

Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions

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Abstract Community of arbuscular mycorrhizal (AM) fungi in a coastal vegetation on Okinawa island in Japan was examined. A sampling plot was established in a colony of *Ipomoea pes-caprae* (Convolvulaceae) on the beach in Tamagusuku, Okinawa Pref, in which eight root samples of *I. pes-caprae* and three root samples each of *Vigna marina* (Leguminosae) and *Paspalum distichum* (Poaceae) were collected. Partial 18S rDNA of AM fungi was amplified from the root samples by polymerase chain reaction (PCR) with primers NS31 and AM1. Restriction fragment length polymorphism analysis with *Hinf*I and *Rsa*I for cloned PCR products revealed that two types of *Glomus* sp., type A and type B, were dominant in the colony. Among them, the fungi of type A were especially dominant near the edge of the colony facing the sea. A phylogenetic analysis showed that the AM fungi of type B are closely related to *Glomus intraradices* and those of type A are nearly related to type B. From the sequence data, it was also found that type A was further divided into two types, type A1 and A2. One

representative strain each of the three types, type A1, A2, and B, propagated from single spore each, was examined for the growth of sorghum (*Sorghum bicolor*) at three different salinity levels, 0, 100, and 200 mM NaCl. At the non-salt-treated condition, the type B fungus was the most effective on shoot growth enhancement of the host plant, whereas at the salt-treated conditions, the type A2 fungus was the most effective. An efficient suppression of Na⁺ translocation into the shoot by the examined AM fungi was found. These results suggested that the AM fungi dominant near the sea are adapted to salt-stressed environment to alleviate the salt stress of host plants.

Keywords Arbuscular mycorrhizal fungi · *Glomus intraradices* · *Ipomoea pes-caprae* · Salinity · *Sorghum bicolor*

Introduction

Coastal herbaceous vegetation is usually consisted of low number plant species that are specifically adapted to the stressed environments such as intermittent drought and salinity, intense insolation and high temperature in summer day, instability of sand, low fertility, etc. It is known that most of these plants have symbiotic associations with arbuscular mycorrhizal (AM) fungi, which contribute to soil nutrient uptake (Smith and Read 1997) and stabilization of sand (Sutton and Sheppard 1976). Many experimental studies also showed that AM fungal association improved the drought tolerance (Nelson and Safir 1982; Busse and Ellis 1985; Davies et al. 1993; Subramanian et al. 1997) and salt tolerance (Al-Karaki 2000; Ruiz-Lozano et al. 1996) of host plants.

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It is well known that AM fungi have broad host range under laboratory conditions. However, some studies suggested that there exist some preferences between host plants and AM fungi, since the effect of symbiosis can be different depending on the combinations (Francis and Read 1995; van der Heijden et al. 1998; Helgason et al. 2002). From stressed environments, furthermore, specific strains of AM fungi tolerant to the environmental conditions were often isolated. For example, Stahl and Smith (1984) reported that *Agropyron smithii* Rydb. colonized with *Glomus microcarpum* Tul. & C. Tul collected from a desert had lower leaf resistance to water vapor loss in an arid condition than that with *G. microcarpum* collected from a more mesic site. The lower leaf resistance means increased stomatal opening. The capability in dry soil condition is recognized as a common adaptation in drought-adapted plant species to gain carbon in such condition (Turner and Kramer 1980). This is an example to show a specific AM fungal strain being more adapted to the dry condition. Therefore, it was expected that some mycorrhizal fungi, which are specifically adapted to the plants or environments, might be found in coastal vegetations.

Molecular identification methods have been often applied to identify the AM fungi in colonizing roots. The DNA primer AM1 designed by Helgason et al. (1998) has high specificity towards the 18S rDNA of AM fungi. In combination with universal primer NS31 (Simon et al. 1992), the AM1 can amplify the partial 18S rDNA (approximately 550 bp) of most AM fungi. This method has been the most applied to investigate the diversity and community of AM fungi in various vegetations (Helgason et al. 1999; Daniel et al. 2001; Kowalchuk et al. 2002; Vandenkoornhuyse et al. 2002; Husband et al. 2002; Yamato et al. 2005; Öpik et al. 2006).

The objective of this study is to examine how the stressed environment affected the community and ability of AM fungi. Community of AM fungi was investigated in coastal vegetation on Okinawa island by a molecular analysis, and AM fungi were propagated from rhizosphere soil of the coastal plants. Furthermore, effect of the isolated fungi on growth and Na content of sorghum (*Sorghum bicolor* var. *saccharatus* Koen) was examined under salt-treated conditions.

Materials and methods

Sampling and soil analyses

In the middle of December 2002, the sampling was conducted in a beach in Tamagusuku, Okinawa Pref. (N 26° 08', E 127° 47'). The mean annual temperature and the annual total precipitation recorded at the nearest meteorological station

(Itokazu Meteorological Station) are 21.0°C and 1,944.0 mm, respectively (average in 1979–2000). In the coast, plant colonies were found in patches, and *Ipomoea pes-caprae* (L.) Sweet (Convolvulaceae) was dominant in most of them. A sampling plot of 100 m² (10×10 m) was established in one plant colony being adjacent to the sea. The plot was set where one side of the plot was on the seaward edge of the plant colony. The plot was almost flat with an altitude less than 1 m. During great wave conditions, it was supposed that the plants in the plot would have seawater spray. In the plot, *I. pes-caprae* was dominantly found with scattered *Vigna marina* (Bur.) Merrill (Leguminosae) and *Paspalum distichum* L. (Poaceae). No other plant species were found in the plot. Two lines were set at the distances of 2.5 and 7.5 m from the seaside edge of the plot, and four sampling positions each for *I. pes-caprae* were set with spacing of 2.5 m on each line (Fig. 1). In total, eight samples of *I. pes-caprae* were obtained. For *V. marina* and *P. distichum*, three sampling positions each were randomly set within the plot (Fig. 1). Roots and soil (10×10×10 cm deep) were collected at each sampling position. For each sample, roots were separated from soil using a 2-mm stainless sieve. The isolated roots were washed thoroughly on a 500-μm stainless sieve with tap water and then fixed and preserved in 50% ethanol at 4°C. The soil samples were put in plastic bags and stored at 10°C for several days until processed.

Soil pH (H₂O) and amount of available phosphate (Truog-P) were analyzed for the soil samples collected with roots of *I. pes-caprae*.

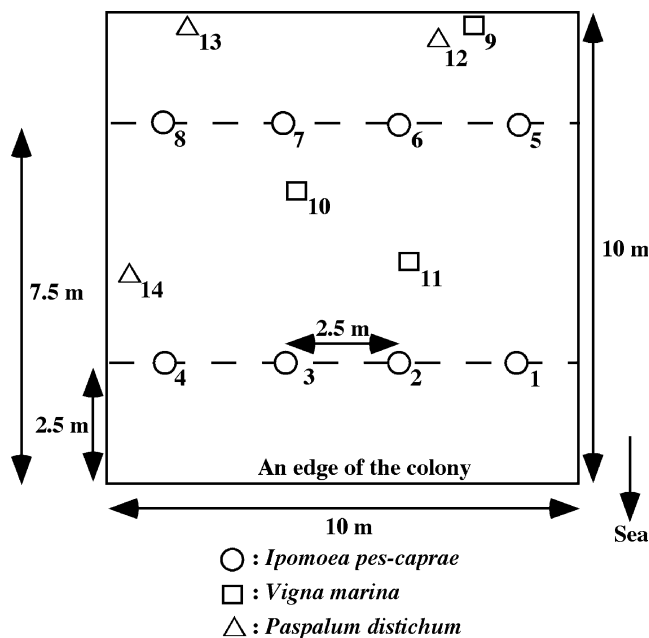


Fig. 1 The sampling plot in a plant colony at a seacoast on Okinawa island. The number in the plot shows the sampling position. The collected host plants in the sampling positions are as follows, 1–8, *I. pes-caprae*; 9–11, *V. marina*, 12–14, *P. distichum*

Molecular analysis

Fine roots, approximately 300 mg in fresh weight, were randomly collected from each root sample, and DNA was extracted using a modified cetyl trimethylammonium bromide 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 of Tris–ethylene diamine tetraacetic acid buffer. Partial fungal 18S rDNA (approximately 550 bp) was amplified by polymerase chain reactions (PCR) from the extracted DNA using TaKaRa Ex Taq™ Hot Start Version (Takara Bio, Otsu, Japan) and primers AM1 and NS31. The PCR reaction mixture contained 5 μl of the extracted DNA sol, 0.75 units of Taq polymerase, 0.25 μM of each primer, 200 μM of each deoxynucleotide triphosphate, and 3 μl of the supplied PCR buffer in 30 μl of the total amount. The PCR reaction 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 manufacture's instructions. For each sample, 12 putative cloned products (white colony) were randomly selected, and then PCR was performed for each of the cloned products using the same primer set, NS31 and AM1. The PCR products were digested independently with *HinfI* and *RsaI* (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 examples of each class using MagExtractor-Plasmid (TOYOBO) according to the manufacture's instructions, and DNA inserts were sequenced using DYEnamic ET dye terminator kit (Amersham Biosciences, Piscataway, NJ, USA) with sequencing primers, M13-47 primer and RV-M primer. For each sequence data, analogous data were obtained from DNA Databank of Japan (DDBJ)–European Molecular Biology Laboratory (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). For the NJ analysis, evolutionary distances were estimated using Kimura's two-parameter model (Kimura 1980), and bootstrap analysis (Felsenstein 1985) was performed with 1,000 replications. The phylogenetic tree was drawn using Treeview (Page 1996).

Propagation of AM fungi

The eight soil samples collected from rhizosphere of *I. pes-caprae* were put together and mixed. The akadama soil (a

granulated volcanic soil with high phosphate absorbing ability), autoclaved at 121°C for 30 min, was mixed with this soil sample mixture at 2:1 (v:v) ratio, and 500 ml of the soil mixture was put into a pot Pao 3.5 (Marumankagaku, Osaka, Japan). At the middle of December 2002, seeds of alfalfa (*Medicago sativa* L.) were sown, and the pot was placed in a greenhouse. Peters soluble fertilizer (25–5–20: N–P–K; WR Grace & Co., Fogelsville, PA, USA) was supplied every 2 weeks at a final N concentration of 100 mg l^{-1} until the fertilizer solution flowed out from the drain holes. After 4 months, propagated spores of AM fungi were isolated by wet sieving method (Gerdemann and Nicolson 1963).

Single-spored pot cultures of AM fungi

Single-spored pot cultures were initiated from the propagated spores using alfalfa as host plants by the method of Brundrett and Juniper (1995) as follows. A germinating spore on nitrocellulose membrane filter square, 5×5 mm, was attached at the root tip of alfalfa seedling, and the seedling was planted into a pot Pao 2.5 (Marumankagaku) containing 120 ml of a soil medium, autoclaved mixture of akadama soil and river sand (1:1, v:v) with pH (H_2O) of 5.3. The pot was placed in a greenhouse, and 50 ml of Peters soluble fertilizer (25–5–20), at a final N concentration of 100 mg l^{-1} , was supplied every 2 weeks. After 3 to 5 months, propagated spores were isolated from the pot cultures by wet sieving method.

Molecular analysis of the single-spored isolates of AM fungi

DNA was extracted from each of the propagated AM fungi using an InstaGene™ DNA Purification Matrix (Bio-Rad, Hercules, CA, USA) as follows. About 20 spores were crushed in 2 μl of supernatant of InstaGene™ Matrix using Pestle & Tubes (Bel-Art Products, Pequannock, NJ, USA). To the suspension of the crushed spores, 50 μl of InstaGene™ Matrix was added, and the mixture was successively heated at 56°C for 30 min and at 100°C for 8 min with a short vortex between the heating processes. After centrifugation at 8,000 rpm for 3 min, the supernatant containing AM fungal DNA was collected. Towards the isolated DNA, the molecular analysis, same as stated above, was applied.

AM fungal inoculums

The single-spored isolates of AM fungi, *Glomus* sp. OGS1, OGS8, and OGS12, were randomly selected from each of the dominantly detected RFLP types. They were propagated with alfalfa as a host plant in pot culture using a pot Pao 3.5

containing 500 ml of autoclaved mixture of akadama soil and river sand (1:1, v:v) for 3 months. During the pot culture, Peters soluble fertilizer (25–5–20), at a final N concentration of 100 mg l⁻¹, was supplied every 2 weeks until the fertilizer solution flowed out from the drain holes. At the same time, nonmycorrhizal pot culture was also prepared under the same conditions. After the cultivation, air-dried and sieved (2 mm) soils were used as inoculums of AM fungi.

Effect of fungal inoculation on plant growth under salinity conditions

As a host plant, sorghum was selected because effects of salinity have been examined for this species (Netondo et al. 2004). This experiment was conducted from late July for 8 weeks in a greenhouse at a temperature of 20–35°C. Into a plastic pot, Pao 3.5 (Marumankagaku), 500 ml of a soil medium, and autoclaved mixture of akadama soil and river sand (1:1, v:v) was filled. The pH (H₂O) of the applied soil medium was 5.3. On the soil medium, a hole of 1.8 cm in diameter and 6 cm in depth was formed by inserting a glass tube and 10 ml of the AM fungal inoculum, the air-dried and sieved pot culture soil, was filled into the hole. For the uninoculated control, an equivalent volume of pot culture soil without AM fungal inoculation was applied to provide a similar microflora. Seeds of sorghum (Takii, Kyoto, Japan) were surface-sterilized with successive immersion into 70% ethanol for 5 min and a sodium hypochlorite solution containing 1% available chlorine for 5 min, and five seeds each were sown in a pot. After the seed sowing, an excess amount of nutrient solution, Peters soluble fertilizer (25–5–20) at a final N concentration of 100 mg l⁻¹, was supplied until the nutrient solution flowed out from the drain holes. After 2 weeks from the seed sowing, seedlings were thinned to one per pot. At this step, the nutrient solution with three concentrations of NaCl, 0, 100, and 200 mM, was supplied until the fertilizer solution flowed out from the drain holes to induce saline-stressed conditions. Plants were watered with tap water as to avoid leaching, and the nutrient solutions with three levels of NaCl were supplied every 2 weeks. Before the nutrient supply, the pots were leached with 1,000 ml of tap water to avoid accumulation of salt and nutrients. The experiment consisted of a randomized block design with two factors, four mycorrhizal inoculations (uninoculated, OGS1, OGA8, and OGS12), and three levels of salt application (0, 100, and 200 mM NaCl). Each treatment consisted of six replicates. The experiment was terminated at 56 days after the seed sowing, and shoots and roots were harvested separately.

The dry weights of the shoots and roots were measured after drying at 70°C for 48 h. The plant material was

ground and digested with concentrated H₂SO₄ and H₂O₂ at a ratio of 5:2 (v/v) at around 180°C. Na concentration in shoot was measured by atomic absorption flame emission spectrophotometer AA-6500F (Shimadzu, Kyoto, Japan).

A subsample of about 0.5 g (fresh weight) of root segments were randomly collected and cut into 1-cm-long pieces. After staining the roots by 0.1% trypan blue in lactoglycerol, AM fungal colonization rate was measured by the grid-line-intersect method (Giovanetti and Mosse 1980).

For shoot dry weight, root dry weight, AM colonization rate, and Na concentration in shoot, analysis of variance was performed on the datasets and means were compared by Tukey's test at the 5% level.

Results

Soil analysis

The soil was alkaline with pH 9.23±0.064 (SD) because it consists of lime sand. The mean available phosphate (Truog-P) was 7.81±0.62 mg kg⁻¹ (SD) indicating the low fertile environment.

RFLP analysis

PCR products of approximately 550 bp were obtained from 13 root samples except for one sample (No. 7) of *I. pes-caprae*. After cloning of the PCR products, at most 12 clones were obtained for each PCR products, which were divided by the RFLP analysis. In total, 142 clones comprised of five RFLP types (type A–E) were obtained (Tables 1 and 2). From the sequence data, it was revealed that type A was further divided into two types, type A1 and A2 (Table 1). The type A fungi were dominantly detected in *I. pes-caprae* and *V. marina*, whereas the type B fungi were dominant in *P. distichum*. In *I. pes-caprae*, dominance of the type A fungi was extremely high in six samples (sample nos. 1–6). Among them, four samples (nos. 1–4) were obtained close to the seaward edge of the plant colony (Table 2). The three other types, type C, D, and E, were also detected in this study, but the frequencies were very low.

Phylogenetic analysis

Some representatives for each RFLP type, 15 for type A, 13 for type B, one each for type C, D, and E, were sequenced, and a NJ phylogenetic tree was obtained from the sequenced data with some data of AM fungi in DDBJ–EMBL–GenBank nucleotide sequence database (Fig. 2). All of the sequence data form a monophyletic clade in each

Table 1 Representative fragment sizes (base pairs) in each restriction fragment length polymorphism type of 18S rDNA PCR products (NS31–AM1) of arbuscular mycorrhizal fungi obtained from coastal plants

RFLP type	Restriction enzyme						Total length
	<i>Hinf</i> I			<i>Rsa</i> I			
A (A1)	142 ^a	49	334	24	129	420	549
A (A2)	142	49	334	24	125	4	420
B	142	383	24		129	420	549
C	191	334	24		125	4	420
D	280	244	24		128	420	548
E	194	334	24		130	422	552

^a The order of the fragments in each enzyme reflects the position of the restriction site in the PCR products

of type A and B, although type A was divided into two subclade, type A1 and A2 (Fig. 2, Table 1). The phylogenetic analysis showed that the type B fungi are closely related to *Glomus intraradices* Schenck Smith, and the type A is also nearly related to the type B.

Propagation and molecular analysis of AM fungi

Whitish spores with diameter of 80–100 µm were propagated from fine roots of *I. pes-caprae* using alfalfa as host plants. In total, 20 single-spored pot cultures were established, which were divided into five cultures of type A and 15 cultures of type B by the RFLP analysis with *Hinf*I and *Rsa*I. For each type, sequences of the partial 18S rDNA were obtained from four cultures each, and the phylogenetic analysis showed that the single-spored isolates form monophyletic clade with those from field root samples in each of the type A and B (Fig. 2). The four cultures in type A was divided into three cultures of type A1 and one culture of type A2 (Fig. 2). For the sequenced single-spored isolates, similarity score among the type A and among the type B each were 99.01–100% and 99.21–100%, respectively. Meanwhile, the similarity score between the two types was lower a little, 97.60–98.21%. This also indicated the validity of the distinction between type A and type B.

Effect of fungal inoculation on plant growth under salinity conditions

Three AM fungal cultures propagated from single spore each were selected as representatives of each RFLP types, OGS1 (type B), OGS8 (type A1), and OGS12 (type A2), and effect on the growth of sorghum was examined under the salt-treated conditions.

Under a non-salt-treated condition, OGS1 showed the highest promoting effect on shoot growth, but this promoting effect was decreased as the salt concentration was increased. Meanwhile, OGS12 was most effective to induce salt tolerance under the salt-treated conditions (Fig. 3).

A similar tendency was also found in root dry weight, though significant difference was not detected (Fig. 3). AM colonization rate was maintained around 80% even under the high salt condition with 200 mM NaCl (Fig. 3). Shoot Na concentrations were decreased by the inoculation of all examined AM fungi under the salt-treated conditions (Table 3), which indicates that the AM fungi suppressed the translocation of Na to the shoot.

Discussion

I. pes-caprae, the dominant plant species in the examined coastal vegetation, is one of the typical coastal herbaceous plant species distributed in tropical to subtropical region (Kitamura et al. 1992). A diverse AM fungal spore, 41 species in six genera, was found in rhizosphere soil of *I. pes-caprae* in an extensive study in coastal sand dunes, Southern India (Beena et al. 2000), which indicates that this

Table 2 Number of clones of each RFLP type obtained from root samples of *I. pes-caprae*, *V. marina* and *P. distichum*

Plant species	Sample No.	RFLP type				
		A	B	C	D	E
<i>Ipomoea pes-caprae</i>	1	9	1	1	0	0
	2	11	0	0	0	0
	3	8	0	0	0	0
	4	10	0	1	0	0
	5	11	1	0	0	0
	6	9	2	0	0	0
	8	4	7	0	1	0
	<i>Vigna marina</i>	9	4	7	0	0
10		10	0	0	0	0
11		12	0	0	0	0
<i>Paspalum distichum</i>	12	5	6	0	0	0
	13	0	11	0	0	0
	14	5	5	0	0	0
Total		98	40	2	1	1

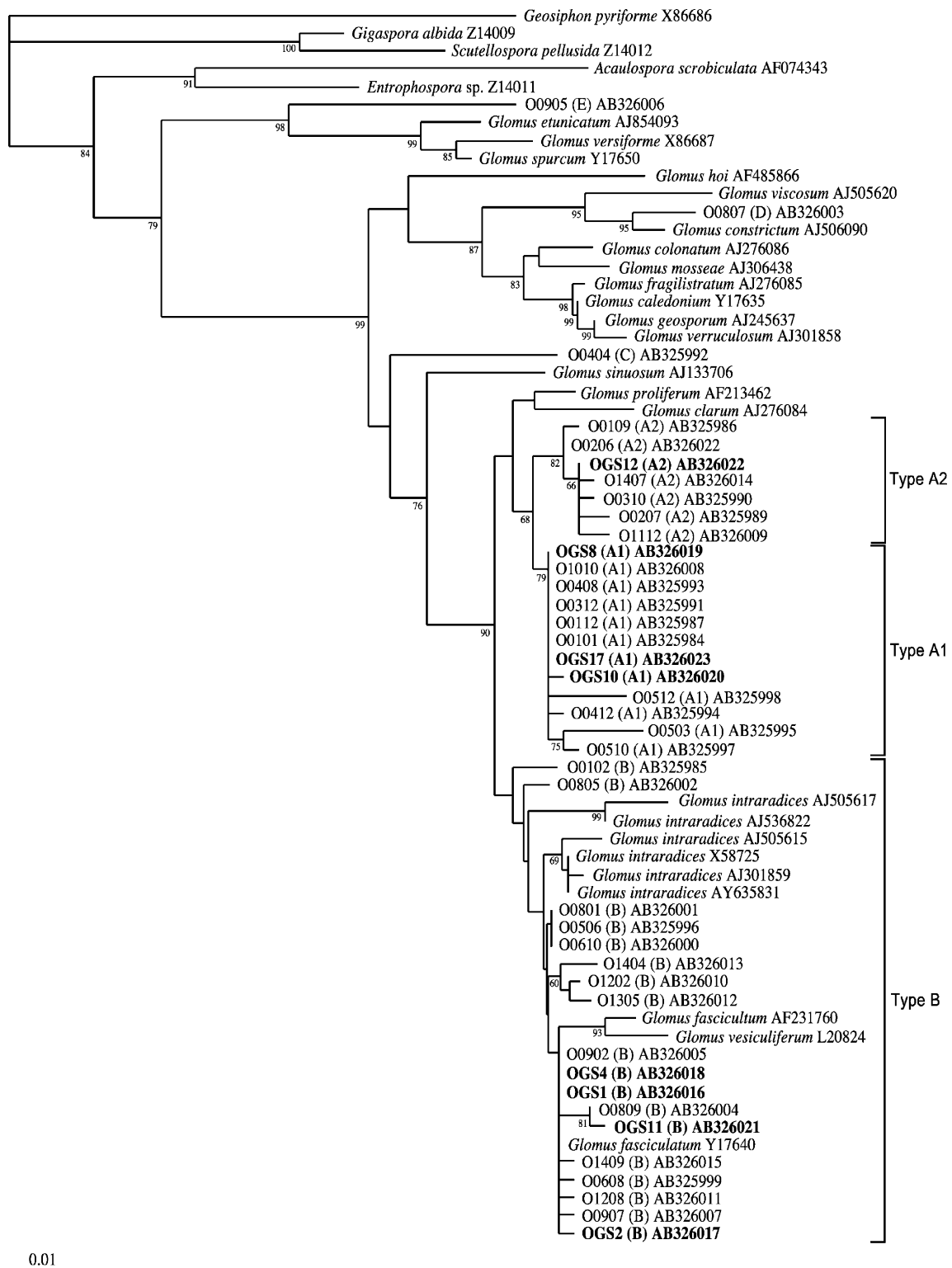
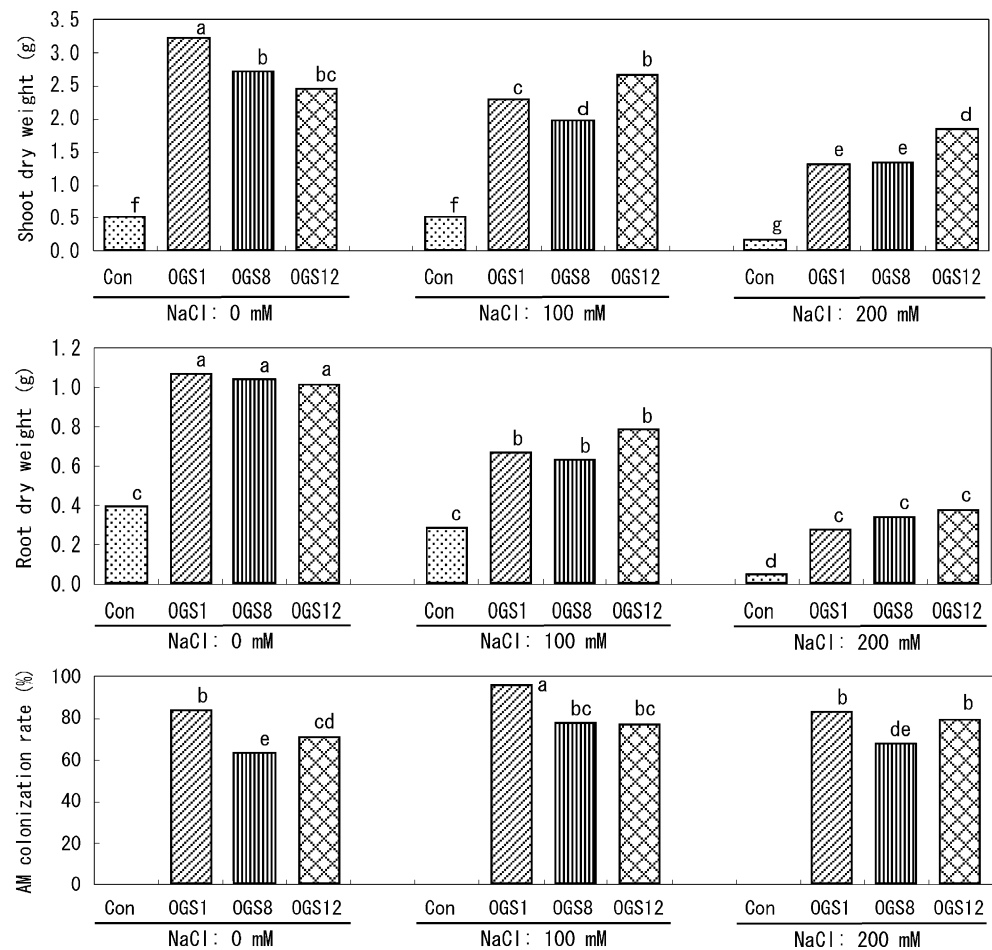


Fig. 2 A neighbor-joining phylogenetic tree based on NS31-AM1 18S rDNA sequences of AM fungi obtained from coastal vegetation on Okinawa island and single-spored cultures originated from the vegetation. The tree is rooted to *Geosiphon pyriforme* (X86686). Bootstrap values are shown where they exceed 60% (1,000 replicates). Group identity (type A–E) related to the RFLP types in Table 1.

Clones obtained from the vegetation were numbered according to the sampling position and clone number. Clones from single-spored cultures were numbered by OGS and pot number, which are shown in bold letters. All sequences obtained in this study have been submitted to DDBJ database (accession numbers AB325984–AB326023)

Fig. 3 Influence of inoculations of arbuscular mycorrhizal (AM) fungi, noninoculated control, OGS1 (type B), OGS8 (type A1), and OGS12 (type A2) and salt applications, 0, 100, and 200 mM of NaCl, on growth of sorghum (*S. bicolor*) and colonization rate of AM fungi. Means with the same letter are not significantly different at $P < 0.05$ (Tukey's test)



plant has high potential to form AM with diverse AM fungal species. Meanwhile, the AM fungi classified into two types, type A and B, in the RFLP analysis were dominantly found in the all examined plants, *I. pes-caprae*, *V. marina*, and *P. distichum* in this study. The phylogenetic analysis revealed that the dominant fungi in type A and B are genetically nearly related each other, and they are identified to be closely or nearly related with *G. intraradices*. This AM fungus is often detected in arid or semiarid environments (Stutz 2000; Muthukumar and Udaiyan 2002; Tao and Zhiwei 2005). Because more diverse AM fungi were detected by the same primer sets, NS31 and AM1, in other studies (Helgason et al. 2002; Husband et al. 2002; Öpik et al. 2003), it was considered that the diversity of AM fungi was low in the examined samples. The low diversity would be caused by the stressed environment of the examined coastal vegetation with intermittent drought and salinity, instability of sand, low fertility, etc. For the drought and salinity, they are much variable because of low water holding capacity of sandy soil. Especially, the salinity would be changeable depending on weather conditions, i.e., high level at having spray of seawater (approximately 500 mM of NaCl) by great waves and lower at having much rainfall.

Almost all AM fungi detected in the roots of *I. pes-caprae* located near the edge of the colony facing the sea was in type A, which indicates that the type A fungi are more adapted to the stressed environment.

The spores of AM fungi propagated from the rhizosphere soil of *I. pes-caprae* were also identified to be in type A or B by the same molecular analysis, which suggested the validity of the molecular analysis to show the dominance of these AM fungi.

Table 3 Influence of inoculations of arbuscular mycorrhizal fungi and salt application on Na concentrations (millimole per kilogram) in shoot of sorghum

AM fungi	Applied NaCl	
	100 mM	200 mM
Con	76.3a	1,133.8a
OGS1	12.9b	163.7b
OGS8	11.2b	83.2b
OGS12	12.9b	84.1b

Mean values in a column followed by the same letter are not significantly different ($p < 0.05$) by Turkey's test

One representative strain in each RFLP type, OGS1 (type B), OGS8 (type A1), and OGS12 (type A2), was examined for the effect on growth of sorghum under the salt-treated conditions. Although many studies reported the decrease of AM colonization under salt-stressed conditions (Poss et al. 1985; Pfeiffer and Bloss 1988; Al-Karaki et al. 2000; Tian et al. 2004), the colonization rates were not decreased even in the high-salinity treatment with 200 mM NaCl in the all examined AM fungi. The effect of OGS1 (type B) on growth of sorghum, which showed the highest growth promotion in the nonsaline treatment, was decreased with increasing salinity. Meanwhile, the growth suppressions were smaller in the type A strains, OGS8 and OGS12. Consequently, the effect of OGS12 (type A) was higher than that of OGS1 (type B) in the salinity treatments.

There was considerable effects found on suppression of Na translocation into the shoot by the examined AM fungi in this study. The ratio of shoot Na concentration with OGS8 (type A) to that of non-AM fungal control was only 14.7% in the medium (100 mM NaCl) and 7.3% in the high-salinity (200 mM NaCl) treatments. The ratios were much lower than those in the other studies that also showed lower shoot Na concentration by AM fungi (Al-Karaki 2000; Giri and Mukerji 2004; Tian et al. 2004). Meanwhile, a similar result to this study was reported by Hatimi (1999). He examined effect of AM fungi isolated from coastal dune on growth and nutrition of *Acacia cyanophylla* Lind. under increasing levels of salinity, in which the ratio of shoot Na concentration with the AM fungi to that without the AM fungi was only 6.1% in a salinity treatment with 1,000 mM NaCl.

In this study, considering the characteristics of the AM fungi with the distribution in the examined vegetation, it was suggested that the AM fungi dominant near the sea were adapted to the environment to alleviate the salt stress of host plants.

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