Two complementary forest-originated *Gigaspora* spp. shifted shoot-to-root ratio for growth improvement in *Cryptomeria japonica* seedlings

Akotchiffor Kevin Geoffroy DJOTAN [*1,3], Yosuke MATSUDA [1,4], Norihisa MATSUSHITA [*2,5], Kenji FUKUDA [2,6]

[1]Mie University, Graduate School of Bioresources, [2]University of Tokyo, Graduate School of Agricultural and Life Sciences

ORCID: [3]https://orcid.org/0000-0002-3726-9826, [4]https://orcid.org/0000-0002-7001-3101, [5]https://orcid.org/0000-0003-3281-8846, [6]https://orcid.org/0000-0002-9980-3107

[*]*Corresponding author, E-mail: geoffroydjotan@yahoo.fr, nmatsushita@g.ecc.u-tokyo.ac.jp*

Supplementary Material and Methods (SMM)

SMM1

Inocula of two *Gigaspora* isolates (DGJPUTLFSSH-4, MAFF 520098; DGJPUTLFSSH-A1, MAFF 520099; LFB-4 and LFB-A1 hereafter) were used in this study. They were propagated using clover (*Trifolium repens* L.) and sorghum (*Sorghum bicolor* (L.) Moench). Based on 385-bp fragments of partial small subunit (SSU) of nuclear ribosomal DNA (rDNA), LFB-4 (accessed PV745562 in GenBank) and LFB-A1 (accessed PV745555 in GenBank) matched with *Gigaspora rosea* T.H. Nicolson & N.C. Schenck and *Gigaspora margarita* W.N. Becker & I.R. Hall, respectively (Djotan 2024). Therefore, we obtained the inocula of the two species, *G. rosea* (MAFF 520062, Strain C1, Pot Ref No. 22-04) and *G. margarita* (MAFF 520052, Strain K-1, Pot Ref No. 22-12), from the National Agricultural Research Organization (NARO) in Japan and used them as references. Inocula of the non-*Gigaspora* AMF taxa F-1 (*Acaulospora longula* Spain & N.C. Schenck, MAFF 520060, Pot Ref No. 22-21), TSU-2 (*Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler, MAFF 520089, Pot Ref No. 22-42), and YC-1 (*Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker, MAFF 520091, Pot Ref No. 22-45) were also obtained from NARO and used in inoculation assays. All inocula consisted of spores, root fragments, and soil passed through 1 mm-mesh sieve. AMF in the inocula provided by NARO originated from agricultural fields, but LFB-4 and LFB-A1 originated from surrounding soils of the *C. japonica* trees E48 and V70 (permanent tree labels at the University of Tokyo Tanashi Forest) collected on December 1st, 2022 (Djotan, 2024). The author propagated LFB-4 and LFB-A1 using *T. repens*. Spore production in LFB-4 and LFB-A1 as assessed from the inocula, was 60 and 30 spores per g of air-dried cultivation soil, respectively. To ensure spore availability for various analyses throughout the study, we established propagation assays using surface sterilized seeds of *T. repens* and *S. bicolor* purchased from Sakata Seed Corporation (Kanagawa, Japan).

SMM2

We performed wet sieving of soil inocula using a slightly modified method of Brundrett et al. (1996) to pick up healthy fresh spores of the isolates LFB-4 and LFB-A1. Spores were placed in a 1.5-mL tube and crushed with a pipette tip containing 100 µL of AE buffer. After a vortex for 10 s, we heated the samples at 98°C for 10 min and cooled them down to room temperature in a Takara Thermal Cycler Dice (Model TP600; Takara, Shiga, Japan). Next, we centrifuged mixtures in a microcentrifuge at 12,000 RPM for five min and transferred 50 µL of the supernatant into new microcentrifuge tubes. The supernatants were 10-fold diluted and used as DNA template for polymerase chain reactions (PCR) performed with a Thermal Cycler Dice (Model TP600). Using the primer set SSUmCf and LSUmBr (Krüger et al., 2009), we amplified *c.* 1400 bp fragments spanning SSU rDNA, the complete ITS region, the complete 5.8S rDNA, and a partial large subunit (LSU) rDNA. PCR mixtures were 10-µL each, containing 2 µL of DNA template, 0.5 µM of each primer, and 5 µL of KOD One PCR Master Mix (Toyobo, Osaka, Japan). Thermal cycling conditions consisted of an initial denaturation at 98°C for 2 min, 30 cycles of denaturation at 98°C for 10 s, annealing at 63°C for 5 s, and extension at 68°C for 10 s followed by a final extension at 68°C for 2 min. We cloned the PCR products using a TA-Enhancer cloning kit (Nippon Gene, Tokyo, Japan) following the manufacturer's instructions. Positive clones were further amplified using KOD One PCR Master Mix (Toyobo) and the primer set U19 (GGT TTT CCC AGT CAC GAC G) / M13R (GCG GAT AAC AAT TTC ACA CAG G) as previously described except that the annealing temperature was set to 58°C. We purified positive clone amplicons with a PCR product cleanup reagent (Exo-SAP IT; Thermo Fisher Scientific, MA, USA) and ran sequencing reactions using the primers M13F (GTA AAA CGA CGG CCA GT, forward) and M13R-pUC (CAG GAA ACA GCT ATG AC, reverse) to label amplicons with a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, CA, USA). Sequencing reaction mixtures were 10-µL each, containing 4.05 µL of PCR-grade water, 1.75 µL of 5×BigDye Buffer, 0.5 µL of BigDye sequencing premix, and 0.25 µM primer (forward or reverse). Cycling settings consisted of 30 cycles of denaturation at 95°C for 10 sec, annealing at 50°C for 5 s, and extension at 60°C for 4 min before cooling down to 4°C. Labelled amplicons were purified with a BigDye Xterminator Purification kit (Applied Biosystems) and sent to Mie University Center for Molecular Biology and Genetics for Sanger sequencing.

Amplicon sequences were visualized in BioEdit version 7.7.1, and only sequences without ambiguity were assembled with a contig assembly program based on a sensitive detection of fragment overlaps within the software (Huang, 1992). The assembled sequences were BLASTed against the National Center for Biotechnology Information (NCBI) database to download similar nucleotide sequences from only type materials for phylogenetic analyses. The assembled sequences were aligned using MAFFT v7.490 (Katoh & Standley, 2013), their phylogenetic positions were inferred using an automatic model finder in IQ-TREE 2 (Minh et al., 2020), and the tree was visualized with FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

To complement molecular identification with morphological features, we further observed and described the morphological features of spores, such as color, shape, size, subtending hyphae, number of spore walls and wall layers, and spore wall thickness, following Błaszkowski (2012). We picked up at least 50 healthy fresh spores of each isolate. Because spore description of the expected species was known to be > 100 µm in diameter, we used a 100-µm mesh size sieve during wet sieving. Purified spore suspension in water was observed under a stereoscopic microscope (SZX16; Olympus, Tokyo, Japan) equipped with a camera (Digital Sight 2000; Nikon, Tokyo, Japan). Spore size measurements were performed on stereoscopic microscope photographs using image analysis software Preview Version 11.0 (1069.5.2). After measurements and observation of further morphological features such as color (Kornerup & Wanscher, 1983), shape, and subtending hyphae, a subset of the spores was mounted on glass slides with covers in lactic acid and the other in a mixture of polyvinyl alcohol lactoglycerol (Omar et al., 1979) and Melzer's reagent (1:1, v/v). The mounted spores were analyzed for their subcellular structures such as number of spore walls and wall layers under a light microscope (BX53, Olympus) equipped with differential interference contrast optics, fitted with a camera (DP74, Olympus). These morphological analyses for species identification (Bentivenga & Morton, 1995), were also carried out on the reference isolates obtained from NARO.

SMM3

Seeds of *C. japonica* (cultivar Ichishi) were obtained from Mie Prefecture Forestry Research Institute (Mie, Japan). Seeds were washed under running tap water for four hours, surface sterilized by soaking into 30% hydrogen peroxide (H_2O_2) for five min twice, incubated on 0.5% GGM in the dark, and

checked every day to remove seed with endophytic fungi that grew out as contaminant. Seeds that germinated without fungal contamination were transferred to autoclaved Akadama soil inside 45-mL Falcon tubes and grown on a plant cultivation rack (CR-620; Tomy, Tokyo, Japan) with a light cycle controller set to 12 hours per day (8:00 - 22:00, light intensity of 65 µmol m^{-2} s^{-1}) in a room set to 20°C. We inoculated the seedlings with LFB-4 and LFB-A1 in a liquid form. Autoclaved ultrapure water was added to the same amount of soil inoculum of each isolate (100 g) and then homogenized by mixing and pipetting with Pasteur pipette, which we used to apply inocula to *C. japonica* seedlings growing each in a Falcon tube after the plant height of the shortest seedlings had reached 2 cm or higher. In this experiment, we prepared five biological replicates and four treatments including the control (no application of AMF). In each of the four groups, seedlings received 10 mL of the mixture containing (1) LFB-4, (2) LFB-A1, (3) 5 mL of LFB-4 and 5 mL of LFB-A1, or (4) 10 mL of water (control). Seedlings were transplanted from Falcon tubes to square plant culture vials 45 days after inoculation and transferred to a growth chamber (KL 1-A2PZ; NK System, Osaka, Japan) set to 25°C with a light cycle controller set to 12 hours of light per day (8:00 - 22:00, light intensity of 65 µmol m^{-2} s^{-1}). The plant culture vials were composed of a semi-transparent container for the growth medium (SPL Incu Tissue, 72×72×100 mm), a transparent container as a cover fitted with air filter disks, and a frame connecting and sealing the containers (Product #: SPL-310070, SPL-310072-40, and SPL-310074; SPL Life Sciences, Pocheon, South Korea). We added a sterilized v/v mixture of Akadama soil and river sand to complement the growth medium in the new vials during transplantation after what the plants were grown for 159 days. To monitor the growth of the seedlings following inoculation, plant height measurements started 45 days after transplantation and were done every three to four weeks until harvests. The seedlings were fertilized with 500-fold dilution of a liquid fertilizer for plant cultivation (Rikidus; Hyponex, Osaka, Japan) twice throughout the cultivation period. After harvest, we amplified *c.* 300 bp of SSU rDNA using primers AMV4.5NF/AMDGR (Sato et al., 2005) to confirm root colonization of LFB-4 and LFB-A1 in *C. japonica* inoculation assays. Reaction mixtures and amplification settings were as described in SMM2 except that annealing was set to 58°C for 5 s.

To check the contribution of AMF isolated from forest ecosystems to crop plants in agricultural systems, we carried out inoculation assays on carrot plants (*Daucus carota* L.) whose seeds were purchased from Sakata Seed Corporation and surface sterilized as previously described. Then, we sowed

113 the seeds together with AMF inocula following the four treatments described above. The growth medium
114 was a sterilized v/v mixture of Akadama soil and river sand in plant culture vials described above. Each
115 treatment was replicated 10 times, and each vial contained 10 carrot plants. They were grown for 140 days
116 (time between seed sowing and plant harvest), and 500-fold dilution of a liquid plant fertilizer (Rikidus) was
117 applied twice throughout the cultivation period.

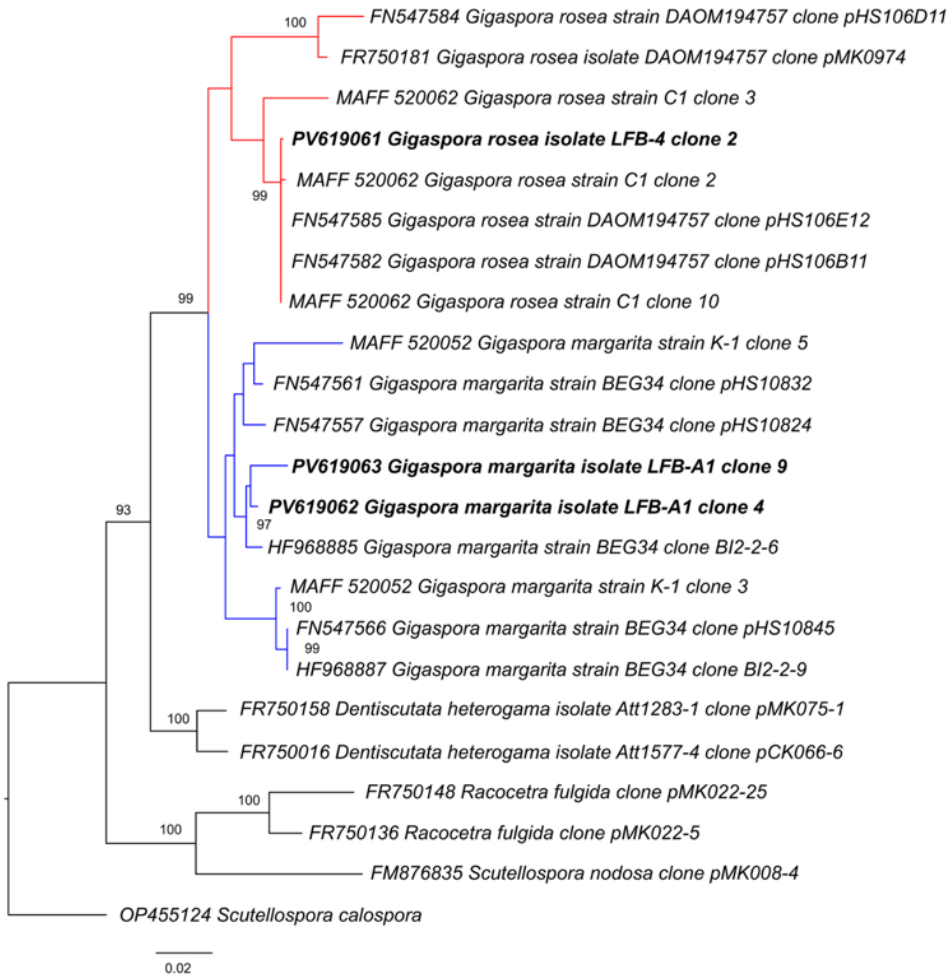
Supplementary Results and Discussions

Identification and description of LFB-4 and LFB-A1

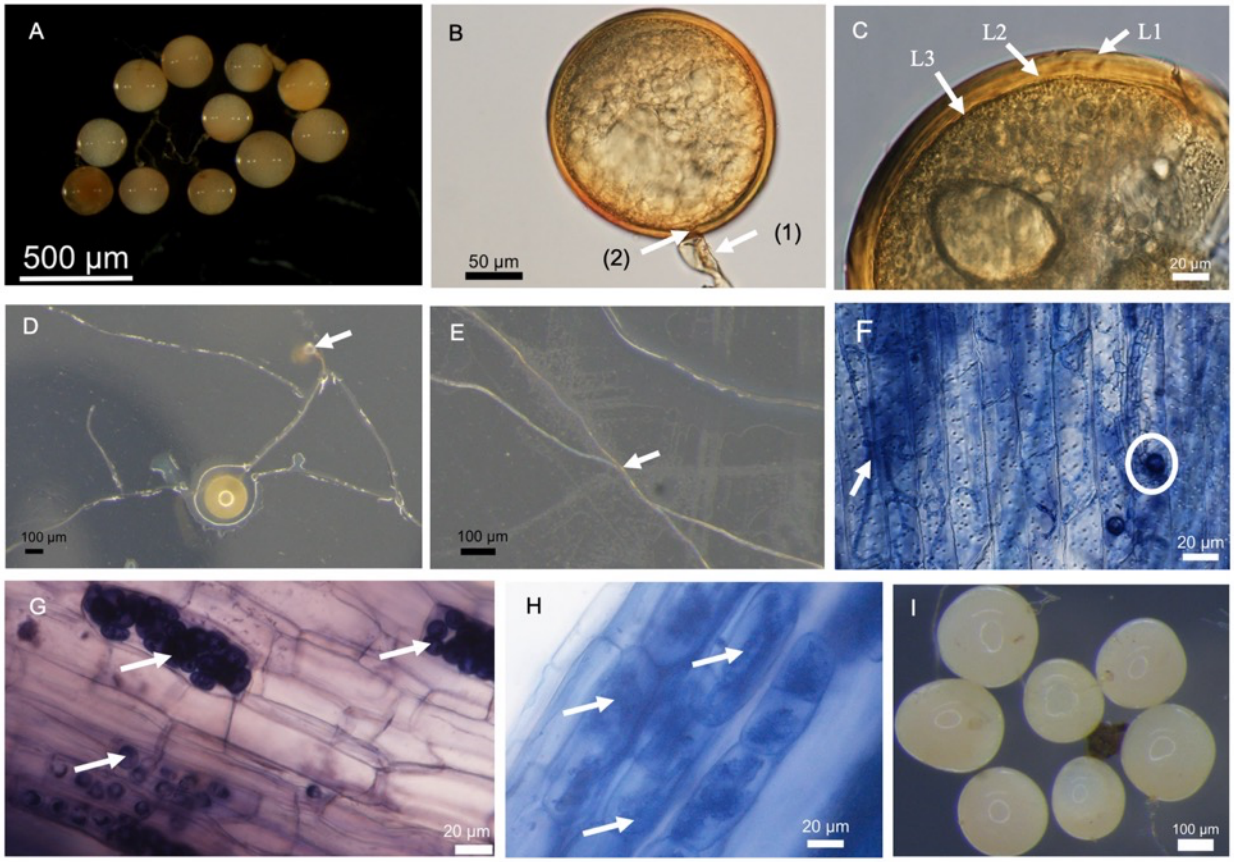
DNA sequences of LFB-4 (GenBank accession PV619061) and LFB-A1 (GenBank accessions PV619062 and PV619063) clustered together with those of the reference fungal strains MAFF 520062 (GenBank accessions PV619064, PV619065, and PV619066) and MAFF 520052 (GenBank accessions PV619067 and PV619068), respectively (Online Resource 1). In both LFB-4 and LFB-A1, globose to subglobose spores were produced singly and blastically at the top of a bulbous sporogenous cell in the soil, inside the plant cells, and on the root surface. Spores had a single wall with three layers (L1, L2, and L3). In LFB-4, spores were yellowish white (4A2) to pinkish white (7A2), and their size was (174–) 235 (-274) µm (Online Resource 2). In LFB-A1, spores were yellowish white (4A2) to sunflower yellow (4A7), and their size was (333–) 424 (-500) µm in diameter (Online Resource 3). Based on the above molecular and morphological data, we identified isolates LFB-4 and LFB-A1 as *G. rosea* and *G. margarita*, respectively.

The SSUmCf-LSUmBr primer pair which amplifies *c.* 1400 bp fragments spanning SSU rDNA, complete ITS region, complete 5.8S rDNA, and LSU rDNA was previously validated for AMF species resolving power (Krüger et al., 2009). Obtaining reference strains from a gene bank (NARO), sequencing them alongside the to-be-identified AMF isolates, and adding only type material sequences available in NCBI for phylogenetic analysis is a non-ambiguous identification strategy that we recommend. Because the genus *Gigaspora* has five species with low morphological variation is low (Bago et al., 1998; Bentivenga & Morton, 1995; Lanfranco et al., 2001; Souza et al., 2004), only morphological identification can be ambiguous and unacceptable in this era of advanced molecular tools, whereas only molecular identification only is unacceptable. For both isolates, the shape, size, color, sporogenous cells, and spore wall layers were as previously described (Bentivenga & Morton, 1995; Błaszkowski, 2012) and INVAM (https://invam.ku.edu accessed 06/15/2025). However, we observed larger *G. margarita* spores (~500 µm in diameter) than previously reported.

Online Resources

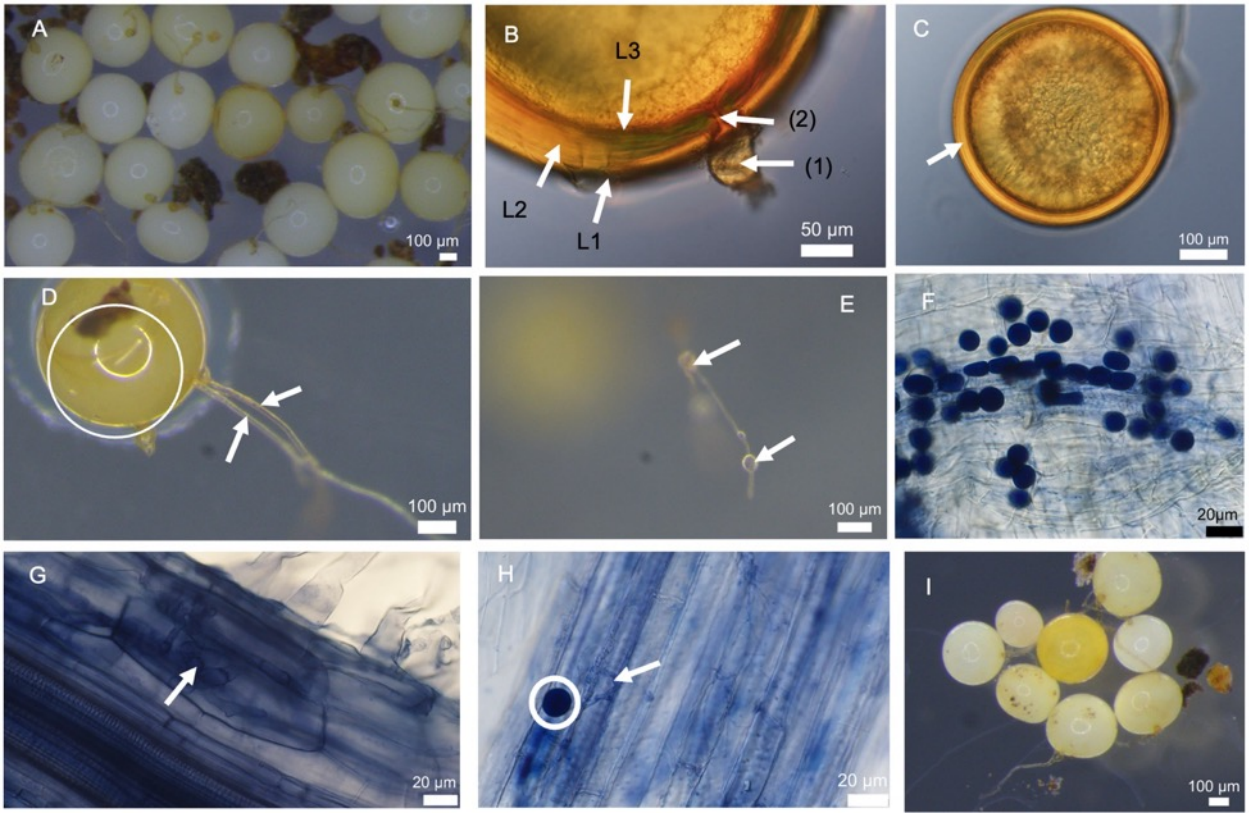


Online Resource **1** Phylogenetic placement of arbuscular mycorrhizal fungal isolates LFB-4 and LFB-A1 along with reference strains of the same species based on *c.* 1400 bp SSUmCf-LSUmBr fragment sequences. Sequences from LFB-4 and LFB-A1 are in bold font. Best model and parameters were selected with automatic model finder in IQ-TREE 2. Ultrafast bootstrap (UFBoot) over 1000 randomizations were performed and bootstrap supports > 90 are shown at the nodes. The input data contained 23 sequences covering 1421 sites spanning a partial small subunit (SSU) nuclear ribosomal DNA (rDNA), the complete internal transcribed spacer (ITS) region, the complete 5.8S rDNA, and a partial large subunit (LSU) rDNA for species resolving power



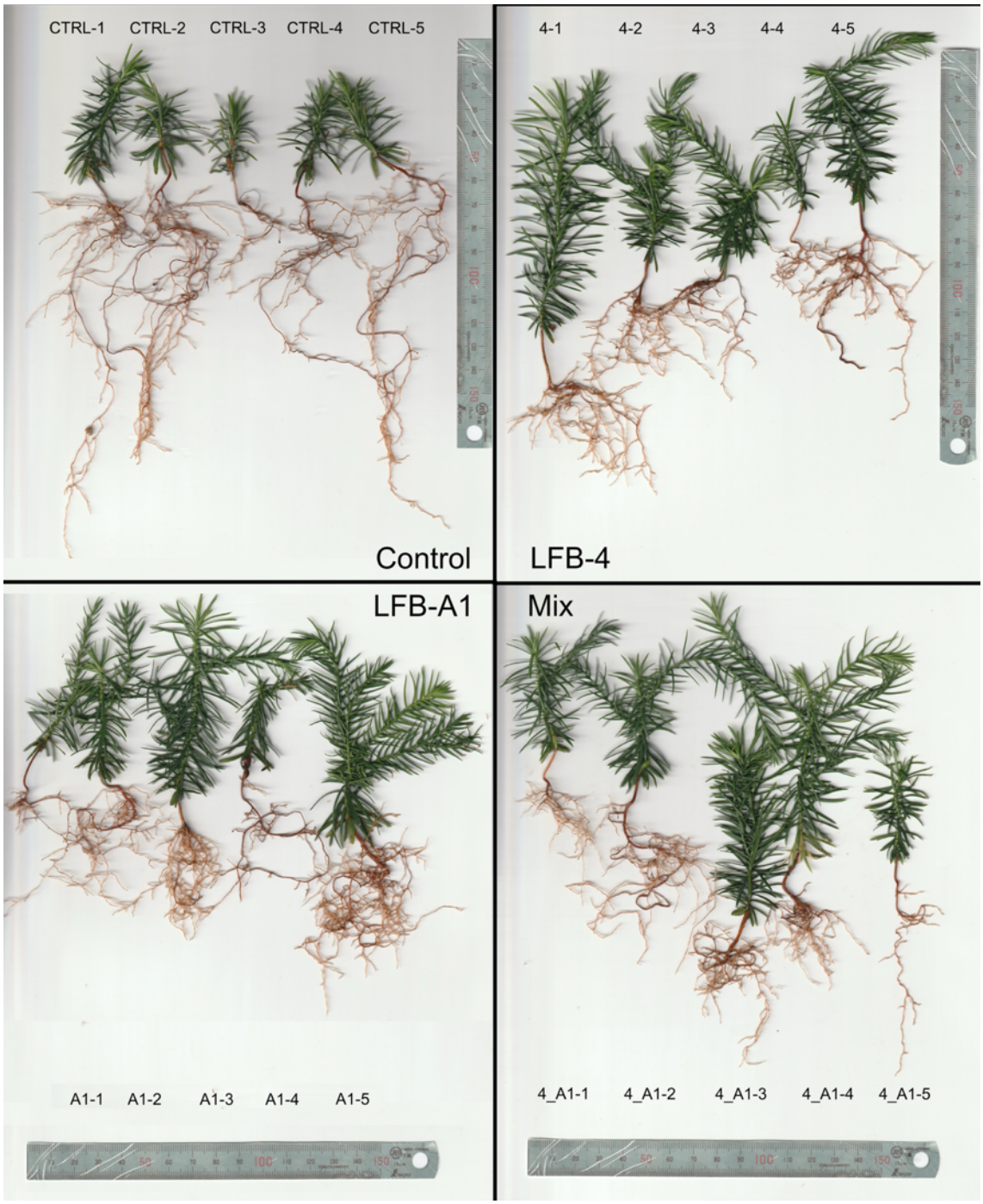
Online Resource 2 Visualization of morphological features in the arbuscular mycorrhizal fungal isolate LFB-4 (*Gigaspora rosea*)

A: Spores observed in water under a stereo microscope; B: Sporogenous cells (1) and plug (2) observed on a spore in lactic acid under a confocal optical microscope; C: Crushed spores in lactic acid showing spore wall layers (L1~3); D: Strains growing asymbiotically from spores incubated on gellan gum medium (arrow indicates an immature spore developed at the tip of the growing mycelium); E: Anastomosis-like mycelia connection in non-symbiotic growth of strains (arrow indicates hyphae fusion); F: Hyphae (arrow) and spores (circle) attached to *Sorghum bicolor* roots; G: Spores produced in *Cryptomeria japonica* root cells (arrows); H: Mycelial coils (arrows) produced in *Trifolium repens* root cells; I: Reference spores in water (MAFF 520062, Strain C1 obtained from the National Agricultural Research Organization, NARO)



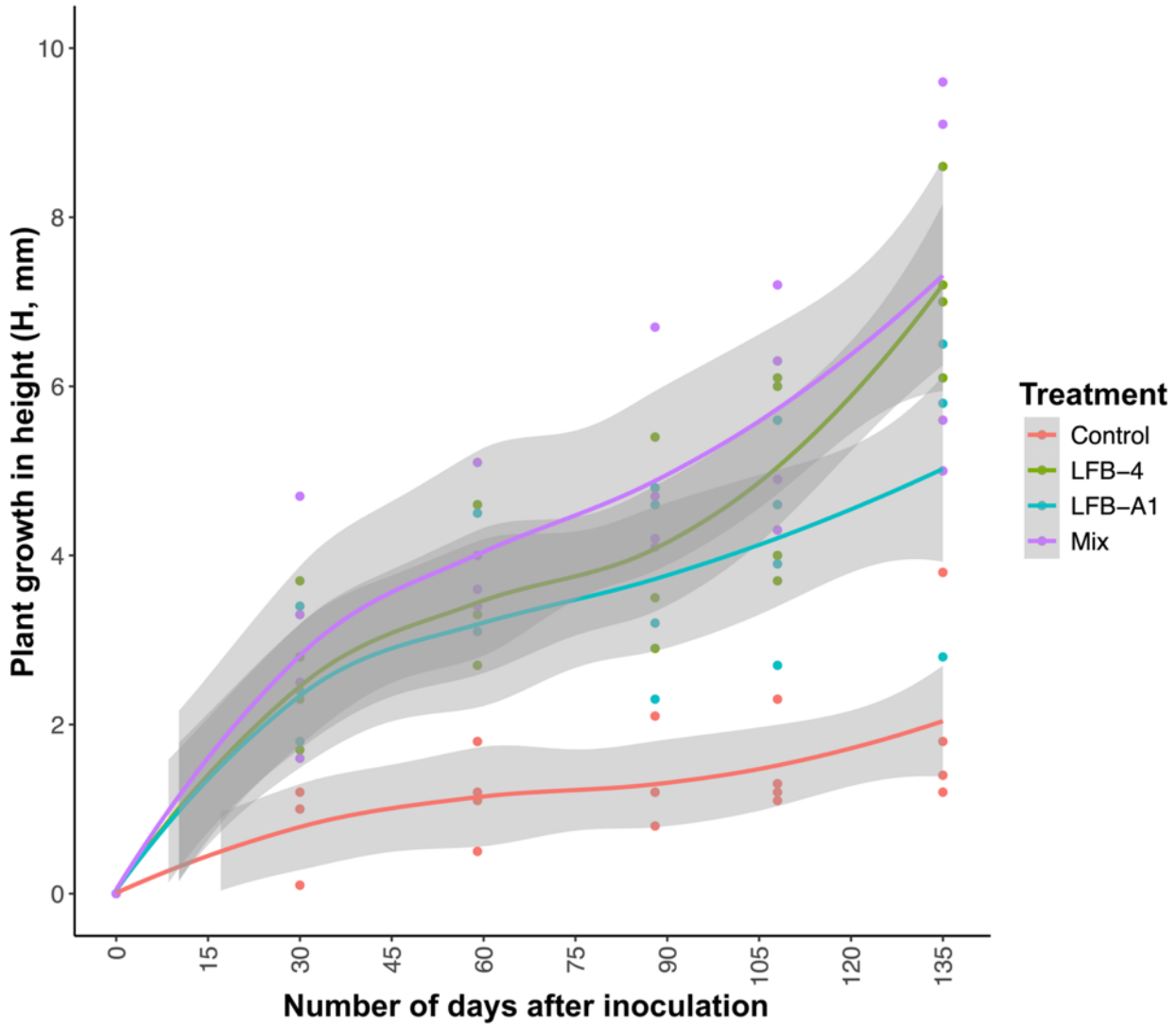
Online Resource 3 Visualization of morphological features in the arbuscular mycorrhizal fungal isolate LFB-A1 (*Gigaspora margarita*)

A: Spores observed in water under a stereo microscope; B: Sporogenous cells (1), the plug (2), and spore wall layers (L1~3) observed on a spore in lactic acid under a confocal optical microscope ; C: A whole spore in lactic acid showing thickness of spore wall layer L2 (arrow); D: Strains growing asymbiotically from spores incubated on gellan gum medium, showing simultaneous multiple germ tubes (arrows); E: Immature spores (arrows) developed intercalary along a mycelium in non-symbiotic growth of strains; F: Spores produced on the surface of fine roots of *Trifolium repens*; G: Mycelial coils (arrow) produced in *Trifolium repens* root cells; H: Hyphae (arrow) and a spore (circle) attached to *Sorghum bicolor* host roots; I: Reference spores in water (MAFF 520052, Strain K-1 obtained from the National Agricultural Research Organization, NARO)



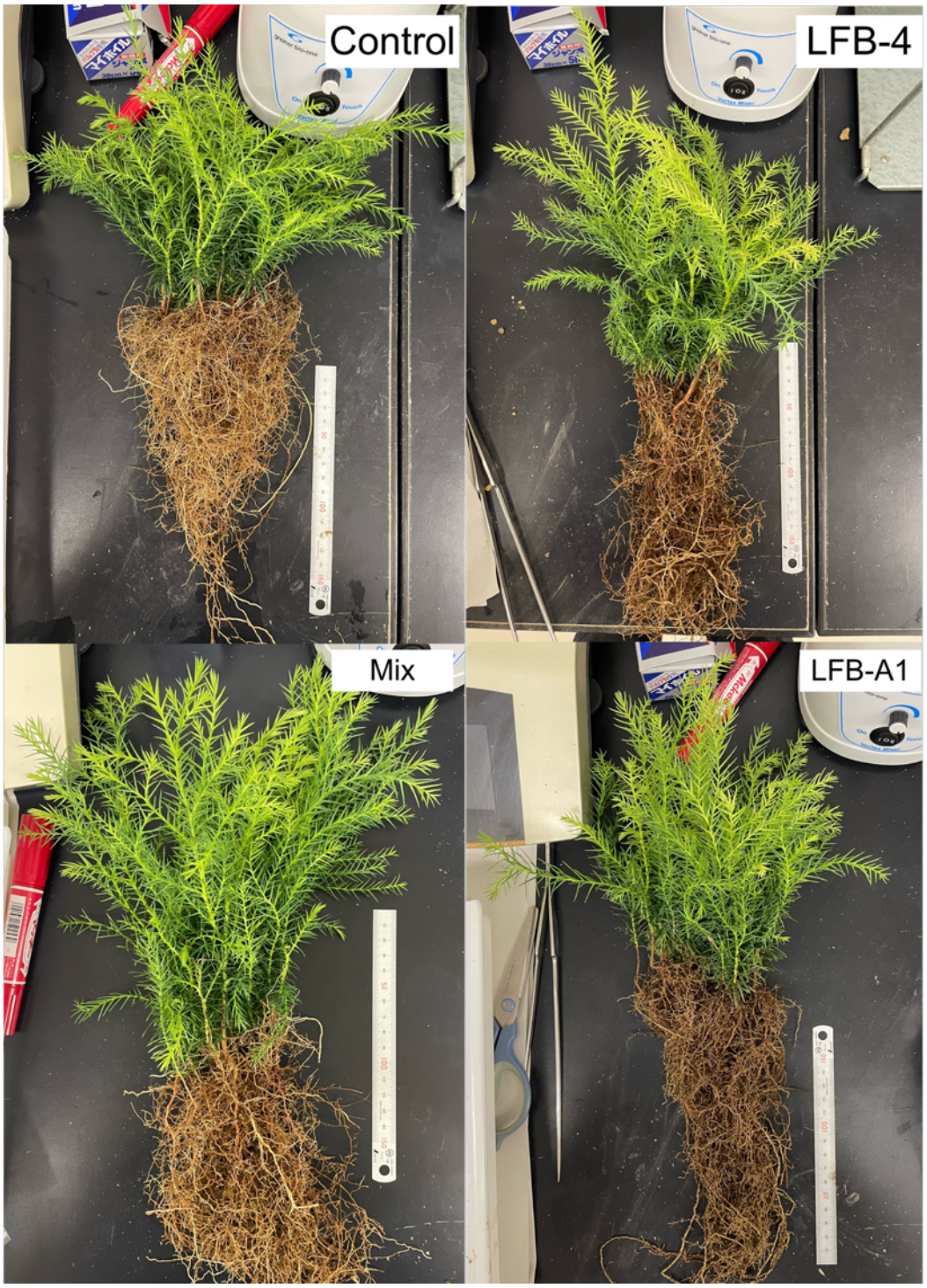
Online Resource 4 *Cryptomeria japonica* seedlings cultivated with and without arbuscular mycorrhizal fungi for 204 days

Control: Not inoculated; Inoculated with LFB-4 (*Gigaspora rosea*), LFB-A1 (*Gigaspora margarita*); Mix is a mixture of LFB-4 and LFB-A1



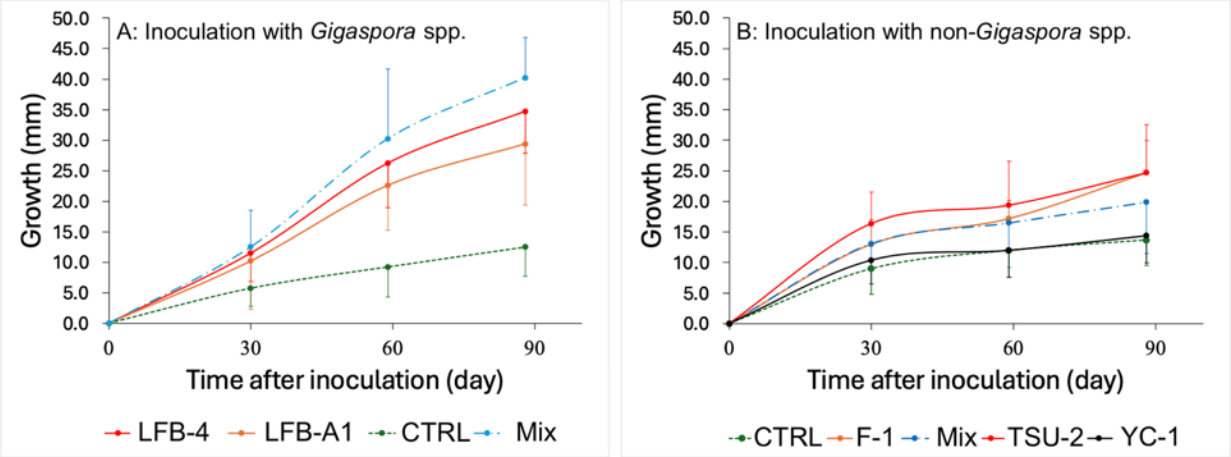
Online Resource 5 Growth in plant height of *Cryptomeria japonica* seedlings cultivated with and without arbuscular mycorrhizal fungi for 204 days

Control: Not inoculated; Inoculated with LFB-4 (*Gigaspora rosea*), LFB-A1 (*Gigaspora margarita*); Mix is a mixture of LFB-4 and LFB-A1



Online Resource 6 *Cryptomeria japonica* seedlings cultivated with and without arbuscular mycorrhizal fungi for 10 months

Control: Not inoculated; Inoculated with LFB-4 (*Gigaspora rosea*), LFB-A1 (*Gigaspora margarita*); Mix is a mixture of LFB-4 and LFB-A1



Online Resource 7 Different mycorrhizal growth response *of Cryptomeria japonica* seedlings cultivated with and without different arbuscular mycorrhizal fungi for 90 days

Control: Not inoculated; Inoculated with LFB-4 (*Gigaspora rosea*), LFB-A1 (*Gigaspora margarita*), F-1 (*Acaulospora longula*), TSU-2 (*Rhizophagus clarum*), YC-1 (*Paraglomus occultum*); Mix is a mixture of LFB-4 and LFB-A1 in (A) and a mixture of F-1, TSU-2, and YC-1 in (B). LFB-4 and LFB-A1 were isolated from a planted forest of *C. japonica*. F-1, TSU-2, and YC-1 which were obtained from the National Agricultural Research Organization (NARO) originated from non-forest ecosystems

Supplementary Tables

Table S1 Confirmation of colonization of *Cryptomeria japonica* roots by the *Gigaspora* isolates LFB-4 and LFB-A1

Arbuscular mycorrhizal fungi treatment [1]	Replicate ID	Root Colonization [2]			Colonization Confirmed by staining [2]	Target Spore and Hyphae content of soils [3]	Polymerase Chain Reactions [4]
		Hyphae	Spores	Coils			
Control	1	0	0	0	Negative	Negative	Negative
Control	2	0	0	0	Negative	Negative	Negative
Control	4	0	0	0	Negative	Negative	Negative
Control	5	0	0	0	Negative	Negative	Negative
LFB-4	1	0	0	0	Negative	3 Spores of LFB-4 + Hyphae	Positive
LFB-4	2	0	>35	0	Positive	5 Spores of LFB-4 + Hyphae	Positive
LFB-4	3	0	0	0	Negative	Hyphae	Positive
LFB-4	5	0	>60	0	Positive	5 Spores of LFB-4 + Hyphae	Positive
LFB-A1	2	0	0	0	Negative	2 Spores of LFB-A1 + Hyphae	Positive
LFB-A1	3	0	0	0	Negative	2 Spores of LFB-A1 + Hyphae	Positive
LFB-A1	4	0	0	0	Negative	5 Spores of LFB-A1 + Hyphae	Positive
LFB-A1	5	0	0	0	Negative	3 Spores of LFB-A1 + Hyphae	Positive
Mix	1	0	<10	2	Positive	2 Spores of LBF-4 + Hyphae	Positive
Mix	2	0	0	0	Negative	1 Spore of LBF-4 + Hyphae	Positive
Mix	3	0	3	3	Positive	1 Spore of LBF-A1 + Hyphae	Positive
Mix	4	0	0	0	Negative	3 Spore of LBF-4 + Hyphae	Positive

[1] Mix refers to co-inoculation of LFB-4 and LFB-A1. [2] Roots of *C. japonica* seedlings cultivated with and without arbuscular mycorrhizal fungi for 204 days were stained with Trypan blue. [3] Spores and hyphae contents were evaluated from 30 g of air-dried experimental soils passed through 1-mm mesh by wet sieving and decanting. [4] A partial small subunit ribosomal fungal DNA was amplified to confirm root colonization of the isolates in *C. japonica*

Table S2 Probabilities associated with one-way analysis of variance and Kruskal-Wallis' test analyzing effects of treatments on soil and plant properties

	AMF inoculation (df = 1)	**Inoculated AMF species (df = 3)**
Soil properties		
pH	ANOVA, F = 0.43, p = 0.52 (ns)	ANOVA, F = 2.68, p = 0.08 (ns)
C (%)	ANOVA, F = 9.01, p = 0.01 (s)	ANOVA, F = 2.86, p = 0.07 (ns)
N (%)	ANOVA, F = 1.58, p = 0.22 (ns)	ANOVA, F = 0.60, p = 0.63 (ns)
C/N	Kruskal-Wallis, p = 0.66 (ns)	Kruskal-Wallis, p = 0.96 (ns)
Plant properties - *Cryptomeria japaonica*		
S/R_{fresh}	Kruskal-Wallis, p = 0.004 (s)	ANOVA, F = 5.22, p = 0.016 (s)
FW_{plant} (mg)	ANOVA, F = 1.11, p = 0.310 (ns)	ANOVA, F = 0.32, p = 0.805 (ns)
FW_{root} (mg)	Kruskal-Wallis, p = 0.275 (ns)	ANOVA, F = 0.76, p = 0.538 (ns)
DW_{shoot} (mg)	ANOVA, F = 4.66, p = 0.049 (s)	Kruskal-Wallis, p = 0.231 (ns)
FW_{shoot} (mg)	ANOVA, F = 7.03, p = 0.019 (s)	Kruskal-Wallis, p = 0.136 (ns)
H (cm)	ANOVA, F = 9.23, p = 0.002 (s)	ANOVA, F = 18.5, p = 0.001 (s)
WC_{shoot} (%)	ANOVA, F = 12.85, p = 0.003 (s)	ANOVA, F = 4.86, p = 0.019 (s)
L_{root} (cm)	Kruskal-Wallis, p = 0.029 (s)	ANOVA, F = 1.70, p = 0.219 (ns)
Plant properties - *Daucus carota*		
FW_{shoot} (mg)	Kruskal-Wallis, p = 0.034 (s)	Kruskal-Wallis, p = 0.183 (ns)
H (cm)	ANOVA, F = 13.11, p = 0.001 (s)	Kruskal-Wallis, p = 0.007 (s)
$FW_{taproot}$ (mg)	Kruskal-Wallis, p = 0.574 (ns)	ANOVA, F = 5.01, p = 0.005 (s)

C: Total carbon; N: Total nitrogen; C/N: C to N ratio; S/R_{fresh}: Fresh shoot to toot weight ratio; FW_{plant}: Plant fresh weight; FW_{root}: Root fresh weight; DW_{shoot}: Shoot dry weight; FW_{shoot}: Shoot fresh weight; H: Plant height; WC_{shoot}: Shoot water content; L_{root}: Total root length; $FW_{taproot}$: Fresh weight of tap root. s and ns indicate significant and non-significant effect of the treatments, respectively

Table S3 Correlations between soil and plant properties in *Cryptomeria japonica* inoculation assays

Variables	H	FW_{plant}	FW_{shoot}	FW_{root}	DW_{shoot}	WC_{shoot}	S/R_{fresh}	L_{root}	pH	N	C
	0.50										
FW_{plant}	(0.051)	-	-	-	-	-	-	-	-	-	-
	0.67	0.92									
FW_{shoot}	(0.005)	(0.000)	-	-	-	-	-	-	-	-	-
	-0.19	0.53	0.29								
FW_{root}	(0.486)	(0.034)	(0.280)	-	-	-	-	-	-	-	-
	0.59	0.94	0.99	0.39							
DW_{shoot}	(0.016)	(0.000)	(0.000)	(0.131)	-	-	-	-	-	-	-
	0.65	0.15	0.35	-0.61	0.22						
WC_{shoot}	(0.006)	(0.589)	(0.185)	(0.013)	(0.423)	-	-	-	-	-	-
	0.68	0.32	0.58	-0.53	0.50	0.63					
S/R_{fresh}	(0.004)	(0.225)	(0.019)	(0.035)	(0.049)	(0.009)	-	-	-	-	-
	-0.27	0.47	0.19	0.88	0.28	-0.58	-0.50				
L_{root}	(0.312)	(0.067)	(0.486)	(0.000)	(0.287)	(0.018)	(0.046)	-	-	-	-
	0.16	-0.12	0.07	-0.24	0.02	0.24	0.37	-0.33			
pH	(0.544)	(0.653)	(0.794)	(0.377)	(0.951)	(0.364)	(0.155)	(0.215)	-	-	-
	0.12	-0.27	-0.16	-0.44	-0.22	0.30	0.05	-0.39	0.14		
N	(0.647)	(0.304)	(0.546)	(0.089)	(0.412)	(0.266)	(0.859)	(0.137)	(0.594)	-	-
	0.43	0.21	0.39	-0.15	0.31	0.55	0.25	-0.23	0.35	0.67	
C	(0.099)	(0.433)	(0.137)	(0.584)	(0.238)	(0.028)	(0.347)	(0.382)	(0.190)	(0.004)	-
	0.08	0.49	0.5	0.46	0.51	0.03	0.09	0.33	0.09	-0.79	-0.10
C/N	(0.773)	(0.055)	(0.051)	(0.076)	(0.043)	(0.919)	(0.735)	(0.214)	(0.752)	(0.000)	(0.706)

S/R_{fresh}: Fresh shoot to root weight ratio; FW_{plant}: Plant fresh weight; FW_{root}: Root fresh weight; DW_{shoot}: Shoot dry weight; FW_{shoot}: Shoot fresh weight; H: Plant height; WC_{shoot}: Shoot water content; L_{root}: Total root length; C: Total carbon; N: Total nitrogen; C/N: C to N ratio. Pearson correlation values were followed by the associated *p*-value in brackets, indicating a significant correlation when < 0.05

References

Bago, B., Bentivenga, S. P., Brenac, V., Dodd, J. C., Piché, Y., & Simon, L. (1998). Molecular analysis of *Gigaspora* (Glomales, Gigasporaceae). *New Phytologist*, *139*, 581–588. https://doi.org/10.1046/j.1469-8137.1998.00212.x

Bentivenga, S. P., & Morton, J. B. (1995). A monograph of the genus *Gigaspora*, incorporating developmental patterns of morphological characters. *Mycologia*, *87*, 719–731. https://doi.org/10.1080/00275514.1995.12026590

Błaszkowski, J. (2012). *Glomeromycota*. W. Szafer Institute of Botany, Polish Academy of Sciences. https://doi.org/10.5555/20123352848

Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture*. Australian Centre for International Agricultural Research

Djotan, A. K. G. (2024). *Diversity and ecology of arbuscular mycorrhizal fungi communities in Cryptomeria japonica and Chamaecyparis obtusa* [University of Tokyo]. Tokyo.

Huang, X. (1992). A contig assembly program based on sensitive detection of fragment overlaps. *Genomics*, *14*, 18–25. https://doi.org/10.1016/S0888-7543(05)80277-0

Katoh, K., & Standley, D. M. (2013). MAFFT: Multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/MOLBEV/MST010

Kornerup, A., & Wanscher, J. H. (1983). *Methuen handbook of colour* (3 ed.). Eyre Methuen

Krüger, M., Stockinger, H., Krüger, C., & Schüßler, A. (2009). DNA-based species level detection of Glomeromycota: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist*, *183*, 212–223. https://doi.org/10.1111/j.1469-8137.2009.02835.x

Lanfranco, L., Bianciotto, V., Lumini, E., Souza, M., Morton, J. B., & Bonfante, P. (2001). A combined morphological and molecular approach to characterize isolates of arbuscular mycorrhizal fungi in *Gigaspora* (Glomales). *New Phytologist*, *152*, 169–179. https://doi.org/10.1046/j.0028-646x.2001.00233.x

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., Lanfear, R., & Teeling, E. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*, 1530–1534. https://doi.org/10.1093/MOLBEV/MSAA015

Omar, M. B., Bolland, L., & Heather, W. A. (1979). A permanent mounting medium for fungi. *Bulletin of the British Mycological Society*, *13*, 31–32

Sato, K., Suyama, Y., Saito, M., & Sugawara, K. (2005). A new primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. *Grassland Science*, *51*, 179–181. https://doi.org/10.1111/j.1744-697X.2005.00023.x

Souza, F. A. d., Kowalchuk, G. A., Leeflang, P., Veen, J. A. v., & Smit, E. (2004). PCR-denaturing gradient gel electrophoresis profiling of inter- and intraspecies 18S rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus *Gigaspora*. *Applied and Environmental Microbiology*, *70,* 1413–1424. https://doi.org/10.1128/AEM.70.3.1413-1424.2004