

## Indetification of Symbiotic Arbuscular Mycorrhizal Fungi in Korea by Morphological and DNA Sequencing Features of Their Spores

Cho, Nam-Seok

School of Forest Resources and Rural Engineering, Chungbuk National University

Kim, Dong-Hun

School of Forest Resources and Rural Engineering, Chungbuk National University

Eom, An-Heum

Dept. of Biology Education, Korea National University of Education

Lee, Jeong-Woo

Mycology Institute, Hankuk Wongun Co. Ltd

他

<https://doi.org/10.5109/9230>

---

出版情報：九州大学大学院農学研究院紀要. 51 (2), pp.201-210, 2006-10-27. Faculty of  
Agriculture, Kyushu University

バージョン：

権利関係：



## Identification of Symbiotic Arbuscular Mycorrhizal Fungi in Korea by Morphological and DNA Sequencing Features of Their Spores

Nam-Seok CHO<sup>1</sup>, Dong-Hun KIM<sup>1</sup>, An-Heum EOM<sup>2</sup>, Jeong-Woo LEE<sup>3</sup>, Tae-Ho CHOI<sup>1</sup>,  
Hee-Yeon CHO<sup>4</sup>, Andrzej LEONOWICZ<sup>5</sup> and Shoji OHGA\*

Laboratory of Forest Resources Management, Division of Forest Ecosystem Management,  
Department of Forest and Forest Products Sciences, Kyushu University,  
Sasaguri, Fukuoka 811–2415, Japan

(Received May 30, 2006 and accepted July 24, 2006)

In order to clarify the diversity of the arbuscular mycorrhizal fungi, 9 individual plant roots and soils were randomly chosen at 27 sites in the general cultivation fields in the Chungbuk- and Chungnam-provinces, middle parts of Korea. In terms of height growth of *Sorghum bicolor*, the soil in Cheongwon site (host plant *Fagopyrum esculentum*) resulted in the best growth, and the order of growth was *Platycodon grandiflorus*, *Miscanthus sinensis*, *Sesamum indicum*, and *Capsicum annuum*. It represents that AM fungi species are having host-specific differences. Concerned to AM fungi colonization of host plant, *Sorghum bicolor*, AM fungi colonization in the roots was an increasing colonization tendency with the increase of culture periods. At the initial culture stage, colonization upto 10 days was rather slow, and afterwards the rates were increased. This colonization increase has leveled off more than 40 days culture. In addition, both morphology-based and molecular approaches of the spores from various soils of Chungbuk- and Chungnam- provinces in Korea were applied to characterize the AM fungi. By following these approaches, in Chungbuk province, total 9 species of 6 genera, such as 3 species of *Glomus*, 1 species of *Paraglomus*, 1 species of *Gigaspora*, 2 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were identified. In Chungnam province, total 6 species of 5 genera, such as 2 species of *Gigaspora*, 1 species of *Glomus*, 1 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were confirmed.

### INTRODUCTION

A mycorrhiza is a symbiotic association between plant and fungus localized in root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources from fungus to plant (Lewis, 1973). The term mycorrhiza (fungus root) was first used by Franke (1934) to describe the long-lived association between plant roots and fungal mycelium (Harley, 1969). Mycorrhiza may be one of the most important but least understood biological associations regulating community and ecosystem functioning (Harley, 1973). Most of the dominant plant species have mycorrhizae, and up to 96% of the root of the dominant species was mycorrhizal (Davidson and Christensen, 1977). These associations occur in most of the angiosperms, all gymnosperms, pteridophytes and some bryophytes (particularly liverworts). In addition, physiological interaction between plants and fungi begin very soon after contact has occurred. In some cases the changes induced in plants could be detrimental to the

general physiology of the plant or even be fatal. These mutualistic associations may result in enhanced survival, nutrient acquisition, reproduction and growth for the component organisms (Smith and Read, 1997). The fungus invades plant root tissues and in general, gains a supply of carbon from the host. Representatives from all the taxonomic groups of fungi (Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina) form mycorrhizal associations (Molina *et al.*, 1997; Lugo and Cabello, 2002). Many of the mycorrhizal fungi are poor competitors in the soil environment, and some are obligate symbionts which are not capable of independent growth either in the natural environment or in vitro cultures. In general, the fungal partner maintains contact with the soil, sometimes forming an extensive mycelial network in the immediate vicinity which may have a very important role in the nutritional interdependence between the partners.

Mycorrhizas are classified (Harley and Smith, 1983) by the structural characteristics at maturity. At present, five kinds of mycorrhiza are known: ectomycorrhiza (EM) (mainly formed in roots of woody plants), arbuscular mycorrhiza (AM), ericoid mycorrhiza, orchid mycorrhiza, and ecto-endomycorrhiza (Peterson and Farquhar, 1994). Arbuscular mycorrhizal (AM) fungi are endomycorrhiza. The name 'vesicular-arbuscular' is derived from the structures formed within the host plant tissues by the invading fungus. After spore germination, hyphae branch to form a fan near the root surface and come in contact with the epidermal cells. And then, hyphae form appressoria and the fungus penetrates between the outermost layers of cells to grow within and

<sup>1</sup> School of Forest Resources and Rural Engineering, Chungbuk National University, Cheongju 361–763, Korea

<sup>2</sup> Dept. of Biology Education, Korea National University of Education, Cheongweon, Korea

<sup>3</sup> Mycology Institute, Hankuk Wogun Co. Ltd., Suweon, Korea

<sup>4</sup> Dental Research Institute, School of Dentistry, UCLA, LA, California 90095, USA

<sup>5</sup> Department of Biochemistry, Marie Curie-Skłodowska University, Lublin 20–031, Poland

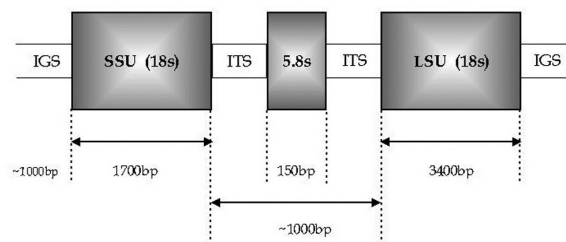
\* Corresponding author (E-mail: ohgasfor@mbox.nc.kyushu-u.ac.jp)

between the cells of the root cortex (Eom *et al.*, 1994; Koske and Gemma, 1989). In outer to middle cortical layers of root the fungus forms vesicles and within cells of the inner cortical layers, nearer to the central stele, the invading fungus forms arbuscules (Morton, 1986; Morton and Benny, 1990). These vesicular–arbuscular forms are the most common and widely occurring mycorrhizal associations. In fact, it has been suggested that up to 25,000 plant species have potential to form vesicular arbuscular mycorrhiza (Law and Lewis, 1983), with representatives largely from crop plants, herbs and tropical trees. These mycorrhizas are agriculturally important and have great economic significance.

AM species is not easily manipulated in natural or laboratory settings. This is biotrophic obligate symbionts (Koske and Polson, 1984), and most of AM fungi either grow poorly or do not grow at all in culture, and also addition of spores or mycelia inoculums rarely results in colonization under non-sterile condition (Danielson and Visser, 1989; Horton and Bruns, 2001). Vegetative structures of these fungi (*i.e.* mycorrhizae and mycelium in the soil) occur largely under ground and are difficult to be tracked and identified. Identification of biological species in the AM fungi was mostly investigated based on the morphological and developmental characteristics of fungal spores (Morton and Benny, 1990). More than 150 AMF species are described based on their spore morphology (Walker and Trappe, 1993), but spore morphotyping requires considerable experience (Clapp *et al.*, 2001; 2002) and spore counts may not reflect the in planta composition of AMF communities (Clapp *et al.*, 1995; Merryweather and Fitter, 1998; Turnau *et al.*, 2001), due to taxon-specific differences between sporulation and root colonization rates.

Therefore, more adequate methods are needed to accurately identify the mycorrhizal fungi. The methodological advance in the study of mycorrhiza identification has been the application of PCR (Mullis and Falloona, 1987; Gardes *et al.*, 1991; Henrion *et al.*, 1992; Lanfranco *et al.*, 1998). PCR-based molecular markers allowing differentiation between species or genotypes have become rapidly indispensable for ecological survey of the diversity of fungi (Lanfranco *et al.*, 1998). However, biochemical and molecular approaches are being incorporated to define and relate taxa in the order Glomales (Simon *et al.*, 1992; Graham *et al.*, 1995; Schüßler *et al.*, 2001; Madan *et al.*, 2002), and to study the diversity of their populations in different soil–plant systems (Clapp *et al.*, 1995; Helgason *et al.*, 1998; Kowalchuk *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002). Most of the recent molecular work has focused on the use of PCR techniques to analyze target sequences within the ribosomal gene cluster (Smith and Read, 1997; van Tuinen *et al.*, 1998; Redecker *et al.*, 2000; Schüßler *et al.*, 2001). Progress in analyzing AMF diversity at species level in planta has recently been made by sequence analysis of the small subunit of the nuclear ribosomal DNA (18S rDNA) (Sanders *et al.*, 1995; Helgason *et al.*, 1998, 1999, Kuhn *et al.*, 2001;

Vandenkoornhuyse *et al.*, 2002). The rDNA has considerable utility to identify distantly related species or groups of related mycorrhizal fungi, and rDNA sequence data are accumulating very fast (Boscot *et al.*, 2000). Thus, interspecies comparisons of the rDNA unit reveal a high degree of conservation in coding regions (18S, 5.8S, 25S and 5S genes) and considerable sequence differences in the spacers (ITS and intergeneric spacer (IGS))(Hijri *et al.*, 1999) (Fig. 1.). Sequence data in the rDNA applied to question in the systematics, phylogeny and ecology of fungi.



**Fig. 1.** Ribosomal DNA gene variability.

IGS; Intergenic spacer, ITS; Internal transcribed spacer, SSU; Small subunit, LSU; Large subunit.

The sequences of rDNA or its related informations made it easy to identify the AM fungal species (Egger, 1995; Bruns *et al.*, 1993; Horton and Bruns, 2001). Much information about the sequences of rDNA with the specific name of species were accumulated in a gene bank of NCBI (<http://www.ncbi.nlm.nih.gov>), and also able to confirm the biological species with the specific primers. Several techniques of polymerase chain reaction (PCR)–randomly amplified polymorphism of DNA (RAPD) and PCR–restriction fragment length polymorphism (RFLP; Gardes and Bruns 1996; Pristsch *et al.*, 1996; 1997) were developed for a last decade by using PCR with DNA polymerase<sup>TM</sup>, including the analyzing the sequences of 18S rDNA by the universal primers (White *et al.*, 1990; Gardes *et al.*, 1991; Kikuchi *et al.*, 2000).

This study was carried out to perform the identification of arbuscular mycorrhizal fungi which are contributed to the biomass production. Those approaches include thorough investigation of physiological and morphological characteristics and molecular biological techniques. Firstly various AM fungi in Chungbuk– and Chungnam– provinces are collected, and their identification was done by morphological and rDNA sequencing characteristics of spores of AM fungi by using designed the specific primers for AM fungi.

## MATERIALS AND METHODS

### Collection of plant roots and soils

Plant roots and soil samples were collected in general cultivation fields in the Chungbuk– (Table 1) and Chungnam–provinces (Table 2), middle parts of Korea. For the mycorrhizal survey 9 individual plant roots were randomly chosen at 27 sites in the two provinces. The samples were taken after removing the

**Table 1.** Collection of plants and its roots (Chungbuk Province)

Sample ID	Collection sites	Host plants
CB02009	Kyowon Univ.	<i>Fagopyrum esculentum</i>
CB02012	Sakyo village	<i>Platycodon grandiflorus</i>
CB01006	Kyowon Univ.	<i>Miscanthus sinensis</i>
CB02040	Songpyeung	<i>Sesamum indicum</i>
CB01007	Kyowon Univ.	<i>Capsicum annuum</i>
CB02020	Boeun-Geosan	<i>Zea mays</i>
CB02015	Daesa-Mungyeong	<i>Capsicum annuum</i>
CB03005	Kyowon Univ.	<i>Glycine max</i>
CB02001	Yongdong	<i>Capsicum annuum</i>
CB02036	Danyang	<i>Glycine max</i>
CB02027	Chungju	<i>Fagopyrum esculentum</i>
CB02055	Mt. Wolak	<i>Allium fistulosum</i>
CB02043	Suanbo	<i>Glycine max</i>
CB02056	Mt. Wolak	<i>Glycine max</i>
CB02057	Mt. Wolak	<i>Capsicum annuum</i>

**Table 2.** Collection of plants and its roots (Chungnam Province)

Sample ID	Collection sites	Host plants
CN02057	Taeon, CN	<i>Capsicum annuum</i>
CN02028	Taeon, CN	<i>Capsicum annuum</i>
CN02032	March hill, CN	<i>Fagopyrum esculentum</i>
CN03025	Buyeo, CN	<i>Glycine max</i>
CN02048	Mt. Chilgap, CN	<i>Glycine max</i>
CN03001	March hill, CN	<i>Zea mays</i>
CN03029	Gongju, CN	<i>Artemisia princeps v. orientalis</i>
CN03022	Seochun, CN	<i>Ipomoea batatas</i>
CN03005	Daechun, CN	<i>Zea mays</i>
CN03002	Boryung, CN	<i>Artemisia princeps v. orientalis</i>
JB02020	Jangsu, JB	<i>Glycine max</i>
JN02018	Mt. Jiri, JN	<i>Sorghum bicolor</i>

top litter layer (5–10 cm) and digging out an appropriate amount of soil close to the roots of the host plant from a depth of 10–15 cm. The samples were collected in triplicate. Soil particles attached to fine feeder roots were removed by generous shaking. The soil samples brought to the laboratory were stored at 4–8°C to sustain the viability of arbuscular mycorrhizal spores.

### Isolations of spores from soils

The AM fungal spores were extracted from the rhizosphere soils by wet sieving and decanting followed by sucrose gradient centrifugation (Sieverding, 1991). After centrifugation, the supernatant was poured through a 50 µm mesh and quickly rinsed with tap water. Spores were grouped, under a dissecting microscope, according to their morphological characteristics and used to identify AM fungi and to initiate cultures of the different native AM fungal isolates.

### Morphological characterization of spores by microscopical observation

In order to assess the colonization, fine roots of host plant were stained with acid fuchsin (Kormaik and McGraw, 1982). Colonization was determined by a grid intersect method (Giovannetti and Mosse, 1980). Colonized areas were classified by 5 different colonization rates, 0–20% = 1, 20–40% = 2, 40–60% = 3, 60–80% = 4 and 80–100% = 5 based on microscopical observa-

tion. After confirming under the light microscope the apparent purity of the cultures of the different AM morphotypes, these were identified to genus and, when possible, to species level. Criteria for morphological spore characterization were mainly based on spore size and colour, wall structure and hyphal attachment (Walker, 1983; Morton and Benny, 1990; Schenk and Perez, 1990; Dodd and Rosendahl, 1996; INVAM, 1997).

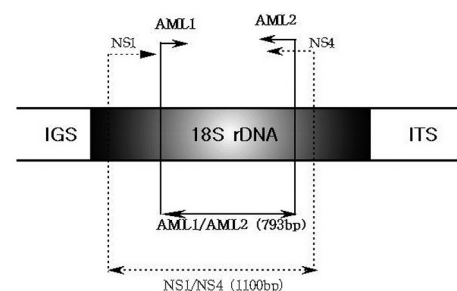
### Molecular characterization (DNA sequencing)

#### Isolation of DNA

Total genomic DNA of the different AM fungal morphotypes was isolated from approximately 50 to 60 spores, which were placed in microcentrifuge tubes containing 40 µl milli Q-water and crushed with a miniature pestle. DNA extraction was done by DNeasy Plant Mini Kit (QIAGEN Science, USA) according to Technical Manual form QIAGEN (Gosselin *et al.*, 1995; Wyss and Bonfante, 1993). The genomic DNA was repeatedly purified with the CTAB extracting buffer until a single band was obtained on gel. The total genomic DNA extracted above was amplified using the primers and conditions listed by Lee and Yoo (2000). The supernatant was frozen at –20°C and used as template for PCR.

#### Polymerase Chain Reaction

SSU rRNA gene sequences were PCR amplified from the purified total DNA by using the universal primer NS1–NS4 and the AM specific primer AML1–AML2 (Auh, 2004) (van Tuinen *et al.*, 1998, Fig. 2). The latter primers were designed based on the small subunit of the nuclear ribosomal DNA (18S rDNA). The 1<sup>st</sup> PCR mixture consisted of 1 µl of universal primer NS1/NS4, 1 µl of the DNA extract, 7 µl of nuclease free water, 10 µl of the PCR master mix (500 unit/ml taq DNA polymerase, 250 µmM dNTP, 1.5 mM MgCl<sub>2</sub>, pH 8.5; Promega Co., USA) in a 20 µl reaction volume. PCR was performed in an automated thermal cycler (PTC–200 Peltier Thermal Cycler, MJ Research, INC. USA) and used to 2<sup>nd</sup> PCR–DNA sample. The 2<sup>nd</sup> PCR was done by using AM fungi specific primer AML1/AML2 (Lee, 2003). The PCR products were separated by electrophoresis in 1.0% agarose gels, stained with ethidium bromide, and visualized by UV trans-illumination. Table 3 represents characteristics of used primers and PCR condition.



**Fig. 2.** 18S ribosomal DNA gene with annealing sites of primer pairs, NS1/NS4 and AML1/AML2 and their approximate DNA lengths (Lee, 2003).

**Table 3.** Characteristics of primers used in this study and conditions of PCR (Lee, 2003)

Primer			PCR conditions
Name	Nucleotide sequences	Tm <sup>a)</sup>	
NS1/NS4 (30 cycles)			
NS1	5'-GTAGTCATATGCTTGTCTC-3'	37.7 °C	95°C (3 min), 40°C (1 min), 72°C (1 min) –1 cycle; 95°C (30 sec), 40°C (1 min), 72°C (1 min, 30 sec), 40°C –28 cycles;
NS4	5'-TTCCGTCAATTCTTTAAG-3'	48.3 °C	95°C (30 sec), 40°C (1 min), 72°C (10 min) –1 cycle
AML1/AML2 (30 cycles)			
AML1	5'-AACTTTTCGATGGTAGGATAGA-3'	47.2°C	95°C (3 min), 47°C (1 min), 72°C (1 min) –1 cycle; 95°C (30 sec), 47°C (1 min), 72°C (1 min) –28 cycles; 95°C (30 sec),
AML2	5'-CCAAACACTTTGGTTTCC-3'	47.1°C	95°C (30 sec), 47°C (1 min), 72°C (10 min) –1 cycle

<sup>a)</sup> Melting temperature was determined using 50 mM salts concentration

### Sequence analysis

The amplification of insert DNA in plasmid was done by automatic sequencer ABIPRISM™ (Perkin-Elmer, USA at Eugenetech Co., Korea). DNA sequences were analyzed by both ways of reverse and forward sequences, and then re-calculated with matching procedures of forward and reverse readings. Reverse sequences were reverse complemented and aligned with forward sequences by GENDOC program. All sequences were submitted to a BLAST search using the GenBank database (<http://www.ncbi.nlm.nih.gov>, Gehrig *et al.*, 1996; Tae, 2000; Tae *et al.*, 2002; Thompson *et al.*, 1994; Saitou and Nei, 1987; Altschul *et al.*, 1990).

## RESULTS AND DISCUSSION

### Growth of *Sorghum bicolor* on various Chungbuk- and Chungnam- provinces soils

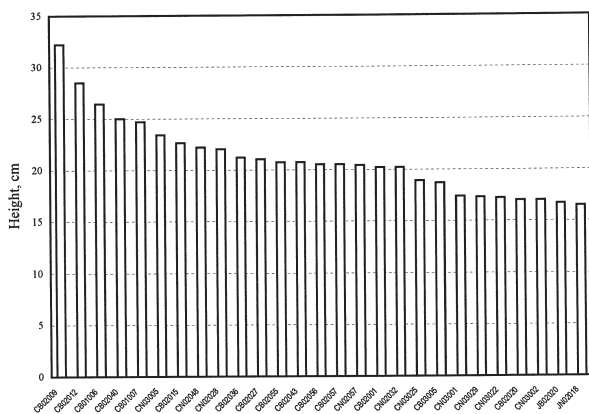
The Growth of *Sorghum bicolor* on various soils collected from Chungbuk- and Chungnam- provinces, weights of seedling, total weights, numbers of seedlings, and their average heights were measured as shown in Table 4. The high growth variability among sampling sites were shown from the data among host plants and sampling sites. Total weights were the highest from the soil growing *Glycine max* in Cheongwon site, and the next soil site growing pepper. In terms of weight growth

**Table 4.** Growth of *Sorghum bicolor* on various collected soils

Sites	Weight/1 seedling g	Total weight G	# of seedlings	Host plant	Height cm
CB02009	0.167	3.33	20	<i>Fagopyrum esculentum</i>	32.2
CB02012	0.210	1.89	9	<i>Platycodon grandiflorus</i>	28.5
CB01006	0.315	3.468	11	<i>Miscanthus sinensis</i>	26.4
CB02040	0.323	1.291	4	<i>Sesamum indicum</i>	25
CB01007	0.501	3.504	7	<i>Capsicum annuum</i>	24.7
CN03005	0.163	2.122	13	<i>Zea mays</i>	23.4
CB02015	0.114	1.367	12	<i>Capsicum annuum</i>	22.6
CN02048	0.366	2.193	6	<i>Glycine max</i>	22.2
CN02028	0.363	2.54	7	<i>Capsicum annuum</i>	22
CB02036	0.142	2.137	15	<i>Glycine max</i>	21.2
CB02027	0.106	1.061	10	<i>Fagopyrum esculentum</i>	21
CB02055	0.165	1.82	11	<i>Allium fistulosum</i>	20.7
CB02043	0.102	1.93	19	<i>Glycine max</i>	20.7
CB02056	0.182	2.372	13	<i>Glycine max</i>	20.5
CB02057	0.152	1.819	12	<i>Capsicum annuum</i>	20.5
CN02057	0.345	2.414	7	<i>Capsicum annuum</i>	20.4
CB02001	0.284	1.418	5	<i>Capsicum annuum</i>	20.2
CN02032	0.178	1.954	11	<i>Fagopyrum esculentum</i>	20.2
CN03025	0.257	2.314	9	<i>Glycine max</i>	18.9
CB03005	1.532	1.532	1	<i>Glycine max</i>	18.7
CN03001	0.161	1.611	10	<i>Zea mays</i>	17.4
CN03029	0.221	1.767	8	<i>Artemisia princeps</i> <i>v. orientalis</i>	17.3
CN03022	0.167	1.002	6	<i>Ipomoea batatas</i>	17.2
CB02020	0.259	2.072	8	<i>Zea mays</i>	17
		1.221	16	<i>Artemisia princeps</i> <i>v. orientalis</i>	17
CN03002	0.076				
JB02020	0.226	2.484	11	<i>Glycine max</i>	16.7
JN02018	0.346	1.039	3	<i>Sorghum bicolor</i>	16.5



of *Sorghum bicolor*, host plants were *Glycine max*, *Capsicum annuum*, *Sorghum bicolor*, *Sesamum indicum*, *Miscanthus sinensis*, and *Fagopyrum esculentum*. On the other hand, in terms of height growth of *Sorghum bicolor*, the soil in Cheongwon site (host plant *Fagopyrum esculentum*) was the best, and the order of height growth *Platycodon grandiflorus*, *Miscanthus sinensis*, *Sesamum indicum*, and *Capsicum annuum*. It refers that AM fungi species are having host-specific differences. Several authors (Lopez-Sanchez and Honrubia, 1992; Michel-Rosales and Valdes, 1996; Helgason *et al.*, 1999; 2002) reported that there was no correlation between the colonization of adjacent plants. Together with the fact that some AM fungi species show host-specific differences (Bever, 2002), the differences in pH are also known to have an impact on spore communities (Porter *et al.*, 1987; Bever, 2002; Michel-Rosales and Valdes, 1996) but not on colonization. This result supports the potentiality of AM inoculation for many crops in Korea of current agronomic interest.



major suborder lacks the ability to form vesicles in roots, AM is now the preferred acronym. The order Glomales is further divided into families and genera according to the method of spore formation. The spores of AM fungi are very distinctive. They range in diameter from 10  $\mu\text{m}$  for *Glomus tenue* to more than 1,000  $\mu\text{m}$  for some *Scutellospora* spp. The spores can vary in color from hyaline (clear) to black and in surface texture from smooth to highly ornamented. *Glomus* forms spores on the ends of hyphae, *Acaulospora* forms spores laterally from the neck of a swollen hyphal terminus, and *Entrophospora* forms spores within the neck of the hyphal terminus. The Gigasporineae are divided into two genera based upon the presence of inner membranous walls and a germination shield (wall structure from which the germ tube can arise) for *Scutellospora* or the absence of these structures for *Gigaspora*.

The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is endomycorrhiza. In this association neither the fungal cell wall nor the host cell membrane are breached. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. The arbuscules are relatively short lived, less than 15 days, and are often difficult to see in field-collected samples.

Other structures produced by some AM fungi include vesicles, auxiliary cells, and asexual spores. Vesicles are thin-walled, lipid-filled structures that usually form in intercellular spaces. Reproductive spores

can be formed either in the root or more commonly in the soil. Spores produced by fungi forming AM associations are asexual, forming by the differentiation of vegetative hyphae. For some fungi (*e.g.*, *Glomus intraradices*), vesicles in the root undergo secondary thickening, and a septum (cross wall) is laid down across the hyphal attachment leading to spore formation, but more often spores develop in the soil from hyphal swellings.

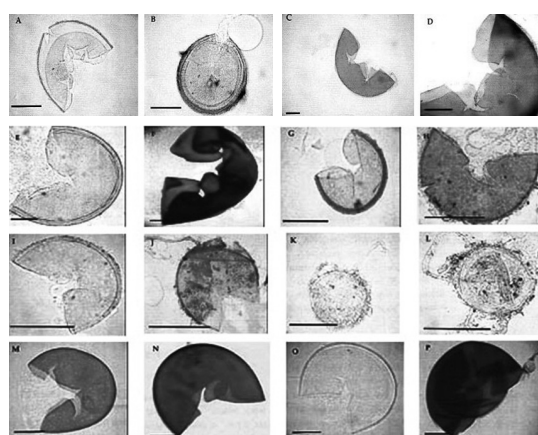
AM fungi spores were obtained from soil samples. As shown in Fig. 6, twelve morphological species were recorded as spores in the soil samples. Within the AM fungi spores, about 18% were with diameter less than 80–100  $\mu\text{m}$  diameter range, 18% in the 100–150  $\mu\text{m}$ , 19% in the 150–200  $\mu\text{m}$  and 45% larger than 200  $\mu\text{m}$ . Some spores were identifiable to species level through their morphological characters (Fig. 6). Among these, *Acaulospora longula*, *Acaulospora leptoticha*, *Glomus etunicatum* and *Gigaspora margarita* were the most commonly encountered species. *Acaulospora longula* was the species most frequently found in the soil samples. Six genera of AM fungi were identified, among which *Acaulospora*, *Gigaspora* and *Glomus* were dominant with frequencies of 57%, 20 and 16%, respectively. The frequencies of AMF from the other three genera were low.

### Molecular characterization (DNA sequencing)

The molecular characterization of AM fungi has been a complicated task in the past because of the considerable degree of variation detected among the copies of ribosomal DNA within single spores, which brought into question the validity of these methods (Calvente *et al.*, 2004). However, recent reports indicated that SSU rRNA sequence analysis is a suitable tool to infer phylogenetic relationships among AM fungi since the expected sequence difference could be more easily linked to a taxonomic entity than the possibly multi-allelic ITS sequences (Schwarzott and Schüßler, 2001).

Both morphology-based and novel molecular approaches of the spores from host plant root-associated soils were applied to characterize the AM fungi spores isolated from root-associated soil. This is the first step to analyse the diversity of AM fungi in the target fields of Chungbuk- and Chungnam- provinces, Korea. By following these approaches, only four distinguishable spore morpho-types, six genera of AM fungi, were found. In Chungbuk province, total 9 species of 6 genera, such as 3 species of *Glomus*, 1 species of *Paraglomus*, 1 species of *Gigaspora*, 2 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were identified. Based on further corroborated by molecular techniques, the six AM fungi morphotypes were genetically characterised by PCR amplification and sequence analysis of a portion of their SSU rRNA to verify their identification.

As shown in Table 5, the accession number for the new sequences, as deposited in the EMBL database are as follows: *Glomus mosseae* (AJ306438), *Paraglomus*



**Fig. 6.** Identified AMF spore based on morphological features (Scale bar 50  $\mu\text{m}$ )

A: *Acaulospora longula*; B: *Achaespora leptoticha*; C: *Gigaspora gigantea*; D: *Gigaspora gigantea*; E: *Gigaspora margarita*; F: *Gigaspora margarita*; G: *Glomus etunicatum*; H: *Glomus etunicatum*; I: *Glomus lamellosum*; J: *Glomus mosseae*; K: *Paraglomus occultum*; L: *Paraglomus* sp.; M: *Scutellospora castanea*; N: *Scutellospora castanea*; O: *Scutellospora weresubiae*; P: *Scutellospora weresubiae*.

**Table 5.** BLAST search on NCBI with analyzed sequences for Identification of AMF (Chungbuk–province)

Isolate	Morphological Identification	Results of BLAST search on NCBI		
		Species	Accession number	Sequence similarity (%)
CB01005–1	<i>Glomus mosseae</i>	<i>Glomus mosseae</i>	AJ306438	713/717 (99%)
CB01005–2	<i>Glomus mosseae</i>	<i>Glomus mosseae</i>	AJ306438	748/755 (99%)
CB01005–4	<i>Glomus mosseae</i>	<i>Glomus. mosseae</i>	AJ306438	635/650 (97%)
CB01006–5	<i>Paraglomus occultum</i>	<i>Paraglomus occultum</i>	AJ276081	743/751 (98%)
CB02009–4	<i>Paraglomus occultum</i>	<i>Paraglomus occultum</i>	AJ276081	693/711 (97%)
CB01006–7	<i>Glomus etunicatum</i>	<i>Glomus etunicatum</i>	Y17644	759/763 (99%)
CB02015–2	<i>Glomus etunicatum</i>	<i>Glomus etunicatum</i>	Y17639	681/686 (99%)
CB02036–3	<i>Glomus etunicatum</i>	<i>Glomus etunicatum</i>	Y17644	724/725 (99%)
CB01006–9	<i>Gigaspora margarita</i>	<i>Gigaspora margarita</i>	AJ567844	332/356 (93%)
CB02020–4	<i>Gigaspora margarita</i>	<i>Gigaspora margarita</i>	AJ567844	450/471 (95%)
CB01007–2	<i>Scutellospora castanea</i>	<i>Scutellospora castanea</i>	AJ418851	220/230 (95%)
CB01007–3	<i>Archaeospora leptoticha</i>	<i>Archaeospora leptoticha</i>	AB047306	708/720 (98%)
CB02012–1	<i>Scutellospora calospora</i>	<i>Acaulospora longula</i>	AJ306439	731/736 (99%)
CB02020–5	<i>Scutellospora weresubiae</i>	<i>Scutellospora weresubiae</i>	AJ306444	701/724 (96%)
CB02040–2	<i>Glomus lamellosum</i>	<i>Glomus etunicatum</i>	Y17639	743/747 (99%)
CB02040–3	<i>Scutellospora heterogama</i>	<i>Glomus lamellosum</i>	AJ276087	744/749 (99%)

**Table 6.** BLAST search on NCBI with analyzed sequences for Identification of AMF (Chungnam–province)

Isolate	Morphological Identification	Results of BLAST search on NCBI		
		Species	Accession number	Sequence similarity (%)
CN–02–032–3	<i>Gigaspora margarita</i>	<i>Gigaspora margarita</i>	AJ567844	450/471 (95%)
CN–02–048–1	<i>Gigaspora gigantea</i>	<i>Gigaspora gigantea</i>	Z14010	392/399 (98%)
CN–03–025–1	<i>Glomus etunicatum</i>	<i>Glomus etunicatum</i>	Y17639	211/213 (99%)
CN–02–028–1	<i>Acaulospora longula</i>	<i>Acaulospora longula</i>	AJ306439	321/332 (96%)
CN–02–028–2	<i>Glomus etunicatum</i>	<i>Glomus etunicatum</i>	Y17644	276/286 (96%)
CN–02–057–1	<i>Acaulospora longula</i>	<i>Acaulospora longula</i>	AJ306439	514/528 (97%)
CN–03–005–1	<i>Gigaspora gigantea</i>	<i>Gigaspora gigantea</i>	Z14010	245/252 (97%)
CN–03–002–1	<i>Gigaspora gigantea</i>	<i>Gigaspora gigantea</i>	Z4010	571/576 (99%)
CN–03–002–5	<i>Archaeospora leptoticha</i>	<i>Archaeospora leptoticha</i>	AB047308	697/712 (97%)
CN–03–005–3	<i>Archaeospora leptoticha</i>	<i>Archaeospora leptoticha</i>	AB047306	708/720 (98%)
CN–02–057–3	<i>Scutellospora weresubia</i>	<i>Scutellospora weresubia</i>	AJ306444	679/684 (99%)

*occultum* (AJ276081), *Glomus etunicatum* (Y17644), *Glomus etunicatum* (Y17639), *Gigaspora Margarita* (AJ567844), *Scutellospora castanea* (AJ418851), *Acaulospora leptoticha* (AB047306), *Acaulospora longula* (AJ306439), *Scutellospora weresubiae* (AJ306444) and *Glomus lamellosum* (AJ276087). Comparisons of the obtained sequences with those present in the database revealed that they show more than 97–99% identity to the SSU rRNA gene of different AM fungal isolates. Only three *Scutellospora calospora*, *Glomus lamellosum*, and *Scutellospora heterogama* did not matched with BLAST search results.

In Chungnam province, total 6 species of 5 genera, such as 2 species of *Gigaspora*, 1 species of *Glomus*, 1 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were identified. As shown in Table 6, the accession number for the new sequences, as deposited in the EMBL database are as follows: *Glomus mosseae* (AJ306438), *Paraglomus occultum* (AJ276081), *Glomus etunicatum* (Y17644), *Glomus etunicatum* (Y17639), *Gigaspora margarita* (AJ567844), *Scutellospora castanea* (AJ418851),

*Archaeospora leptoticha* (AB047306), *Acaulospora longula* (AJ306439), *Scutellospora weresubiae* (AJ306444) and *Glomus lamellosum* (AJ276087). Comparisons of the obtained sequences with those present in the database revealed that they show more than 97–99% identity to the SSU rRNA gene of different AM fungal isolates. Only three *Scutellospora calospora*, *Glomus lamellosum*, and *Scutellospora heterogama* did not matched with BLAST search results.

## CONCLUSIONS

This study was carried out to perform the collection of AM fungi from various sites of Chungbuk– and Chungnam– provinces in Korea, and identification of arbuscular mycorrhizal fungi which are associated to symbiotic relation with plants including crops. Those approaches include thorough investigation of physiological and morphological characteristics and molecular biological techniques.

In terms of height growth of *Sorghum bicolor*, the soil in Cheongwon site (host plant *Fagopyrum esculen-*



*tum*) resulted in the best growth, and the order of height growth was *Platycodon grandiflorus*, *Miscanthus sinensis*, *Sesamum indicum*, and *Capsicum annuum*. It refers that AM fungi species are having host-specific differences. Concerned to AM fungi colonization of host plant, *Sorghum bicolor*, AM fungi colonization in the roots were observed by Microscope, and there was some increasing colonization tendency with the increase of culture periods. At the initial culture stage, colonization upto 10 days was rather slow, and afterwards the rates were increased. This colonization increase has leveled off more than 40 days culture.

Both morphology-based and molecular approaches of the spores from various soils of Chungbuk- and Chungnam- provinces in Korea were applied to characterize the AM fungi. By following these approaches, in Chungbuk province, total 9 species of 6 genera, such as 3 species of *Glomus*, 1 species of *Paraglomus*, 1 species of *Gigaspora*, 2 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were identified. In Chungnam province, total 6 species of 5 genera, such as 2 species of *Gigaspora*, 1 species of *Glomus*, 1 species of *Scutellospora*, 1 species of *Acaulospora*, and 1 species of *Archaeospora*, were confirmed.

In conclusion, this study emphasizes the importance of exploring and exploiting the natural diversity of AM fungi, particularly in Chungbuk- and Chungnam- provinces, Korea, as a starting point to formulate inoculants to be applied for the production of appropriate plant varieties with optimized quality.

## ACKNOWLEDGMENTS

This study was carried out mainly with the Grants of the Bioindustry Research Projects, Chungbuk Province (2004–2007), and partly supports by Heuk-Salim Institute, the Brain Pool Scientist Invitation Program of KOFST-KOSEF(2004) and the Visit Research Program of KOSEF-JSPS (2005).

## REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman 1997 Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, **25**: 3389–3402
- Auh J. K. 2004 Identification of arbuscular mycorrhizal fungi in Korean ginseng (*Panax ginseng*) by 18S rDNA sequencing. MS Dissertation, Korea National University of Education, Cheongwon, Korea
- Bever, J. D. 2002 Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil*, **244**: 281–290
- Bruns, T. D. and M. Gardes 1993 Molecular tools for the identification of ectomycorrhizal fungi–taxon specific oligonucleotide probes for suilloid fungi. *Molecular Ecology*, **2**: 233–242
- Boscot, F., J. C. Munch, J. Y. Charosset and M. Gardes 2000 Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. *Microbiology Reviews*, **24**: 606–607
- Calvente, R. *et al.* 2004 Detection of AM fungi by ribosomal DNA of single spores sequencing. *Applied Soil Ecology*, **26**: 11–19
- Clapp, J. P., A. Rodriguez and J. C. Dodd 2001 Inter- and intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. *New Phytol.*, **149**: 539–554
- Clapp, J. P., A. Rodriguez and J. C. Dodd 2002 Glomales rRNA diversity—all that glistens is not necessarily glomalean? *Mycorrhiza*, **12**: 269–270
- Clapp, J. P., J. P. W. Young, J. W. Merryweather and A. H. Fitter 1995 Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytol.*, **130**: 259–265
- Danielson, R. M. and S. Visser 1989 Host response to inoculation and behavior of introduced and indigenous ectomycorrhizal fungi of jack pine grown on oil-sands tailing. *Can. J. Forest Res.*, **19**: 1412–1421
- Davidson, D. E. and M. Christensen 1977 Root-microfungal and mycorrhizal associations in a shortgrass prairie. In: *The Belowground Ecosystem: A Synthesis of Plant-associated Processes*, ed. J. K. Marshall, pp. 279–287. Colorado State University Press, Collins, CO
- Dodd, J. C. and S. Rosendahl 1996 The BEG Expert System—a multimedia identification system for arbuscular mycorrhizal fungi. *Mycorrhiza*, **6**: 275–278
- Egger, K. N. 1995 Molecular analysis of ectomycorrhizal fungal communities. *Can. J. Bot.*, **77**: 11–21
- Eom, A. H., S. S. Lee, T. K. Ahn and M. W. Lee 1994 Ecological Roles of Arbuscular Mycorrhizal Fungi in Two Wild Legume Plants. *Mycoscience*, **35**: 69–78
- Francke, H. L. 1934 Beiträge aus Kenntnis der Mykorrhiza von *Monotropa hypopithys* L. Analyse und Synthese der Symbiose. *Flora* 129, pp. 1–59
- Gardes, M. and T. D. Bruns 1996 ITS-RFLP matching for identification of fungi. In: *Methods in Molecular Biology: Species Diagnostics Protocols*, Vol. 50 (Clapp, J., ed.). pp. 177–186. Umana Press, Totowa, NJ
- Gardes, M., T. J. White, J. A. Fortin, T. D. Bruns and J. W. Taylor 1991 Identification of indigenous and introduced symbiotic in ectomycorrhizae by amplification of the nuclear and mitochondrial ribosomal DNA. *Can. J. Bot.*, **69**: 180–190
- Gehrig, H., A. Schussler and M. Kluge 1996 *Geosiphon pyriforme*, a fungus forming endocytobiosis with Nostoc (Cyanobacteria), is an ancestral member of the Glomales: Evidence by SSU rRNA analysis. *J. Mol. Evol.*, **43**: 71–81
- Giovannetti, M. and B. Mosse 1980 An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *The New Phytologist*, **84**: 489–500
- Gosselin, L., R. Jobidon and L. Bernier 1995 Assessment of genetic variation within *Chondrostereum purpureum* from Quebec by random amplified polymorphic DNA analysis. *Mycol. Res.* **99**: 151–158
- Graham, J. H., N. C. Hodge and J. B. Morton 1995 Fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. *Appl. Environ. Microbiol.*, **61**: 58–64
- Harley, J. L. 1969 *The biology of mycorrhiza*. Leonard Hill, London
- Harley, J. L. 1973 Symbiosis in ecosystems. *J. Nat. Sci. Council. Sri Lanka* **1**: 31–48
- Harley, J. L. and S. E. Smith 1983 Mycorrhizal symbiosis. Academic Press, pp. 267–295. London, UK
- Helgason, T., T. J. Daniell, R. Husband, A. H. Fitter and J. P. W. Young 1998 Ploughing up the wood-wide web? *Nature*, **394**: 431
- Helgason, T., A. H. Fitter and J. P. W. Young 1999 Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. *Mol. Ecol.*, **8**: 659–666
- Helgason, T., J. W. Merryweather, J. Denison, P. Wilson, J. P. W. Young and A. H. Fitter 2002 Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *J. Ecol.*, **90**: 371–384

- Henrion, B., F. Le Tacon and F. Martin 1992 Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RNA genes. *New Phytol.*, **122**: 289–298
- Hijri, M., M. Hosny, D. van Tuinen and H. Dulieu 1999 Intraspecific ITS polymorphism in *Scutellospora castanea* (Glomales, Zygomycota) is structured within multinucleate spores. *Fungal Genet. Biol.*, **26**: 141–151
- Horton, T. R. and T. D. Bruns 2001 The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Mol. Ecol.*, **10**: 1855–1871
- INVAM 1997 <http://www.invam.caf.wvu.edu/>
- Kikuchi, K., N. Matsushita, A. Guerin-Laguette, A. Ohta and K. Suzuki 2000 Detection of *Tricholoma matsutake* by specific ITS primers. *Mycol. Res.*, **104**: 1427–1430
- Kormaik, P. P. and A. C. McGraw 1982 Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck, N. C. (Ed.), "Methods and Principles of Mycorrhizal Research". The American Phytopathological Society, St. Paul, USA, pp. 37–45
- Koske, R. E. and J. N. Gemma 1989 A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research*, **92**: 486–505
- Koske, R. E. and W. R. Polson 1984 Are VA mycorrhizae required for sand dune stabilization. *Bio. Science*, **34**: 420–424
- Kowalchuk, G. A., F. A. De Souza and J. A. V. Veen 2002 Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch coastal sand dunes. *Mol. Ecol.*, **11**: 571–581
- Kuhn G., M. Hijiri and I. R. Sanders 2001 Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature*, **414**: 745–748
- Lanfranco, L., Perotto, S., and Bonfante, P. 1998. Applications of PCR for studying the biodiversity of mycorrhizal fungi. In: "Applications of PCR in Mycology" (eds Bridge, P. D., Arora, D. K., Reddy, C. A., and Elander, R. P.), pp. 107–124. CAB International, UK
- Law, R. and D. H. Lewis 1983 Biotic environments and the maintenance of sexsome evidence from mutualistic symbioses. *Biological J. of the Linnaean Society*, **20**: 249–276
- Lee J. K. 2003 Molecular biological identification of arbuscular mycorrhizal fungi collected from plant roots. MS Dissertation, Korea National University of Education, Cheongwon, Korea
- Lee, S. S. and J. Y. Yoo 2000 Identification of the orchid Mycorrhizal Fungi isolated from the roots of Korean Native Orchid. *Mycobiol.*, **25**: 17–26
- Lewis, D. H. 1973 Concepts in fungal nutrition and the origin of biotrophy. *Biological Reviews* **48**: 261–278
- Lopez-Sanchez, M. E. and M. Honrubia 1992 Seasonal variation of vesicular-arbuscular mycorrhizae in eroded soils from southern Spain. *Mycorrhiza*, **2**: 33–39
- Lugo, M. A. and M. N. Cabello 2002 Native arbuscular mycorrhizal fungi (AMF) from mountain grassland (Cordoba, Argentina) I. Seasonal variation of fungal spore density. *Mycologia*, **94**: 579–586
- Madan, R., C. Pankhurst, B. Hawke and S. Smith 2002 Use of fatty acids for identification of AM fungi and estimation of the biomass of AM spores in soil. *Soil Biol. Biochem.*, **34**: 125–128
- Merryweather, J. and A. Fitter 1998 The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. *New Phytologist*, **138**: 117–129
- Michel-Rosales, A. and M. Valdés 1996 Arbuscular mycorrhizal colonization of lime in different agroecosystems of the dry tropics. *Mycorrhiza*, **6**: 105–109
- Molina, R. 1997 Special Forest Products: Integrating Social Economic, and Bioogical Considerations into Ecosystem Management. In Creating A Forestry for The 21st Century. The Science of Ecosystem Management. Edited by K. A. Kohm and J. F. Franklin. Island Press. Washington, D. C. 315–336
- Morton, J. B. 1986 Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. *Mycologia*, **82**: 92–207
- Morton, J. B. 1988 Taxonomy of VA mycorrhizal fungi: classification, nomenclature and identification. *Mycotaxon*, **32**: 267–324
- Morton, J. B. and G. L. Benny 1990 Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon*, **37**: 471–492
- Mullis, K. B. and F. A. Faloona 1987 Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, **155**: 335–350
- Peterson, R. L. and M. L. Farquhar 1994 Mycorrhizas—Integrated development between roots and fungi. *Mycologia*, **86**: 311–326
- Porter, W. M., A. D. Robson and L. K. Abbott 1987 Field survey of the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *J. Applied Ecology*, **24**: 659–662
- Pritsch, K. and F. Buscot 1996 Biodiversity of ectomycorrhizas from morphotypes to species. Proceedings of the 4th European Symposium on Mycorrhizae. In: "Mycorrhizas in Integrated Systems from Genes to Plant Development" (Azcon-Aguilar, C. and J. M. Barea Eds.), pp. 9–14. Office for Official Publications of the European Communities, Luxembourg
- Pritsch, K., H. Boyle, J. C. Munch and F. Buscot 1997 Characterization and identification of black alder mycorrhizas by PCR/RFLP analysis of the rDNA internal transcribed spacer (ITS). *New Phytol.*, **137**: 357–369
- Redecker, D., J. B. Morton and T. D. Bruns 2000 Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. *Mycologia*, **92**: 282–285
- Saitou, N. and M. Nei 1987. The Neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol. Biol. Evol.*, **4**: 406–425
- Sander, I. R., M. Alt, K. Groppe, T. Boller and A. Wiemken 1995 Identification of ribosomal DNA polymorphisms among and within spores of the Glomales—application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist*, **130**: 419–427
- Schenk, N. C. and Y. Perez 1990 Manual for Identification of VA Mycorrhizal Fungi. Synergistic Publications, Gainesville, Florida, pp. 250.
- Schwarzott, D. and A. Schüßler 2001 A simple and reliable method for SSU rRNA gene DNA extraction, amplification and cloning from single AM fungal spores. *Mycorrhiza*, **10**: 203–207
- Schüßler, A., D. Schwarzott and C. Walker 2001 A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycol. Res.*, **105**: 1413–1421
- Sieverding, E. 1991 Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche GTZ. GmbH Eschborn, pp. 371.
- Simon, L., M. Lalonde and T. D. Bruns 1992 Specific amplification of 18S fungal ribosomal genes from VA endomycorrhizal fungi colonising roots. *Appl. Environ. Microbiol.*, **58**: 291–295
- Smith, S. E. and D. J. Read 1997 Mycorrhizal Symbiosis. Academic Press, London, 605 pp.
- Stutz, J. C. and J. B. Morton 1996 Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Can. J. Bot.*, **74**: 1883–1889
- Tae M. S. 2000 Molecular and morphological diversity of arbuscular mycorrhizal fungi collected at Mt. Wolak. MS Dissertation, Korea National University of Education, Cheongwon, Korea
- Tae, M. S., A. H. Eom and S. S. Lee 2002 Sequence analyses of PCR amplified partial SSU of Ribosomal DNA for Identifying Arbuscular Mycorrhizal Fungi in Plant roots. *Mycobiol.*, **30**: 13–17
- Thompson, J. D., D. G. Higgins and T. J. Gibson 1994 CLUSTAL

- W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.*, **22**: 4673–4680
- Turnau, K., P. Ryszka, V. Gianinazzi-Pearson and D. van Tuinen 2001 Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza*, **10**: 169–174
- van Tuinen, D., E. Jacquot, B. Zhao, A. Gollotte and P. V. Gianinazzi 1998 Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology*, **7**: 879–887
- Vandenkoornhuyse, P., R. Husband, T. J. Daniell, I. J. Watson, J. M. Duck, A. H. Fitter and J. P. W. Young 2002 Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Mol. Ecol.*, **11**: 1555–1564
- Walker, C. 1983 Taxonomic concepts in the Endogonaceae: spore wall characteristics in species descriptions. *Mycotaxon*, **18**: 443–455
- Walker, C. and J. M. Trappe 1993 Names and epithets in the Glomales and Endogonales. *Mycol. Res.*, **97**: 339–344
- White, T. J., T.D. Bruns, S. Lee and J. Taylor 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., D. H. Gelfand, J. J. Sninsky and T. J. White (eds.) *PCR Protocols: a Guide to Methods and Applications*. pp. 315–322. Academic Press, New York.
- Wyss, S. and P. Bonfante 1993 Amplification of genomic DNA of arbuscular mycorrhizal (AM) fungi by PCR using short arbitrary primers. *Mycol. Res.*, **97**: 1351–1357