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SHORT NOTE

Rutaceae sampled from Germany, Malta, and Mallorca (Spain) are associated with AMF clustering with *Glomus hoi* Berch & Trappe

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Abstract Six Rutaceae species collected from natural habitats (Malta, Mallorca (Spain), and Tenerife (Spain)) and the Botanical Garden in Marburg were examined with respect to mycorrhizal structures and fungal identity. All species have the same gross colonization pattern of arbuscular mycorrhiza (AM) with distinct intracellular and intercellular phases but show remarkable differences in details, especially in terms of the extent of the intracellular phase. The associated AM fungi, identified using molecular methods, cluster together with *Glomus hoi* Berch & Trappe, although the plants were collected from very distant locations.

Keywords Arbuscular mycorrhiza · Rutaceae · Fungal identity · Colonization pattern

Introduction

The Rutaceae (Sapindales/Rosids II) consist of about 1,815 species in 161 genera (Stevens 2001 onwards) with *Citrus* L. being the best known and agriculturally most important. There are few works on mycorrhizal structures in Rutaceae (Janse 1896; McLuckie and Burges 1932; Rayner 1933; Johnston 1949; Yamato and Iwasaki 2002; Hawley and Dames 2004; Muthukumar et al. 2006), the latter four of which are survey investigations listing the mycorrhizal status without structural details. On the basis of these investigations, Smith and Smith (1997) and Dickson et al. (2007) list the Rutaceae under those families in which both *Arum*- and *Paris*-type of arbuscular mycorrhiza (AM) may occur. The present study presents the detailed pattern of mycorrhizal colonization in seven Rutaceae species and first indications for a specificity with regard to the fungal partners in this family. The structures found are integrated into the continuum of AM symbioses postulated by Dickson (2004).

There are many studies trying to improve the yield of *Citrus* plantations including tests of increased resistance against pathogens using fungal inoculants (e.g., Menge et al. 1978; Graham and Fardelmann 1986). A possible specificity of Rutaceae regarding their fungal endophyte, hence, may be of high economic relevance.

Materials and methods

Seven species of Rutaceae, *Cneorum tricoccon* L., *Cneorum pulverulentum* Vent., *Ruta chalepensis* L., *Dictamnus albus* L., *Orixa japonica* Thunb., *Ptelea trifoliata* L. and *Phellodendron amurense* Rupr. were collected and fixed as indicated in Table 1.

Parts of the collected roots were stained with trypan blue and squashed after Phillips and Hayman (1970) in order to check their colonization status. For a detailed structural examination, 12–20 fine roots per specimen were dehydrated in an ascending ethanol series and then embedded in Unicryl™ (Brithish Biocell Int.). Series of 4-μm sections (transvers and longitudinal) stained with Toluidine Blue O (1 g of Toluidine Blue O plus 1 g of sodium tetraborate in 100 ml of $Aqua_{dest}$; after Krause 1927) were prepared and mounted on slides in Corbit-Balsam. Full series of slides were investigated in order to reconstruct the three-dimen-

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Table 1 Collection sites and fixation procedures

Species (number of specimens collected)	Collection date	Location	Collection sites	Fixation
Cneorum tricoccon (6)	March 20 and 21, 2005	Mallorca	Garrigue, *Quercus ilex* forest, *Pinus halepensis* forest	Ethanol (70%), CTAB
Cneorum pulverulentum (3)	March 21, 2006	Punta de Teno, Tenerife		Ethanol (70%)
Ruta chalepensis (4)	March 30 and 31, 2006	Gozo, Malta	Garrigue	Ethanol (70%), silica gel
Dictamnus albus (2)	Aug 11, 2006	Marburg, Germany	Botanical Garden	Ethanol (70%), liquid nitrogen
Orixa japonica (1)	July 21, 2006	Marburg, Germany	Botanical Garden	Ethanol (70%), liquid nitrogen
Ptelea trifoliata (1)	July 21, 2006	Marburg, Germany	Botanical Garden	Ethanol (70%)
Phellodendron amursense (1)	July 21, 2006	Marburg, Germany	Botanical Garden	Ethanol (70%)

CTAB *N*-cetyl-*N*,*N*,*N*-trimethyl-ammonium-bromide

sional colonization pattern. Light microscopy was done using a Leica DMRB microscope equipped with a digital photo device (Leica DFC280).

For every specimen of *C. tricoccon*, *R. chalepensis*, *O. japonica*, and *D. albus*, one primary root piece of about 3 cm length was used to extract DNA following the CTAB-protocol of Doyle and Doyle (1990). Fungal 18S rDNA was amplified through polymerase chain reaction (PCR; Saiki et al. 1985) using the fungus specific primers AM1 (Helgason et al. 1998) and NS31 (Simon et al. 1992). The PCR was carried out with the following profile: initial denaturation for 3 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 60°C, 40 s at 72°C, and a final extension for 10 min at 72°C. After purifiying the PCR products with a NucleoSpin Extract II Kit (Macherey-Nagel), they were directly sequenced without prior cloning by the didesoxynucleotide chain termination method (Sanger et al.

Table 2 NCBI accession numbers and authors of the species of Fig. 2

Species	NCBI accession number	Author(s)
Endogone pisiformis	X58724	Simon et al. (1992)
Glomus fasciculatum	Y17640	Schüßler et al. (2001b)
Glomus geosporum	AJ245637	Schüßler et al. (2001b)
Glomus caledonium	Y17635	Schüßler et al. (2001b)
Glomus coronatum	AJ276086	Schüßler et al. (2001b)
Glomus mosseae	U96141	Vandenkoornhuyse and Leyval (1998)
Glomus hoi	AF485888	Helgason et al. (2002)
Glomus intraradices	AJ536822	Calvente et al. (2003)
Glomus proliferum	AF213462	Declerck et al. (2000)
Glomus versiforme	AJ276088	Schüßler et al. (2001b)
Glomus cf. etunicatum	Y17644	Schüßler et al. (2001b)
Glomus vesiculiferum	L20824	Simon et al. (1993a)
Glomus verruculosum	AJ301858	Schwarzott et al. (2001)
Glomus luteum	AJ276089	Schüßler et al. (2001b)
Glomus claroideum	AJ276075	Schüßler et al. (2001b)
Glomus lamellosum	AJ276087	Schüßler et al. (2001b)
Gigaspora albida	Z14009	Simon et al. (1993b)
Gigaspora gigantean	Z14010	Simon et al. (1993b)
Acaulospora scrobiculata	AJ306442	Schüßler et al. (2001a)
Glomus sp. endophyte *Orixa*1	EU518486	Appelhans et al. (present study)
Glomus sp. endophyte *Ruta*1	EU518487	Appelhans et al. (present study)
Glomus sp. endophyte *Dictamnus*1	EU518488	Appelhans et al. (present study)
Glomus sp. endophyte *Dictamnus*2	EU518489	Appelhans et al. (present study)
Glomus sp. endophyte *Cneorum*2	EU518491	Appelhans et al. (present study)
Glomus sp. endophyte *Cneorum*3	EU518490	Appelhans et al. (present study)
Glomus sp. endophyte *Cneorum*4	EU518492	Appelhans et al. (present study)

1977) using an ABI 377 (Applied Biosystems, Rodgau–Jügesheim, Germany).

The sequences were edited with the Sequencher™ (Gene Codes Corporation, Ann Arbor, MI, USA) program. Sequences were aligned by hand using BioEdit (Hall 1999). A nucleotide–nucleotide Basic Local Alignment Search Tool search revealed the highest sequence similarities to a number of undescribed *Glomus* species as well as *Glomus hoi*. The phylogenetic analysis was carried out using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) including the species listed in Table 2.

A Bayesian Analysis was performed, using gamma distribution rate variation among sites, ten million generations of the MCMC chains in two independent runs, trees sampled every 100 generations. The first 25,000 trees were discarded as burn-in as the analysis then reached stationarity. All other trees sampled were used to calculate a strict consensus tree.

Results

Colonization of the outer cortex layers (intracellular phase)

The fungus enters a primary root by penetrating a single rhizodermis cell (penetration point) with little coiling therein and proceeds directly into the following outer cortex cells. Here, the hyphae start to coil, branch, and spread into several cells in longitudinal, tangential, and radial direction. Up to this point, the colonization is exclusively intracellular and no signs of hyphal degradation were seen. This intracellular phase differs among the

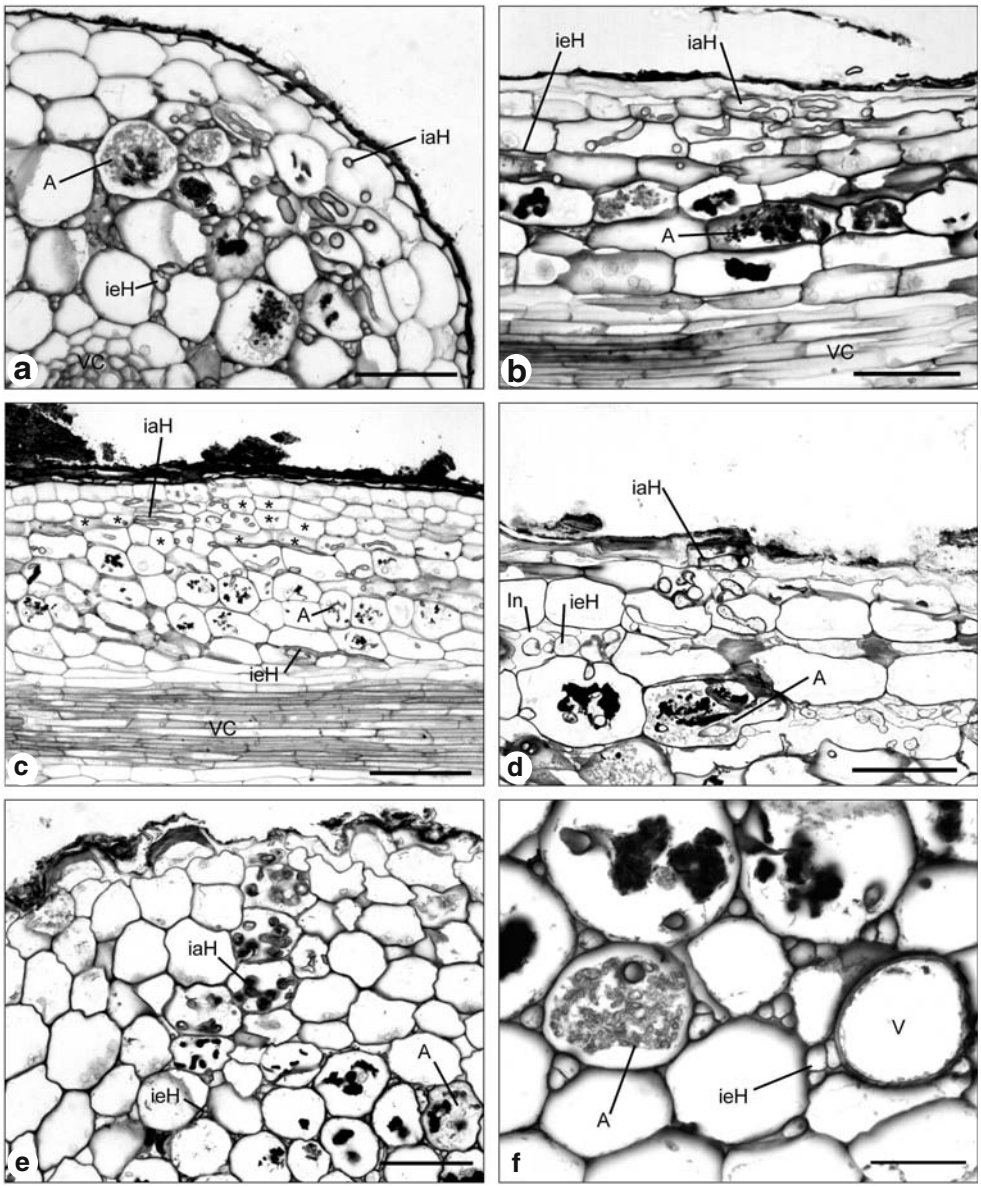

Fig. 1 Differences in colonization pattern of AM fungi in roots of Rutaceae. **a**, **b** *Ruta chalepensis* (**a** = transverse section, **b** = longitudinal section). The intracellular phase comprises four cells in radial, four cells in longitudinal, and five cells in tangential direction (**a** transverse section, **b** longitudinal section). **c** *Cneorum pulverulentum* (longitudinal section). The intracellular spread of mycorrhization covers eight cells in longitudinal direction. The *asterisks* indicate those cells in which intracellular hyphae are found in subsequent sections. **d** *Dictamnus albus* (longitudinal section) The intracellular phase is limited to two cells in each direction. **e** *Orixa japonica* (transverse section). Only a single row of cells is colonized in the intracellular phase. **f** *Ruta chalepensis* (transverse section). Intercellular spaces are closely packed with AM hyphae. *A* = arbuscule; *iaH* = intracellular hypha; *ieH* = intercellular hypha; *In* = intercellular space; *V* = vesicle; *VC* = vascular cylinder. *Scales*: (**a**, **b**, **d**, **e**) = 50 μm, (**c**) = 100 μm, (**f**) = 20 μm

species. In *C. tricoccon*, *R. chalepensis*, *Ph. amursense*, and *Pt. trifoliata*, the extention of the intracellular phase is about four to five cells in longitudinal and tangential direction per penetration point, with three to four cells (including the rhizodermis) in radial direction (Fig. 1a, b). In *C. pulverulentum*, the intracellular phase is extended to up to eight cells in longitudinal direction (Fig. 1c), whereas the spread in tangential and radial direction is the same as in the former species. In contrast, the intracellular phase of *D. albus* is reduced to two cells of colonization in longitudinal and tangential as well as in radial direction (Fig. 1d). A special form was found in *O. japonica*, where the intracellular phase is mostly confined to a single row of up to six cells colonized in radial direction (Fig. 1e). Here, branching of the colonizing hyphae is reduced.

Colonization of the inner cortex layers (intercellular phase)

After having colonized a species specific number of outer cortex layers, the hyphae transist into the intercellular spaces. There, they spread in longitudinal, tangential, and inner radial direction, forming lateral branches which penetrate single cells and develop arbuscules (e.g., Fig. 1a, f). These arbuscules are terminal structures, characterized by eventual finely dichotomous branching and, thus, are typical for the *Arum*-type AM. Vesicles (Fig. 1f) develop intercellularly and, like the arbuscules, are confined to the inner cortex layers. In the specimen of *C. pulverulentum* and *Ph. amurense* of our material, hyphae occurred singly or in pairs in an intercellular space, whereas the intercellular spaces of the other species were mostly closely packed with numerous hyphae (Fig. 1f). In contrast to the specific colonization pattern of the outer cortex, a specificity of this feature is not certain but also could be a phenomenon of colonization density.

Identity of the AM fungi

The extraction and sequencing of fungal DNA succeeded in three (out of six) specimens of *C. tricoccon*, in one (out of four) for *R. chalepensis*, and in all specimen of *D. albus* and *O. japonica.* The sequencing data, although without cloning, were unambiguous, speaking for the colonization of only a single fungus species per root preparation. Despite

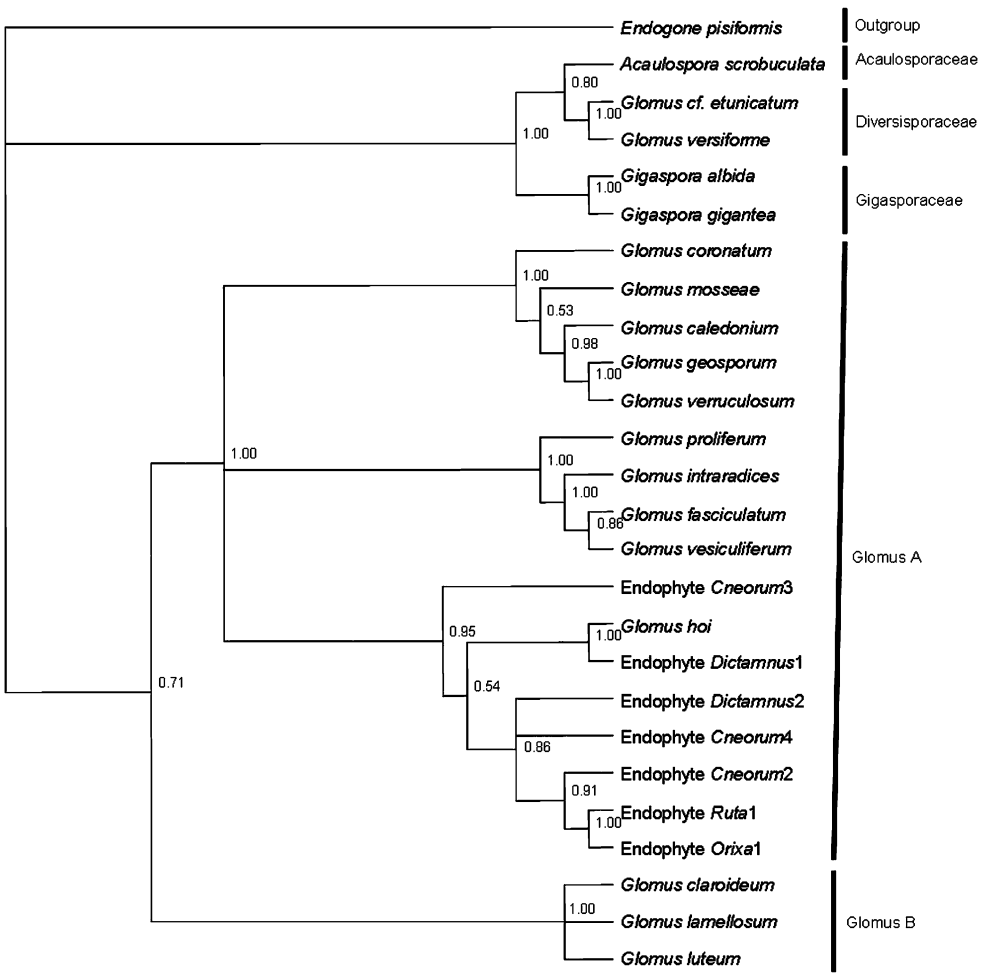

Fig. 2 Strict consensus tree of the NS31-AM1 region of the SSU rRNA from the Endophytes in the Rutaceae species and relevant reference species. Posterior probabilities of the Bayesian analysis are shown on the branches. NCBI accession numbers and names of authors from the reference species are given in Table 2

the large distances between the collection sites (up to 1,600 km apart), the sequencing of partial 18S rDNA showed that the fungi cluster with *G. hoi* Berch & Trappe, which is part of the *Glomus* A lineage (Schüßler et al. 2001a; Schwarzott et al. 2001). The monophyly of the group of *G. hoi* and the endophytes in Rutaceae is strongly supported by 0.95 posterior probability (Fig. 2).

Discussion

AM structures used to be classified into the predominantly intercellular *Arum*-type or the exclusively intracellular *Paris*-type (Gallaud 1905; Smith and Smith 1997). More recently, Dickson (2004) stated a continuum of structures between *Arum*- and *Paris*-type, which can be amended by the aberrant mycorrhizal colonization pattern found in mycoheterotrophic plants (e.g., Imhof 2003, 2007). Considering Dicksons (2004) classification, the AM patterns described here fit best into the "intermediate 1"-type, provided that "intracellular hyphae in the outer cells" also means coiled intracellular hyphae, which is not explicitly mentioned by Dickson (2004). The present findings in Rutaceae prove that the intracellular phase, even within the "intermediate 1"-type, can differ greatly in extent as well as in pattern, strongly corroborating the notion of a structural continuum in AM. This is also reflected in the published mycorrhizal surveys, which ambiguously report *Arum*- (Yamato and Iwasaki 2002; Muthukumar et al. 2006) as well as *Paris*-type (Johnston 1949; Hawley and Dames 2004) for Rutaceae. In fact, species with a limited intracellular phase may easily be categorized as "*Arum*-type," especially when only investigated using the quick but less detailed squeezing method of Phillips and Hayman (1970). Interestingly, the description of *Acronychia* sp. by Janse (1896) indicates what we today call "intermediate 1," whereas McLuckie and Burges (1932) report an early intercellular colonization in *Eriostemon crowei* (both Rutaceae). Rayner (1933), working on *Citrus*, only mention "intercellular as well as intracellular infection." If we consider the arbuscules to be the functionally most important interface between fungus and plant in AM (Harrison et al. 2002; Balestrini and Lanfranco 2006), the inner cortex colonization is crucial for the symbiosis in Rutaceae, whereas the intracellular (outer cortex) phase only link the extraradical mycelium with the intercellular phase developing the arbuscules. The often reported phenomenon of arbuscules developed close to the central cylinder of a root (e.g., Noldt and Bauch 2001; Russell et al. 2002) is plausible, since there the exchanged nutrients and carbohydrates can be most easily relocated. Since the coiled intracellular hyphae of the investigated Rutaceae do not disintegrate, as they sometimes do in *Paris*-type AM (e.g., McGee et al. 1999; Imhof 2001), they can actually serve as a permanent connection to the rhizosphere. The decreasing longitudinal extent of outer cortex colonization from *C. pulverulentum* over *C. tricoccon* and *D. albus* to *O. japonica* may be interpreted as a focusing of hyphal development towards the inner cortex. In other words, compared to the other Rutaceae under investigation, the colonization pattern observed in *O. japonica* represents the shorter, more efficient way to reach the essential symbiotic interface.

All fungal DNA extracted from seven roots of Rutaceae in this investigation is nearly identical, although the plants have been collected from location as distinct as Malta, Mallorca (Spain) and Marburg (Germany), as well as from Garrigue, Mediterranean forests, and Central European soils. This may be interpreted as a first hint of mycorrhizal specificity within the Rutaceae, which, however, should be backed up through further investigations. Specificity with regard to their mycorrhizal partners is so far only known for mycoheterotrophic plants (Bidartondo and Bruns 2002; Bidartondo et al. 2002; Selosse et al. 2002; Franke et al. 2006; Merckx and Bidartondo 2008).

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References

Balestrini R, Lanfranco L (2006) Fungal and plant gene expression in arbuscular mycorrhizal symbiosis. Mycorrhiza 16:509–524 Medline. DOI 10.1007/s00572-006-0069-2

Bidartondo MI, Bruns TD (2002) Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups. Mol Ecol 11:557–569. Medline. DOI 10.1046/j.0962-1083.2001.01443.x

Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Dominguez L et al (2002) Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature 419:389–392. Medline. DOI 10.1038/nature01054

Calvente R, Cano C, Ferrol N, Concepcion AA, Jose BM (2003) 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. DOI 10.1016/j.apsoil.2003.10.009

Declerck S, Cranenbrouck S, Dalpe Y, Seguin S, Grandmougin-Ferjani A, Fontaine J et al (2000) *Glomus proliferum* sp. nov.: a description based on morphological, biochemical, molecular and monoxenic cultivation data. Mycologia 92(6):1178–1187. DOI 10.2307/3761485

Dickson S (2004) The *Arum-Paris* continuum of mycorrhizal symbioses. New Phytol 163(1):187–200. DOI 10.1111/j.1469-8137.2004.01095.x

Dickson S, Smith FA, Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after

Gallaud, where next. Mykorrhiza 17:375–393. DOI 10.1007/s00572-007-0130-9

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Franke T, Beenken L, Döring M, Kocyan A, Agerer R (2006) Arbuscular mycorrhizal fungi of the *Glomus*-group A lineage (Glomerales; Glomeromycota) detected in myco-heterotrophic plants from tropical Africa. Mycol Prog 5:24–31. DOI 10.1007/s11557-006-0500-2

Gallaud I (1905) Études sur les mycorhizes endotrophes. Rev Gen Bot 17:7–496

Graham JH, Fardelmann D (1986) Inoculation of *Citrus* with root fragments containing chlamydospores of the mycorrhizal fungus *Glomus intraradices*. Can J Bot 64:1739–1744. DOI 10.1139/b86-233

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago trunculata* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413–2429. Medline. DOI 10.1105/tpc.004861

Hawley GL, Dames JF (2004) Mycorrhizal status of indigenous tree species in a forest biome of the Eastern Cape, South Africa. S Afr J Sci 100(11, 12):633–637

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide-web. Nature 394:431. Medline. DOI 10.1038/28764

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J Ecol 90(2):371–384. DOI 10.1046/j.1365-2745.2001.00674.x

Imhof S (2001) Subterranean structures and mycotrophy of the achlorophyllous *Dictyostega orobanchoides* (Hook.) Miers (Burmanniaceae). Rev Biol Trop 49:237–245

Imhof S (2003) A dorsiventral mycorrhizal root in the achlorophyllous *Sciaphila polygyna* (Triuridaceae). Mycorrhiza 13:327–332 Medline. DOI 10.1007/s00572-003-0255-4

Imhof S (2007) Specialized mycorrhizal colonization pattern in achlorophyllous *Epirixanthes* spp. (Polygalaceae). Plant Biol 9:786–792. Medline. DOI 10.1055/s-2007-965613

Janse JM (1896) Les endophytes radicaux de quelques plantes javanaises. Ann Jard Bot Buitenzorg. 14:53–212

Johnston A (1949) Vesicular-arbuscular mycorrhiza in sea-island cotton and the tropical plants. Trop Agric 26:118–121

Krause R (1927) Enzyklopädie der Mikroskopischen Technik, 3rd edn. Urban and Schwarzenberg, Berlin

McGee PA, Bullock S, Summerell BA (1999) Structure of mycorrhizae of the Wollemi Pine (*Wollemia nobilis*) and related Araucariaceae. Aust J Bot 47:85–95. DOI 10.1071/BT97064

McLuckie J, Burges A (1932) Mycotrophism in the Rutaceae. I. The mycorrhiza of *Eriostemon crowei* F.v.M. Proc Linn Soc N S W 57:291–312

Menge JA, Johnson ELV, Platt RG (1978) Mycorrhizal dependency of several *Citrus* cultivars under three nutrient regimes. New Phytol 81:553–559. DOI 10.1111/j.1469-8137.1978.tb01628.x

Merckx V, Bidartondo MI (2008) Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. Proc R Soc Lond B Biol Sci 275:1029–1035. DOI 10.1098/rspb.2007.1622

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(1):11–24. Medline. DOI 10.1007/s00572-006-0077-2

Noldt G, Bauch J (2001) Colonization of fine roots of mahogany (*Swietenia macrophylla* King) by vesicular-arbuscular mycorrhizal fungi under plantation conditions in Central Amazon. J Appl Bot 75:168–172

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–162

Rayner MC (1933) Mycorrhiza in the genus *Citrus*. Nature 131:399–400. DOI 10.1038/131399b0

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. Medline. DOI 10.1093/bioinformatics/btg180

Russell AJ, Bidartondo MI, Butterfield BG (2002) The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. New Phytol 156:283–295. DOI 10.1046/j.1469-8137.2002.00504.x

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA et al (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354. Medline. DOI 10.1126/science.2999980

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74 (12):5463–5467. Medline. DOI 10.1073/pnas.74.12.5463

Schüßler A, Schwarzott D, Walker C (2001a) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421. DOI 10.1017/S0953756201005196

Schüßler A, Gehrig HH, Schwarzott D, Walker C (2001b) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105:5–15. DOI 10.1017/S0953756200003725

Schwarzott D, Walker C, Schüßler A (2001) *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Mol Phylogenet Evol 21(2):190–197 Medline. DOI 10.1006/mpev.2001.1007

Selosse M-A, Weiß M, Jany J-L, Tillier A (2002) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. Mol Ecol 11:1831–1844. Medline. DOI 10.1046/j.1365-294X.2002.01553.x

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58(1):291–295. Medline

Simon L, Levesque RC, Lalonde M (1993a) Identification of endomycorrhizal fungi colonizing roots by fluorescent single-strand conformation polymorphism-polymerase chain reaction. Appl Environ Microbiol 59(12):4211–4215. Medline

Simon L, Bousquet J, Levesque RC, Lalonde M (1993b) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69. DOI 10.1038/363067a0

Smith FA, Smith SE (1997) Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol 137:373–388. DOI 10.1046/j.1469-8137.1997.00848.x

Stevens PF (2001) Angiosperm Phylogeny Website. Version 8, June 2007. Accessed at http://www.mobot.org/MOBOT/research/APweb

Vandenkoornhuyse P, Leyval C (1998) SSU rDNA sequencing and PCR fingerprinting reveal genetic variation within Glomus mosseae. Mycologia 90(5):791–797. DOI 10.2307/3761320

Yamato M, Iwasaki M (2002) Morphological types of arbuscular mycorrhizal fungi in roots of understory plants in Japanese deciduous broadleaved forests. Mycorrhiza 12:291–296. Medline. DOI 10.1007/s00572-002-0187-4