B. A. and Skipper, H. A. 1982. Methods for the recovery and quantitative estimation of propagules from soil., pp. 29-35, *In* Schenck, N. C., ed. Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minn.

Eom, A. H., Hartnett, D. C. and Wilson, G. W. T. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* **122**: 435-444.

_____, _____, _____ and Figge, D. A. H. 1999. The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. *American Midland Naturalist* **142**: 55-70.

Helgason, T., Fitter, A. H. and Young, J. P. W. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising Hyacinthoides non-scripta (bluebell) in a seminatural woodland. *Molecular Ecology* **8**: 659-666.

_____, Daniell, T. J., Husband, R., Fitter, A. H. and Young, J. P. W. 1998. Ploughing up the wood-wide web? *Nature* **394**: 431.

Koske, R. E. and Gemma, J. N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**: 486-505.

Newsham, K. K., Fitter, A. H. and Watkinson, A. R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* **83**: 991-1000.

Redecker, D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10**: 73-80.

_____, Thierfelder, H., Walker, C. and Werner, D. 1997. Restriction analysis of PCR-amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the order glomales. *Applied and Environmental Microbiology* **63**: 1756-1761.

Simon, L., Lalonde, M. and Bruns, T. D. 1992. Specific amplification of 18S fungal ribosomal genes from vesicular- arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology* **58**: 291-295.

Smith, S. E. and Read, D. J. 1996. Mycorrhizal symbiosis. 2nd ed. Academic Press, London.

Tae, M. S., Eom, A.-H. and Lee, S. S. 2002. Sequence analysis of PCR amplified partial SSU ribosomal DNA for identifying arbuscular mycorrhizal fungi in plant roots. *Mycobiology* **30**: 13-17.

van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf, E. R., Boller, T., Wiemken, A. and Sanders, I. R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69-72.

Vandenkoornhuyse, P., Husband, R., Daniell, T., Watson, I., Duck, J., Fitter, A. and Young, J. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology* **11**: 1555-1564.