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FULL PAPER

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Community analysis of arbuscular mycorrhizal fungi in a warm-temperate deciduous broad-leaved forest and introduction of the fungal community into the seedlings of indigenous woody plants

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Abstract A community of arbuscular mycorrhizal (AM) fungi was investigated in a warm-temperate deciduous broad-leaved forest using a molecular analysis method. Root samples were obtained from the forest, and DNA was extracted from the samples. Partial 18S rDNA of AM fungi were amplified from the extracted DNA by polymerase chain reaction using a universal eukaryotic primer NS31 and an AM fungal-specific primer AM1. After cloning the PCR products, 394 clones were obtained in total, which were divided into five types by restriction fragment length polymorphism (RFLP) with *Hin*fI, *Rsa*I, and *Hsp*92II. More than 20% of the clones were randomly selected from each RFLP type and sequenced. Phylogenetic analysis showed that all the obtained clones belonged to *Glomus* but could not be identified at species level. Topsoil of the forest containing plant roots was inoculated to nonmycorrhizal seedlings of indigenous woody plants, *Rhus javanica* var. *roxburghii* and *Clethra barvinervis*, to introduce the community of AM fungi into the seedlings. Among these five RFLP types, four types were detected from both seedlings, which indicates that the AM fungal community in the forest root samples was introduced at least partly into the seedlings. Meanwhile, an additional four types that were not found in the forest root samples were newly detected in the seedlings, these types were closely related to one another and close to *G. fasciculatum* or *G. intraradices*. It is expected that a community of indigenous diverse AM fungi could be introduced into target fields by planting these mycorrhizal seedlings.

Key words Arbuscular mycorrhiza · *Clethra barvinervis* · Community structure · Deciduous broad-leaved forest · *Rhus javanica* var. *roxburghii*

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Introduction

The majority of terrestrial plants have symbiotic associations with arbuscular mycorrhizal (AM) fungi and benefit from these associations through enhanced uptake of mineral nutrients, especially phosphate, protection from pathogens, and improved water status (Smith and Read 1997). It is well known that AM fungi have a broad host range under pot culture conditions. However, recent studies revealed that the effects of AM fungi on host plants are different depending on the combination of plants and fungi, which suggests that each individual fungus is functionally distinct (Francis and Read 1995; Streitwolf-Engel et al. 1997; van der Heijden et al. 1998a; Helgason et al. 2002). Furthermore, it was shown that not only do AM fungi affect the increase of host plant biodiversity (Grime et al. 1987; Gange et al. 1993), but also the diversity of AM fungi relates to host plant biodiversity (van der Heijden et al. 1998b). These studies suggest the significance of the AM fungal community in a natural ecosystem.

A community of AM fungi has been hitherto estimated from the relative abundance of spores in soil. However, this method is not always appropriate to examine fungal composition in an ecological context because sporulation activity differs greatly depending on the AM fungi (Morton et al. 1995), host plants (Giovannetti et al. 1988; Bever et al. 1996), and season (Gemma and Koske 1988). It is therefore necessary to evaluate the identity and quantity of hyphae other than the spores of AM fungi, but morphological features of intraradical hyphae are usually insufficient to identify the AM fungi at species level.

Molecular analysis methods have been recently developed to identify the AM fungi in colonizing roots. The DNA primer AM1 designed by Helgason et al. (1998) has strong specificity toward the 18S rDNA of most AM fungi. The multiple colonization of AM fungi is separated into classes through cloning and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the numbers of each class can be used as an approximate estimate of their proportion in the roots (Husband

et al. 2002). These methods has been applied to reveal the community structure of AM fungi in arable fields (Daniell et al. 2001), grasslands (Vandenkoornhuyse et al. 2002), and several types of forests (Helgason et al. 1998, 1999, 2002; Husband et al. 2002).

The ecosystems of secondary deciduous broad-leaved forests in Japan are composed of diverse plant species where often some vulnerable or endangered species are found (Takeuchi et al. 2003). With the appreciation of biodiversity in these broad-leaved forests, restorations of such forests have been recently attempted by local governments in Japan. In such attempts, it is recommended to plant seedlings of indigenous strains originated from local vegetation (Tsumura and Iwata 2003).

Although most of the tree species forming the forest canopy are associated with ectomycorrhizal fungi, most of the other trees, shrubs, and understory herbaceous plants are associated with AM fungi (Yamato and Iwasaki 2002), which suggests that the AM fungal community has a strong relationship to plant diversity in the ecosystem. However, our knowledge about the community of AM fungi in the ecosystem has been quite limited.

Some of the selected AM fungi that were commercialized by several private companies have been applied in agriculture and reforestation (Suzui et al. 2000; Marumoto et al. 1999). However, in attempting natural forest restoration, application of diverse indigenous AM fungi would be more desirable because the diversity of AM fungi relates to host plant biodiversity (van der Heijden et al. 1998b). To apply diverse AM fungi to fields, seedlings of woody plants colonized by diverse AM fungi can be used as vectors for the fungal introduction. After planting of such vector plants, it is expected that the diverse indigenous AM fungi would proliferate in a wide area for a long time with the expanding root systems of their host plants.

In this study, as the first step, community structure of AM fungi in a deciduous broad-leaved forest was investigated by the molecular identification method. Introduction of the AM fungal community to pot seedlings of the woody plants was then attempted and subsequently assessed by the same molecular identification method.

Materials and methods

Sampling

Roots and soil samples were collected from a secondary deciduous broad-leaved forest in Mizuho-cho, Funai, Kyoto Prefecture in June 2002 (35°12′ N, 135°23′ E, 210 m above sea level). The annual mean temperature and the annual total precipitation recorded at the nearest meteorological station (Sonobe Meteorological Station) are 14.0°C and 1265.7 mm, respectively (averages in 1979–2000). The soil property of the forest is loam. The vegetation was that of a secondary forest including Japanese red pine, but most of the red pine trees have already died because of pine wilt disease caused by the pine wood nematode (*Bursaphelenchus xylophilus*), which is a typical phenomenon extensively spread among secondary forests in the western part of Japan (Mamiya 1988). In the forest, a 100 m^2 (10 × 10 m) sampling plot was established in which 29 plant species belonging to 24 genera in 18 families were found (Table 1). Among them, 17 species belonging to 16 genera in 15 families are regarded to be associated with AM fungi (Table 1). The sampling plot was divided into 25 subplots of 4 m^2 (2 m × 2 m), and two core soil samples each containing plant roots (100 ml in volume, 5 cm in depth) were collected after removing the litter layers at the center in all the subplots,

Molecular analysis

One of the core samples from each of 25 subplots was applied to molecular analysis to examine the community structure of AM fungi. Isolated plant roots were washed on a stainless sieve (pore size, 500 μm) under running tap water to remove the attached soil and debris. All the fine roots except the ectomycorrhizal and ericaceous ones were collected and freeze-dried in each root sample. DNA was extracted from each of the freeze-dried root samples (90–361 mg, 169 mg on average) using the CTAB method (Weising et al. 1995). After an additional purification using the Toyobo DNA Purification Kit (Toyobo, Tokyo, Japan), the DNA was dissolved in 50 μl TE buffer [10 mM Tris-HCl pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA)]. Partial fungal 18S rDNA (~550 bp) was amplified by PCR from 5 μl of the extracted DNA solution using TaKaRa Ex Taq Hot Start Version (Takara Bio, Otsu, Japan) with a universal eukaryotic primer NS31 (Simon et al. 1992) and the AM fungal-specific primer AM1 (Helgason et al. 1998). The reactions were performed on a TaKaRa PCR Thermal Cycler 480 (Takara Bio) using 200 μM of each dNTP, 0.25 μM of each primer, and the supplied PCR buffer. PCR conditions were initial denaturation step at 94°C for 2 min, a following step of 30 cycles at 94°C for 20 s, 58°C for 30 s, and 72°C for 45 s, then a final elongation step at 72°C for 5 min. The PCR products were cloned using a pT7Blue Perfectly Blunt Cloning Kit (Novagen, Madison, WI, USA) according to the manufacturer's instructions. For each sample, 18 putative cloned products (white colony) were randomly selected, then PCR was performed for each of the cloned products using the same primer set, NS31 and AM1. The cloned PCR products were digested independently with *Hin*fI, *Rsa*I (Toyobo), and *Hsp*92II (isoschizomer of *Nla*III) (Promega KK, Tokyo, Japan), and the fragment sizes were detected by microchip electrophoresis system SV1210 (Hitachi Electronics Engineering, Tokyo, Japan), which allowed classifying the cloned PCR products into several RFLP types. Randomly selected clones of each RFLP type were purified by MagExtractor–Plasmid (Toyobo) according to the manufacturer's instructions and sequenced using a DYEnamic ET dye terminator kit (Amersham Biosciences, Piscataway, NJ, USA) with the sequencing primers M13–47 primer and RV-M primer. All the sequence data were subjected to BLAST searches (Altshul et al. 1997), and analogous data were downloaded

Table 1. The plant species in tree layer (>2 m), shrub layer (0.5 < 2.0 m), and understory (<0.5 m) found in the examined plot with their mycorrhizae

Tree (>2 m)

Species	Family	Height (cm)	DBH (cm)	Mycorrhizal type
Quercus variabilis	Fagaceae	1550	16.5	ECM
Quercus variabilis	Fagaceae	1200	11.5	ECM
Quercus variabilis	Fagaceae	1150	10.5	ECM
Ilex pedunculosa	Aquifoliaceae	1500	20.0	AM
Ilex pedunculosa	Aquifoliaceae	905	8.5	AM
Ilex pedunculosa	Aquifoliaceae	702	5.8	AM
Ilex pedunculosa	Aquifoliaceae	487	2.8	AM
Ilex pedunculosa	Aquifoliaceae	382	5.0	AM
Clethra barvinervis	Clethraceae	1100	9.5	AM
Clethra barvinervis	Clethraceae	957	7.2	AM
Clethra barvinervis	Clethraceae	917	10.0	AM
Clethra barvinervis	Clethraceae	902	9.3	AM
Clethra barvinervis	Clethraceae	846	6.0	AM
Clethra barvinervis	Clethraceae	354	3.1	AM
Acer crataegifolium	Aceraceae	898	5.3	AM
Quercus serrata	Fagaceae	865	8.5	ECM
Lyonia ovalifolia var. *elliptica*	Ericaceae	832	6.7	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	650	6.5	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	624	4.1	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	567	3.8	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	556	4.0	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	479	5.6	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	435	2.4	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	410	1.9	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	401	1.9	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	395	2.8	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	342	3.5	ERM
Lyonia ovalifolia var. *elliptica*	Ericaceae	306	1.2	ERM
Rhus trichocarpa	Anacardiaceae	655	3.5	AM
Rhus trichocarpa	Anacardiaceae	499	2.3	AM
Rhus trichocarpa	Anacardiaceae	454	2.1	AM
Rhus trichocarpa	Anacardiaceae	400	1.8	AM
Rhus trichocarpa	Anacardiaceae	310	2.0	AM
Prunus grayana	Rosaceae	653	4.6	AM
Vaccinium oldhami	Ericaceae	396	3.0	ERM
Vaccinium oldhami	Ericaceae	326	1.8	ERM
Vaccinium oldhami	Ericaceae	264	1.5	ERM
Vaccinium oldhami	Ericaceae	252	1.6	ERM
Vaccinium oldhami	Ericaceae	240	1.2	ERM
Vaccinium oldhami	Ericaceae	225	2.2	ERM
Vaccinium oldhami	Ericaceae	224	1.1	ERM
Vaccinium oldhami	Ericaceae	224	1.4	ERM
Vaccinium oldhami	Ericaceae	224	1.0	ERM
Rhododendron reticulatum	Ericaceae	346	2.1	ERM
Rhododendron macrosepalum	Ericaceae	298	1.8	ERM
Rhododendron macrosepalum	Ericaceae	289	1.6	ERM
Rhododendron macrosepalum	Ericaceae	284	1.3	ERM
Rhododendron macrosepalum	Ericaceae	271	1.6	ERM
Rhododendron macrosepalum	Ericaceae	219	1.0	ERM
Rhododendron macrosepalum	Ericaceae	215	1.1	ERM
Eurya japonica	Theaceae	280	2.5	AM
Eurya japonica	Theaceae	236	2.5	AM
Eurya japonica	Theaceae	219	1.1	AM

Table 1. *Continued*

Shrub (0.5 < 2.0 m)		
Species	Family	Mycorrhizal type
Ilex crenata	Aquifoliaceae	AM
Lyonia ovalifolia var. *elliptica*	Ericaceae	ERM
Pieris japonica	Ericaceae	ERM
Rhododendron macrosepalum	Ericaceae	ERM
Rhododendron reticulatum	Ericaceae	ERM
Vaccinium oldhami	Ericaceae	ERM
Eurya japonica	Theaceae	AM
Understory (<0.5 m)		
Species	Family	Mycorrhizal type
Acer crataegifolium	Aceraceae	AM
Rhus trichocarpa	Anacardiaceae	AM
Ilex pedunculosa	Aquifoliaceae	AM
Ilex crenata	Aquifoliaceae	AM
Eleutherococcus sciadophylloides	Araliaceae	AM
Abelia spathulata	Caprifoliaceae	AM
Clethra barvinervis	Clethraceae	AM
Chamaecyparis obtusa	Cupressaceae	AM
Lyonia ovalifolia var. *elliptica*	Ericaceae	ERM
Pieris japonica	Ericaceae	ERM
Rhododendron kaempferi	Ericaceae	ERM
Rhododendron macrosepalum	Ericaceae	ERM
Vaccinium japonicum	Ericaceae	ERM
Vaccinium oldhami	Ericaceae	ERM
Castanea crenata	Fagaceae	ECM
Quercus serrata	Fagaceae	ECM
Quercus variabilis	Fagaceae	ECM
Tripterospermum japonicum	Gentianaceae	AM
Lindera umbellata	Lauraceae	AM
Disporum smilacinum	Liliaceae	AM
Heloniopsis orientalis	Liliaceae	AM
Smilax china	Liliaceae	AM
Magnolia salicifolia	Magnoliaceae	AM
Ardisia japonica	Myrsinaceae	AM
Cymbidium goeringii	Orchidaceae	OM
Platanthera minor	Orchidaceae	OM
Prunus grayana	Rosaceae	AM

The species in tree layer are shown with their height and diameter at breast height
The mycorrhizal types were judged from the plant families
DBH, diameter at breast height; ECM, ectomycorrhiza; AM, arbuscular mycorrhiza; ERM, ericoid mycorrhiza; OM, orchid mycorrhiza

from the GenBank database. For all the sequenced data, or for some of the sequenced and downloaded data, multiple sequence alignments were carried out using Clustal W version 1.82 (Thompson et al. 1994). The aligned sequences were analyzed by the neighbor joining method (Saitou and Nei 1987) using Neighbor in the PHYLIP version 3.5c package (Felsenstein 1993), and the topology was tested with 1000 bootstrap trials for each of the phylogeny. The phylogenetic trees were drawn using Treeview (Page 1996).

Pot culture of woody plants

The other core samples from each of 25 subplots were used as inoculum of AM fungi for pot cultures of seedlings of *Rhus javanica* var. *roxburghii* (Anacardiaceae) and *Clethra barvinervis* (Clethraceae). The roots in the core samples were cut into pieces about 1 cm in length, and 25 soil samples including the root pieces were then put together and mixed. One volume (100 ml) of the soil sample and four volumes (400 ml) of autoclaved mixture of Akadama soil and river sand (1:1, v:v) were well mixed and stuffed into each pot (500 ml). One seedling each of *R. javanica* var. *roxburghii* or *C. barvinervis* without AM colonization, grown from surface-sterilized seeds for 60 days in an autoclaved mixture of Akadama soil and river sand (1:1, v:v), was transplanted into each pot. Five replicates of pot cultures for each of the two plant species were prepared. The plants were grown in a greenhouse, with an application of Hyponex liquid fertilizer (6-6-6) (Hyponex Japan, Tokyo, Japan) at a final N concentration of 60 mg/l, until the solution flowed out from the drain holes, every 2 weeks. After 3 months from the transplanting, three core soil samples (2 cm in diameter, 7 cm in depth) were collected from each

Table 2. Representative fragment sizes (base pairs) in each restriction fragment length polymorphism (RFLP) types of arbuscular mycorrhizal (AM) fungal 18S rDNA polymerase chain reaction (PCR) products (NS31-AM1)

RFLP type[a]	*Hin*fI				*Rsa*I			*Hsp*92II				Total length
A	526[b]	23			123	4	422	160	26	71	296	549
B	143	383	23		127	422		253	296			549
C	143	380	23		127	419		111	142	293		546
D	143	383	23		549			253	296			549
E	143	382	23		549			111	142	295		548
P1	143	49	334	23	127	422		111	142	296		549
P2	143	49	334	23	127	422		253	296			549
P3	143	49	334	23	549			111	142	296		549
P4	142	49	334	23	549			252	296			548

[a] The DNA sequences in RFLP type A–E were obtained from forest root samples and those in type P1–P4 were newly obtained from pot seedlings; the clones whose fragment sizes are different at several base pairs are regarded as the identical RFLP type
[b] The order of the fragments in each enzyme reflects the position of the restriction site in the PCR product

Table 3. Number of clones of each RFLP type obtained from each subplot

RFLP type	No. of subplot																								Total in each RFLP type
	1	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
A	4	7	2	–	4	2	1	1	4	2	3	–	–	3	8	3	2	–	1	–	–	2	7	16	72
B	3	–	–	–	–	5	1	1	–	–	3	–	3	–	4	1	1	–	2	6	2	2	–	–	34
C	4	4	–	7	6	2	4	6	7	8	3	9	9	4	3	7	9	9	7	–	12	3	1	–	124
D	2	1	14	1	2	8	–	–	–	2	1	–	1	1	2	–	3	–	2	4	2	5	5	–	56
E	5	6	–	9	4	–	10	7	7	5	5	7	3	7	1	4	2	6	4	7	1	4	4	–	108
Total in each subplot	18	18	16	17	16	17	16	15	18	17	15	16	16	15	18	15	17	15	16	17	17	16	17	16	394

pot using a cork borer. The three core samples were put together and all fine roots were collected. DNA was then extracted from each root sample by the CTAB method. The molecular analysis, as already described, was applied for the extracted DNA to examine the community structure of the AM fungi in the pot seedlings.

Results

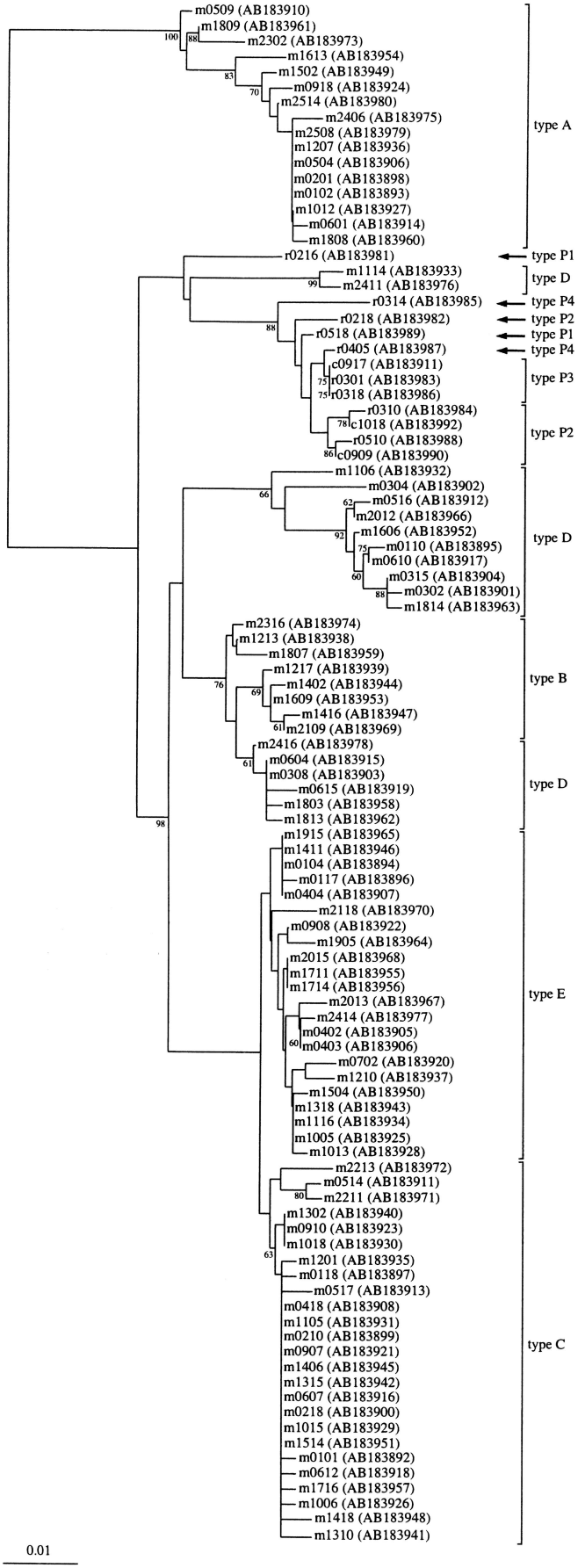
Community structure of AM fungi in the broad-leaved forest

Each of the core soil samples collected from a subplot generally contained three to five kinds of plant roots except ericoid mycorrhizal and ectomycorrhizal roots, all of which were examined together. A PCR product of ~550 bp was obtained from all the root samples except from one subplot (no. 8). After cloning of the PCR products, 18 clones at most were obtained from each PCR product and subjected to further RFLP analysis. Among the RFLP types obtained, those with only 1 clone were excluded from further analysis, because it is possible that these low-frequency types might be chimeras from different DNA templates in the PCR (Bradley and Hillis 1997). In total, 394 clones were obtained and divided into five RFLP types, A, B, C, D, and E (Table 2). At least four RFLP types were found in 17 subplots among the 24 subplots examined (Table 3).

More than 20% of the clones in each RFLP type, which correspond to 89 clones in total, were randomly selected and sequenced. The neighbor-joining phylogenetic analysis for all the sequenced clones showed that the clones in each of four types, A, B, C, and E, formed each monophyletic clade (Fig. 1). This result indicates that the grouping of the clones by RFLP with *Hin*fI, *Rsa*I, and *Hsp*92II was generally valid. Another neighbor-joining phylogenetic tree was drawn with the data of some representatives from each monophyletic clade and some of the analogous data in GenBank database obtained by the BLAST searches (Fig. 2). This second phylogenetic tree showed that all the clones obtained in this study belonged to *Glomus* (Fig. 2). However, none of the clones could be identified at species level, because no clones formed a monophyletic clade with the identified species.

Community structure of AM fungi in pot cultures

All the 10 pot seedlings were successfully colonized by AM fungi and subjected to molecular analysis. The analysis showed that the four RFLP types B, C, D, and E found in



the forest samples were also detected in the seedlings (Table 4). Among them, types B, C, and D were predominantly detected. Meanwhile, four additional RFLP types, types P1, P2, P3, and P4, which were not found in the forest samples, were newly detected. Some representatives were randomly selected in those four types, and 12 clones in total were sequenced. Phylogenetic analysis with these clones revealed that the four newly detected types are closely related to one another and were found to be close to *G. intraradices* and *G. fasciculatum* (see Figs. 1, 2). Considerable variation was found in the composition of the RFLP types among the pot seedlings, even in the same host plant species.

Discussion

In this intensive study in the 100 m^2 forest plot, the 394 clones obtained were divided into five RFLP types, types A–E. Among the 24 subplots, at least four RFLP types were found in 17 subplots, which suggested that each of the AM fungi in the five RFLP types was widely distributed in the plot.

All the AM fungi in the five RFLP types belonged to *Glomus*, and four types, excepting type A, were closely related, which indicates that the diversity of AM fungi was lower than that found in other extensive studies in forests using the same analysis method (Helgason et al. 1998, 1999, 2002; Husband et al. 2002; Öpik et al. 2003). The low diversity of AM fungi may be a characteristic of this kind of forest, although the diversity of plant species with AM colonization was not low. It is also possible that some AM fungi cannot be detected in this study because of the unsuitability of the primer AM1 to the sequence in Archaeosporaceae, Paraglomaceae (Daniell et al. 2001), and some *Glomus* species (Schüßler et al. 2001). On the other hand, Kubota et al. (2005) found spores of AM fungi belonging to *Paraglomus*, *Acaulospora*, *Glomus*, and *Gigaspora* in a Japanese secondary forest. Further extensive study would be therefore required to reveal the diversity of AM fungi in Japanese deciduous broad-leaved forests.

The phylogenetic analysis showed that most of the examined clones obtained in this study did not form a

Fig. 1. Neighbor-joining phylogenetic tree based on NS31–AM1 18S rDNA sequences of arbuscular mycorrhizal (AM) fungi obtained from forest root samples and pot seedlings. The tree is rooted to *Acaulospora laevis* (AF131041), which were removed from the figure because of space limitation. Group identity (type A–E, P1–P4) relates to the restriction fragment length polymorphism (RFLP) types. The DNA sequences in types A–E were obtained from forest root samples and those in types P1–P4 were newly obtained from pot seedlings. Clone identifiers relate to origin (*m*, forest sample; *r*, seedling of *Rhus javanica* var. *roxburghii*; *c*, *Clethra barvinervis*), subplot number or pot number, and clone number. Bootstrap values are shown where they exceed 60% (1000 replicates). All sequences have been submitted to GenBank database (accession numbers AB183892–AB183992)

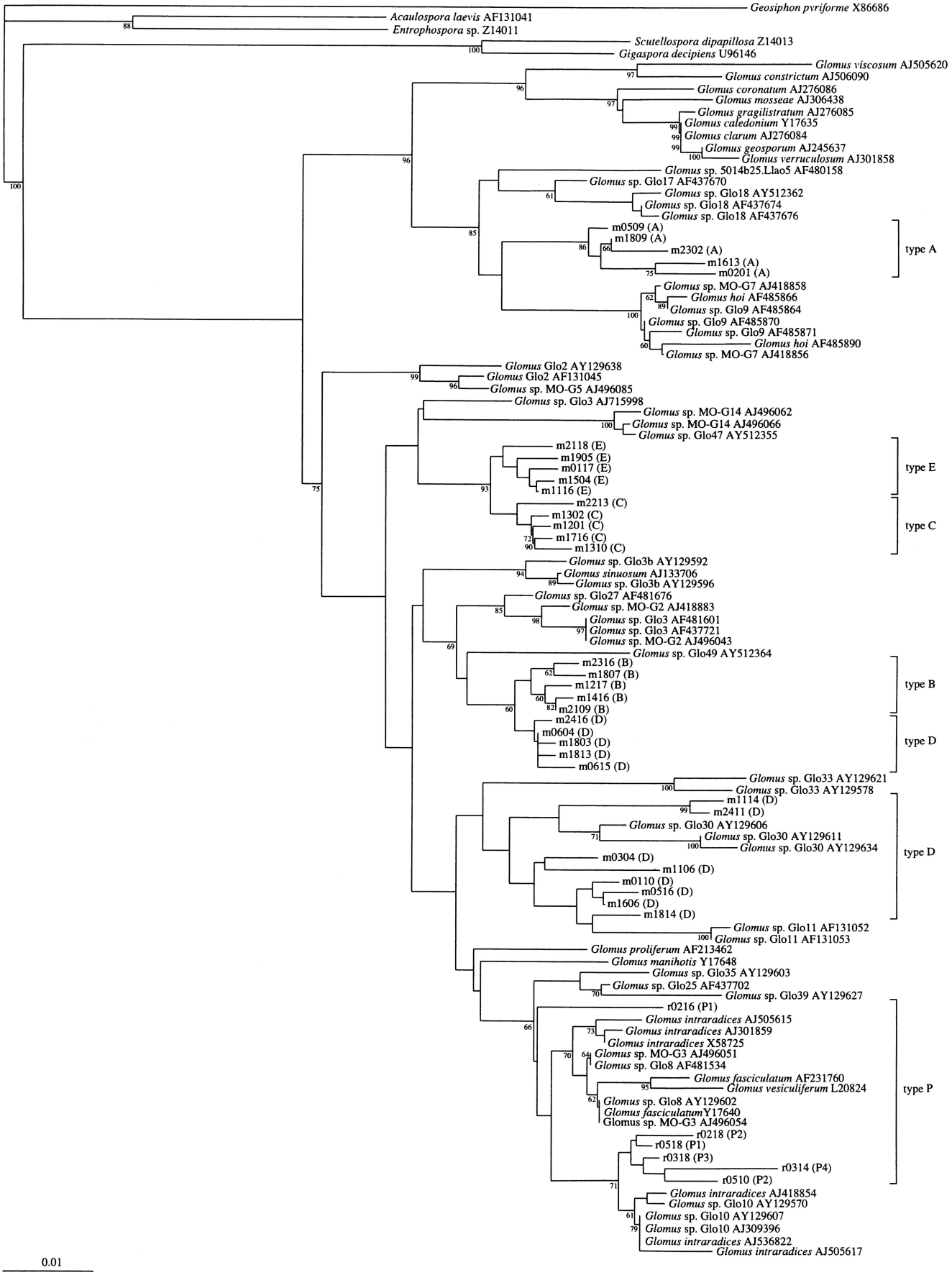


Fig. 2. Neighbor-joining phylogenetic tree based on NS31–AM1 18S rDNA sequences of AM fungi obtained in this study and from GenBank database. The tree is rooted to the *Geosiphon pyriforme* (X86686). Group identity and clone identifiers are described in Fig. 1. Bootstrap values are shown where they exceed 60% (1000 replicates). Accession numbers are given for sequences published previously

Table 4. Number of clones of each RFLP type obtained from each pot seedling

RFLP type	Seedling no.[a]										Total of each RFLP type
	r1	r2	r3	r4	r5	c6	c7	c8	c9	c10	
A	–	–	–	–	–	–	–	–	–	–	0
B	5	3	2	1	5	6	11	5	1	–	39
C	3	12	1	15	–	5	1	6	1	13	57
D	8	1	4	1	6	5	5	6	2	3	41
E	1	–	–	–	–	–	–	–	1	–	2
P1	–	1	–	–	1	–	–	–	–	–	2
P2	–	1	7	–	4	–	–	–	12	1	25
P3	–	–	3	–	–	–	–	–	1	–	4
P4	–	–	1	1	–	–	–	–	–	–	2
Total in each subplot	17	18	18	18	16	16	17	17	18	17	172

[a] The identifiers of the pot seedlings relate to plant species (r, *Rhus javanica* var. *roxburghii*; c, *Clethra barvinervis*) and seedling number

monophyletic clade with the sequences from GenBank database. This result might be caused by insufficiency of the available data of AM fungi in GenBank, but it is also likely that some unknown AM fungi exist in the forest. Most of the AM fungal sequences in GenBank database analogous to those in types A and D were also obtained from forest plant roots (Helgason et al. 2002; Husband et al. 2002; Öpik et al. 2003), which suggests that some of these AM fungi could be specifically adapted to the forest environments.

Among the five RFLP types found in the forest root samples, four types were detected from the pot culture seedlings of *R. javanica* var. *roxburghii* and *C. barvinervis*. However, type A was not detected in the seedlings, probably because of the low inoculum potential or low affinity to those two host plant species. The composition of the RFLP types was quite various among the seedlings in each plant species; therefore, the effect of the host plant species on the AM fungal community introduced was not clear. The high variation in the RFLP types may be caused by the lack of uniformity of the inocula contained in the soil and roots. In the analysis of AM fungi in the pot seedlings, some clones, of types P1, P2, P3, and P4, were newly detected, all of which are closely related with *G. intraradices* and *G. fasciculatum.* Probably the fungi of these clones could not be found in the forest root samples because of their low level of colonization, but propagated well in the seedlings in pot cultures, which indicates that these fungi may have aptitude to the environment of the pot seedlings.

The *R. javanica* var. *roxburghii* is a well-known pioneer tree species, and *C. barvinervis*, which was growing in the sampling plot, is often found near mountain ridges. These two plant species are, therefore, considered to have some tolerance for arid environments and be appropriate to be planted in reforestation. Many recent studies suggested the significance of the diverse AM fungi on diverse plant communities (Grime et al. 1987; Gange et al. 1993; van der Heijden et al. 1998b). In this study, community of AM fungi in the forest plot was roughly introduced into the pot culture seedlings of these woody plants. Therefore, it is expected that planting these mycorrhizal seedlings could lead to the introduction of the AM fungal community to the planted fields.

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