Mycoscience (2009) 50:100–105
DOI 10.1007/s10267-008-0459-8

FULL PAPER

Masahide Yamato · Shiho Ikeda · Koji Iwase

Community of arbuscular mycorrhizal fungi in drought-resistant plants, *Moringa* spp., in semiarid regions in Madagascar and Uganda

Received: December 3, 2007 / Accepted: September 15, 2008

Abstract The community structure of arbuscular mycorrhizal (AM) fungi in the roots of drought-resistant trees, *Moringa* spp., was examined in semiarid regions in Madagascar and Uganda. Root samples were collected from 8 individuals of *M. hildebrandtii* and 2 individuals of *M. drouhardii* in Madagascar and from 21 individuals of *M. oleifera* in Uganda. Total DNA was extracted from the root samples, and partial nSSU rDNA of AM fungi was amplified using a universal eukaryotic primer NS31 and an AM fungal-specific primer AM1. The PCR products were cloned and divided by restriction fragment length polymorphism (RFLP) analysis with *Hin*fI and *Rsa*I. Some representatives in each RFLP types were sequenced, and a neighbor-joining phylogenetic analysis was conducted for the obtained sequences with analogous sequences of AM fungi. The RFLP and phylogenetic analyses showed that AM fungi closely related to *Glomus intraradices* or *G. sinuosum* were detected in many samples. The AM fungal groups frequently detected in the *Moringa* spp. might be widely distributed species in semiarid environments.

Key words Arbuscular mycorrhizal fungi · *Glomus intraradices* · *Moringa drouhardii* · *Moringa hildebrandtii* · *Moringa oleifera*

Introduction

Moringa spp. comprise a single genus in a small family Moringaceae and are known to be drought-resistant trees. They were originally distributed in semiarid tropical and subtropical areas along the Indian Ocean from India to southern Africa, including Madagascar. *Moringa* spp., 13 species in total, are divided into three life forms, i.e., bottle trees, slender trees, and tuberous shrubs (Olson and Carlquist 2001). Their drought resistance is attributed to having water-storing organs, i.e., the bloated trunks of bottle trees and tubers in the slender trees and shrubs.

The slender tree species *M. oleifera* Lam. is a fast-growing tree and can grow to 6–7 m in 1 year even in areas with less than 400 mm annual precipitation (Odee 1988). This plant has many valuable properties because of the high content of protein in the leaves and of oil in the seeds (Makkar and Becker 1997). It is also known that ground seeds can be used as a coagulant for water purification (Jahn 1988). Recently, this tree has been planted in semiarid tropical areas for multipurpose use, e.g., foods, supplements, edible oil, and forage.

It is well known that most terrestrial plants have symbioses with mycorrhizal fungi. Colonization of arbuscular mycorrhizal (AM) fungi has been reported in *Moringa* spp. (Panwar and Vyas 2002; Muthukumar et al. 2006). The AM fungal association contributes to soil nutrient uptake and disease resistance of host plants and stabilization of sand (Smith and Read 1997). Many experimental studies also showed that AM fungi improved the drought tolerance of host plants (Nelson and Safir 1982; Busse and Ellis 1985; Davies et al. 1993; Subramanian et al. 1997).

AM fungi have a broad host range under laboratory conditions. It has also been shown that the growth of plants could differ depending on the strains or composition of AM fungi (van der Heijden et al. 1998; Moora et al. 2004; Munkvold et al. 2004). The community structure of AM fungi would, therefore, affect the growth of plants in natural environments.

To analyze communities of AM fungi in roots, molecular methods have recently been applied in which DNA sequences of nuclear small subunit ribosomal RNA gene (nSSU rDNA), rDNA internal transcribed spacer (rDNA ITS), and nuclear large subunit ribosomal RNA gene (nLSU rDNA) have been examined (Öpik et al. 2006). Among

M. Yamato (✉)
Environment Department, The General Environmental Technos, Co., Ltd., 1-3-5 Azuchimachi, Chuou-ku, Osaka 541-0052, Japan
Tel. +81-774-21-5001; Fax +81-774-21-5005
e-mail: yamato_masahide@kanso.co.jp

S. Ikeda
Forest Development Technological Institute, Kyoto, Japan

K. Iwase
Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, Tottori, Japan

them, a part of nSSU rDNA amplified by primer pairs, a universal eukaryotic primer NS31 (Simon et al. 1992), and an AM fungal-specific primer AM1 (Helgason et al. 1998), were the most utilized (Helgason et al. 1999; Daniell et al. 2001; Husband et al. 2002; Vandenkoornhuyse et al. 2002; Öpik et al. 2003; Oba et al. 2004; Douhan et al. 2005; Yamato and Iwase 2005; Yamato et al. 2008).

Because *Moringa* spp. are fast-growing and drought-resistant tree species in a semiarid environment, the symbioses with AM fungi would be important for their growth. In the present study, we examined the community of AM fungi colonizing in roots of *Moringa* spp. in semiarid regions in Madagascar and Uganda by the molecular identification method using primers NS31 and AM1.

Materials and methods

Sampling.

The samplings were conducted in the middle of December 2005 in Madagascar and in the middle of January 2006 in Uganda. The examined trees are listed with sampling sites in Table 1. In Madagascar, eight trees of *M. hildebrandtii* Engler and two trees of *M. drouhardii* Jumelle were examined. The *M. hildebrandtii* trees examined were planted in villages, whereas the *M. drouhardii* trees were wild ones. They are bottle trees with fat trunks, and the biggest specimen of *M. hildebrandtii* was 249 cm in diameter at breast height (DBH). In Uganda, 21 trees of *M. oleifera* were examined. They were all planted for multipurpose use such as foods, edible oil, and forage. The annual precipitations of the sampling sites are 475–724 mm in Madagascar and 975–1474 mm in Uganda, according to the Food and Agriculture Organization (FAO) country profiles and mapping information system (http://www.fao.org/countryprofiles).

For each tree, three soil cores containing roots (50 cm × 50 cm × 20 cm deep) were randomly sampled at 50 cm apart from the tree base. The soil cores collected from a single tree were put together into a plastic bag and well mixed. The roots of *Moringa* spp., which were easily recognized by their special smell, were isolated and washed thoroughly on a 500-µm stainless sieve with tap water, and then fixed and preserved in 50% ethanol.

Soil analysis

Soil water content, soil pH (H_2O), and amount of available phosphate (Truog-P) (Truog 1930) were analyzed for each soil sample.

Table 1. Location and profile of *Moringa* (M.) trees examined in this study

Tree no.	Sampling date (d/m/y)	Location	Latitude	Longitude	Altitude (m)	Species	Tree height (m)	DBH (cm)
Madagascar								
M1	18/12/2005	Andranomena	S20 11 10.9	E44 25 30.6	22	*M. hildebrandtii*	6.7	58.0
M2	18/12/2005	Morondava	S20 17 47.6	E44 18 09.1	4	*M. hildebrandtii*	15.7	249.0
M3	18/12/2005	Antandrokosy	S20 18 53.6	E44 23 26.1	23	*M. hildebrandtii*	11.4	125.2
M4	19/12/2005	Saint Augastin	S23 33 11.9	E43 45 49.0	12	*M. hildebrandtii*	6.1	75.5
M5	20/12/2005	Sakaraha	S22 54 33.7	E44 31 45.0	472	*M. hildebrandtii*	11.1	62.7
M6	20/12/2005	Andranolava	S22 37 45.7	E44 41 34.9	612	*M. hildebrandtii*	12.7	93.9
M7	20/12/2005	Andranolava	S22 36 49.2	E44 40 45.7	586	*M. hildebrandtii*	12.8	108.6
M8	21/12/2005	Saint Augastin	S23 31 11.6	E43 45 08.4	15	*M. drouhardii*	6.6	93.6
M9	21/12/2005	Saint Augastin	S23 31 11.3	E43 45 07.2	12	*M. drouhardii*	5.6	91.4
M10	21/12/2005	Saint Augastin	S23 27 59.7	E43 45 57.2	15	*M. hildebrandtii*	6.7	41.1
Uganda								
U1	12/01/2006	Wakiso	N0 23 54.6	E32 29 21.8	1168	*M. oleifera*	7.0	8.5
U2	12/01/2006	Mukono	N0 20 21.5	E32 43 13.9	1213	*M. oleifera*	4.5	7.0
U3	12/01/2006	Mukono	N0 20 21.5	E32 43 13.9	1213	*M. oleifera*	5.7	9.2
U4	13/01/2006	Masaka	S0 10 23.8	E31 45 13.6	1230	*M. oleifera*	4.3	6.0
U5	13/01/2006	Masaka	S0 10 23.8	E31 45 13.6	1230	*M. oleifera*	5.9	10.4
U6	14/01/2006	Tororo	N0 41 16.2	E34 06 36.2	1164	*M. oleifera*	4.8	13.3
U7	14/01/2006	Tororo	N0 41 16.2	E34 06 36.2	1164	*M. oleifera*	4.8	14.6
U8	14/01/2006	Tororo	N0 41 16.2	E34 06 36.2	1164	*M. oleifera*	5.0	12.3
U9	14/01/2006	Tororo	N0 40 39.1	E34 09 12.8	1152	*M. oleifera*	5.1	10.1
U10	14/01/2006	Tororo	N0 40 39.1	E34 09 12.8	1152	*M. oleifera*	5.0	14.3
U11	14/01/2006	Tororo	N0 39 31.9	E34 08 54.4	1203	*M. oleifera*	6.3	12.9
U12	15/01/2006	Jinja	N0 25 43.0	E33 09 36.5	1213	*M. oleifera*	5.0	11.6
U13	16/01/2006	Kajansi	N0 09 01.7	E32 32 46.5	1176	*M. oleifera*	4.0	7.5
U14	17/01/2006	Mukono	N0 27 10.9	E32 45 39.6	1189	*M. oleifera*	8.9	27.4
U15	17/01/2006	Matuga	N0 27 21.7	E32 32 34.0	1210	*M. oleifera*	5.1	12.1
U16	18/01/2006	Nakasongola	N1 18 54.9	E32 27 58.7	1088	*M. oleifera*	3.8	16.2
U17	18/01/2006	Nakasongola	N1 18 48.4	E32 27 24.0	1108	*M. oleifera*	5.3	10.9
U18	19/01/2006	Mpigi	N0 13 35.0	E32 19 14.0	1234	*M. oleifera*	6.0	13.2
U19	19/01/2006	Mpigi	N0 13 35.0	E32 19 14.0	1234	*M. oleifera*	6.5	18.5
U20	19/01/2006	Mpigi	N0 14 12.5	E32 19 07.3	1270	*M. oleifera*	5.9	16.4
U21	19/01/2006	Mpigi	N0 14 12.5	E32 19 07.3	1270	*M. oleifera*	6.5	17.3

DBH, diameter at breast height

Molecular phylogenetic analysis

Fine roots, about 300 mg in fresh weight, were randomly selected from each root sample, and DNA was extracted using a modified cetyltrimethylammonium bromide CTAB method (Weising et al. 1995). After an additional purification using Mag Extractor–Plant Genome (Toyobo, Tokyo, Japan), the DNA was dissolved in 50 µl TE buffer. Partial fungal nSSU rDNA (~550 bp) was amplified by polymerase chain reaction (PCR) from the extracted DNA using Takara Ex Taq Hot Start Version (Takara Bio, Otsu, Japan) with primers NS31 and AM1. The reaction mixture for PCR contained 5 µl extracted DNA solution, 0.75 units Taq polymerase, 0.25 µM each primer, 200 µM each dNTP, and 3 µl supplied PCR buffer in 30 µl of the total amount. The PCR was performed on a TaKaRa PCR Thermal Cycler 480 (Takara Bio) as follows: initial denaturation step at 94°C for 2 min, following a step of 30 cycles at 94°C for 20 s, 58°C for 30 s, and 72°C for 45 s, then the 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 instruction. For each root sample, 18 clones were randomly selected, and PCR was performed using the same primer set, NS31 and AM1. Aliquots of each of the PCR products were digested with *Hin*fI or *Rsa*I (Toyobo), and restriction fragment length polymorphism (RFLP) types were determined using microchip electrophoresis system SV1210 (Hitachi Electronics Engineering, Tokyo, Japan). Plasmid DNA was extracted from several samples in each RFLP type using MagExtractor-Plasmid (Toyobo) according to the manufacture's instructions, and DNA inserts were sequenced using a DYEnamic ET dye terminator kit (Amersham Biosciences, Piscataway, NJ, USA) with sequencing primers M13-47 and RV-M. A capillary sequencer MegaBACE1000 (Amersham Biosciences) was used for the sequencing. For each sequence, analogous data were obtained from the DDBJ/EMBL/GenBank database, and multiple sequence alignment and neighbor-joining (NJ) phylogenetic analysis (Saitou and Nei 1987) were carried out using CLUSTAL W version 1.83 (Thompson et al. 1994). The alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S2253. For the NJ analysis, evolutionary distances were estimated using Kimura's two-parameter model (Kimura 1980), and bootstrap analysis (Felsenstein 1985) was performed with 1000 replications. The phylogenetic tree was drawn using Treeview (Page 1996).

Results

Soil analysis

The physical and chemical properties of the examined soil samples are shown in Table 2. Many soil samples were wet because the sampling was carried out in the rainy season. However, some samples in Madagascar and the eastern part of Uganda showed rather low water content, less than 5%. Some soil samples in Madagascar were calcareous with relatively higher pH values, more than 8. The available phosphate (Truog-P) in the examined soil samples varied greatly, from 8.1 to 1328.1 mg P kg^{-1} soil. Excrement of livestock would have affected this high phosphate level.

Table 2. Properties of soil samples collected with roots of *Moringa* spp.

Tree no.	Water content (%)	pH (H_2O)	Available phosphate (mg P kg^{-1})
Madagascar			
M1	4.0	7.6	22.1
M2	5.9	8.5	402.0
M3	11.1	6.8	108.5
M4	6.6	8.2	130.8
M5	8.9	7.0	34.2
M6	8.7	7.6	74.4
M7	9.0	8.1	383.6
M8	7.0	8.2	42.2
M9	8.1	8.0	128.4
M10	8.2	8.3	10.0
Uganda			
U1	10.8	5.8	34.8
U2	17.2	5.6	11.3
U3	14.4	6.9	31.3
U4	12.2	6.5	9.6
U5	14.8	5.9	13.0
U6	4.1	6.5	17.7
U7	7.6	7.0	16.3
U8	3.9	7.7	12.3
U9	14.4	6.7	136.1
U10	10.4	6.6	93.3
U11	14.4	7.7	1328.1
U12	10.9	6.4	18.0
U13	12.3	6.8	54.2
U14	15.9	7.6	70.8
U15	16.3	6.1	13.1
U16	3.4	8.0	183.2
U17	4.8	6.9	86.7
U18	19.7	6.9	83.7
U19	17.3	6.9	73.1
U20	13.2	5.6	8.1
U21	8.6	7.4	29.6

Molecular phylogenetic analysis

PCR products of about 550 bp were obtained from 31 root samples, 10 from *M. hildebrandtii* and *M. drouhardii* in Madagascar, and 21 from *M. oleifera* in Uganda. In total, 450 clones were obtained, which were divided into 14 RFLP types. In total, 70 clones were sequenced, and the obtained data were phylogenetically analyzed (Fig. 1). The AM fungi in the clades A, B, C, and D included several RFLP types, respectively. Clade A (A1, A2, A3) and clade B (B1, B2) were detected in many examined samples (Table 3). Actually, AM fungi in clade A and clade B were detected in 27 and 23 root samples, respectively, in the 31 samples examined.

The phylogenetic analysis of AM fungi (see Fig. 1) showed that the clade A fungi were closely related to *Glomus intraradices* N.C. Schenck & G.S. Sm., and the

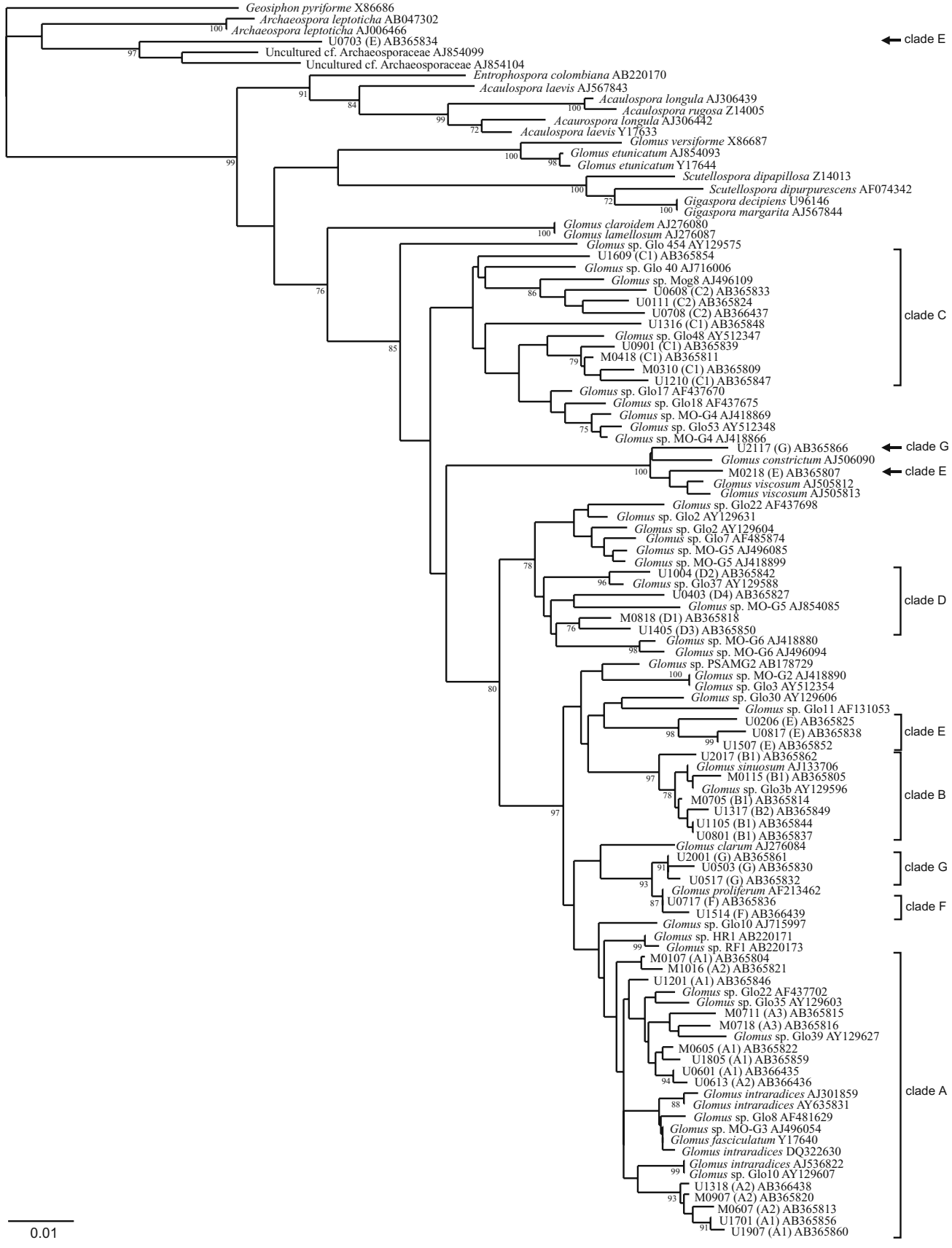

Fig. 1. A neighbor-joining phylogenetic tree based on NS31-AM1 nuclear small subunit (nSSU) rDNA sequences of arbuscular mycorrhizal (AM) fungi obtained in this study and the DDBJ/EMBL/GenBank database. The tree is rooted to *Geosiphon pyriforme* (X86686). Group identity (type A1, A2, A3, B1, B2, C1, C2, D1, D2, D3, D4, E, F, G) relates to the restriction fragment length polymorphism (RFLP) types shown in Table 3. Clone identifiers relate to tree number and clone number. Bootstrap values are shown where they exceed 70% (1000 replicates). A *scale* is shown to infer the evolutionary distances. Accession numbers are given for all sequences

Table 3. Number of clones of each restriction fragment length polymorphism (RFLP) type obtained from each *Moringa* tree

RFLP type	Number of clones of each RFLP type in the following tree no.																										Total
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21	
A	15	16	10	4		15	8	9	14	2	12	4	10		3	11	3	3	10	5	7	5	3		3		9	11	15	1	1	209
B	1		2	5	17	1	4	3	1	2	3		2	5	7			5	3	4	2		13	6	1		5	2		7	6	107
C			5	7				3		10	1					3	2	2	2		1	2				6	1	1				46
D								1	1					6					1	1				2							1	13
E		1										8		4	2	1	1	2		1	1	4			3	5	1		1			35
F				1							1	1		1	3		6								8					6	1	28
G												1			3		2								3					3		12
Total	16	17	17	17	17	16	12	16	16	14	17	14	12	16	18	15	14	12	16	11	11	11	16	8	18	11	16	14	16	17	9	450

clade B fungi were closely related to *G. sinuosum* (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck, but clade C had no close relationship with described species. Clades E and G were found to be paraphyletic in relationship in the phylogenetic tree. Because the fungi in clades E, F, and G were detected more often in the samples from Uganda than those from Madagascar, AM fungal species diversity may be higher in Uganda samples (see Table 3). The fungi in clade F and the majority of clade G were closely related to *G. proliferum* Dalpé & Declerck (Fig. 1).

Discussion

Although the samplings were conducted in the rainy season, some soil samples showed water content less than 5%. High temperature and strong sunshine would bring dry conditions. It is supposed that the examined trees would have suffered from more serious arid conditions in the dry season. For the survival and growth of trees in the stressed environments, the water-storing organs, the bloated trunks or the tubers, would have an important role. In all the examined trees, colonization of AM fungi was detected. The AM fungi also may have some role for growth and drought tolerance of the host plants.

AM fungi in clade A, frequently detected in the examined samples, were phylogenetically close to *G. intraradices*. This AM fungal species is often found in arid and semiarid environments (Stutz et al. 2000; Muthukumar and Udaiyan 2002; Tao and Zhiwei 2005). Clade B fungi, the second most frequent, were close to *G. sinuosum*, which have also been found in some arid and semiarid environments (Muthukumar and Udaiyan 2002; Calvente et al. 2004). Meanwhile, a global analysis of AM fungal communities examined by rDNA sequences showed that *G. intraradices* is a cosmopolitan species in various environments, not only in the dry environments, but also in mesic environments, forests, grasslands, and arable fields (Öpik et al. 2006).

Functional diversification among isolates in the same AM fungal species has recently been suggested from the different effects on plant growth (Munkvold et al. 2004). Under water-stressed conditions, some AM fungi adapt to such environmental conditions. For example, Stahl and Smith (1984) reported that two strains of *G. microcarpum* Tul. & C. Tul, collected from a desert and a mesic site, showed different effect on leaf resistance to water vapor loss in *Agropyron smithii* Rydb. That is, the strain from the desert induced lower leaf resistance in an arid condition than another one from a mesic site. The lower leaf resistance means increased stomatal openings, which is a common adaptation of plants to gain carbon in arid conditions (Turner and Kramer 1980).

For the AM fungi found in such water-stressed environments, it would be interesting to examine their ability to improve host plant growth in such an environment. For AM fungal strains of the cosmopolitan species, furthermore, it is interesting to know whether they have adaptability to the various environmental conditions or whether they are

functionally differentiated to have an aptitude to stressed conditions.

Acknowledgments This work was partly supported by the Ministry of Economy, Trade and Industry, Japan. We express our gratitude to Dr. Raharimalala Fidele in Antananarivo University in Madagascar and Mr. Opiro Lakuma Kenneth in Homeopathy & Herbal Center in Uganda for their help in collecting *Moringa* samples.

References

Busse MD, Ellis JR (1985) Vesicular-arbuscular mycorrhizal (*Glomus fasciculatum*) influence on soybean drought tolerance in high phosphorus soil. Can J Bot 63:2290–2294

Calvente R, Cano C, Ferrol N, Azcon-Aguilar C, Barea JM (2004) Analysing natural diversity of arbuscular mycorrhizal fungi in olive tree (*Olea europaea* L.) plantations and assessment of the effectiveness of native fungal isolates as inoculants for commercial cultivars of olive plantlets. Appl Soil Ecol 26:11–19

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. FEMS Microbiol Ecol 36:203–209

Davies FT, Potter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P-concentration-response in gas exchange and water relations. Physiol Plant 87: 45–53

Douhan GW, Petersen C, Bledsoe CS, Rizzo DM (2005) Contrasting root associated fungi of three common oak-woodland plant species based on molecular identification: host specificity or non-specific amplification? Mycorrhiza 15:365–372

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

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

Helgason T, Fitter AH, Young JPW (1999) Molecular diversity of arbuscular mycorrhizal fungi colonizing *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. Mol Ecol 8:659–666

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Jahn SAA (1988) Using *Moringa* seeds as coagulants in developing countries. J Am Water Works Assoc 80:43–50

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Makkar HPS, Becker K (1997) Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. J Agric Sci 128:311–322

Moora M, Öpik M, Sen R, Zobel M (2004) Native arbuscular mycorrhizal fungal communities differently influence the seedling performance of rare and common *Pulsatilla* species. Funct Ecol 18:554–562

Munkvold L, Kjöller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. New Phytol 164:357–364

Muthukumar T, Udaiyan K (2002) Arbuscular mycorrhizal fungal composition in semi-arid soils of Western Ghats, southern India. Curr Sci 82:624–628

Muthukumar T, Senthilkumar M, Rajangam M, Udaiyan K (2006) Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Mycorrhiza 17:11–24

Nelson CE, Safir GR (1982) Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154:407–413

Oba H, Shinozaki N, Oyaizu H, Tawaraya K, Wagatsuma T, Barraquio WL, Saito M (2004) Arbuscular mycorrhizal fungal communities associated with some pioneer plants in the lahar area of Mt. Pinaturbo, Philippines. Soil Sci Plant Nutr 50:1195–1203

Odee D (1988) Forest biotechnology research in drylands of Kenya: the development of *Moringa* species. Dryland Biodivers 2:7–8

Olson ME, Carlquist S (2001) Stem and root anatomical correlations with life form diversity, ecology, and systematics in *Moringa* (Moringaceae). Bot J Linn Soc 135:315–348

Öpik M, Moora M, Liira J, Kõljalg U, Zobel M, Sen R (2003) Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils. New Phytol 160:581–593

Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J Ecol 94:778–790

Page RDM (1996) An application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Panwar J, Vyas A (2002) AM fungi: a biological approach towards conservation of endangered plants in Thar desert, India. Curr Sci 82:576–578

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Simon LM, Lalonde TD, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonising roots. Appl Environ Microbiol 58:291–295

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, San Diego

Stahl PD, Smith WK (1984) Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithii*. Mycologia 76:261–267

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1997) Effect of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. Can J Bot 75:1582–1591

Stutz JC, Copeman R, Martin CA, Morton JB (2000) Pattern of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. Can J Bot 78:237–245

Tao L, Zhiwei Z (2005) Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China. Appl Soil Ecol 29:135–141

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position sequence gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Truog E (1930) The determination of the readily available phosphorus of soils. J Am Soc Agron 22:874–882

Turner NC, Kramer PJ (1980) Adaptation of plants to water and high temperature stress. Wiley, New York

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555–1564

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091

Weising K, Nybom H, Wolff K, Meyer W (1995) DNA fingerprinting in plants and fungi. CRC Press, Florida

Yamato M, Iwase K (2005) 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. Mycoscience 46:334–342

Yamato M, Ikeda S, Iwase K (2008) Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa Island and effect of the isolated fungi on growth of sorghum under salt-stressed conditions. Mycorrhiza 18:241–249