THE MYCORRHIZAL FUNGAL ASSOCIATIONS OF THE VULNERABLE *SOPHORA FRASERI* (FABACEAE)

Downie, A.F., Le Brocque, A.F. and Dearnaley, J.D.W.*

Australian Centre for Sustainable Catchments, The University of Southern Queensland, Toowoomba 4350, Australia.
**Author for correspondence: Fax +617 4631 1530; Email: dearns@usq.edu.au*

Abstract

Sophora fraseri Benth. is a vulnerable Australian shrub that occurs on rainforest margins in south-east Queensland and northern New South Wales. The mycorrhizal fungi that colonise roots of *S. fraseri* are not known although other members of the Fabaceae are typically colonised by arbuscular mycorrhizal (AM) fungi from the *Glomus* genus. In this study we have sampled the roots of *S. fraseri* plants growing at 4 different sites in south-east Queensland. The fungal community of the roots of the plants was assessed via DNA extraction, polymerase chain reaction (PCR) amplification with AM fungal-specific primers, cloning and sequencing. These analyses showed that roots of *S. fraseri* contained a number of non-AM fungal species including *Elsinoë* and *Phoma* and four mycorrhizal *Glomus* taxa, two of which are related to *G. intraradices* and two others which are currently unnamed.

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Introduction

Sophora fraseri (Brush-Sophora or Necklace pod) (Figure 1) is a rare Australian Fabaceae shrub that is threatened by human activities including land clearing, fire and invasion of exotic weeds (Department of Environment and Climate Change 2005). *S. fraseri* is only found on rainforest margins in south-east Queensland and northern New South Wales (Nicholson & Nicholson 2000) and is at risk of local extinctions as existing populations are small (Department of Environment and Climate Change 2005). There are recovery plans for the species (Department of Environment and Climate Change 2005) and the plant is currently being grown horticulturally as a safeguard against population losses in the natural state.

Mycorrhizas are mutual symbiotic associations that exist between plants and fungi, probably occurring in over 80% of angiosperm families (Brundrett 2002). Plant growth is enhanced by mycorrhizal interactions with an increased access to soil resources, including the ability to trap water and alleviate nutrient stress by obtaining greater amounts of phosphorous, nitrogen, copper, nickel and zinc (Smith & Read 1997). Other advantages include increased resistance to parasitic organisms (Azcón-Aguilar & Barea 1997) as well as tolerance to environmental stresses such as salinity, drought and heavy metal contamination (Cantrell & Linderman 2001, Auge 2001, Hildebrandt *et al.* 1999). Mycorrhizal fungi thrive on the carbohydrates released by host plants. It is estimated that up to 20 percent of the total carbon fixed by the host plants may be used by mycorrhizal fungi (Graham 2000).

The majority of plant families have at least one type of mycorrhizal association (for exceptions see Brundrett 2002 and references cited therein). There are a number of different mycorrhizal types including arbuscular mycorrhizas, ectomycorrhizas, orchid mycorrhizas and ericoid mycorrhizas (Brundrett 2004). Of these associations, arbuscular mycorrhizas are the most widespread and important, colonizing the majority of all vascular plants (Schüßler *et al.* 2001).

Figure 1. Close up of flowering branch of *S. fraseri*. Scale is approximately 1 cm.

The main AM fungal taxa recorded in plants in the family Fabaceae are those from the large *Glomus* genus. In France different species of *Medicago* were associated with a variety of *Glomus* spp. (Sykorova *et al.* 2007, Pivato *et al.* 2007) while in Germany *Trifolium repens* associated with both *G. mosseae* and *G. caledonium* (Geue & Hock 2004). In Australia, although a number of studies have recorded AM colonisation in a variety of native Fabaceae (e.g., McGee 1986, Bellgard 1991, Brundrett & Abbott 1991, Torpy *et al.* 1999) few studies have identified the fungal partners of such plants.

Molecular methods based on PCR amplification of taxonomically important DNA regions are currently being utilized for AM fungal identification (Ahulu *et al.* 2006; Vallino *et al.* 2006). Targeted sequences include the large subunit (LSU), the small subunit (SSU) and the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA) genes (Pivato *et al.* 2007, Ahulu *et al.* 2006, Renker *et al.* 2006, Vallino *et al.* 2006, Redecker *et al.* 2007). The ITS regions, which are located between the SSU and 5.8S rRNA coding regions and between the 5.8S and LSU coding regions and are found in all known nuclear rRNA genes of eukaryotes, have a high level of genetic diversity (Kjoller & Rosendahl 2000, Redecker & Raab 2006), even within single AM fungal spores (Sanders *et al.* 1995). Nuclear LSU and SSU rDNA sequences evolve slowly, have small amounts of within species diversity and are the sequences of choice when identifying AM fungi, particularly in environmental samples (Pivato *et al.* 2007, Ahulu *et al.* 2006; Vallino *et al.* 2006, Wubet *et al.* 2006).

The first specific primer designed for AM fungi was VANS1 (Simon *et al.* 1992). This primer did not amplify all AM fungi as it was only based on three SSU sequences (Redecker 2002). A primer known as AM1 was created by Helgason *et al.* (1998) and was designed to match all SSU RNA sequences that were known at the time for the Glomales. Vallino *et al.* (2006) warned that the primer may not be specific for all AM fungal taxa as though it amplified most Glomeromycota taxa (i.e., Glomaceae, Acaulosporaceae, Diversisporaceae and Gigasporaceae), it did not match the highly divergent SSU sequences of the basal families; Archaeosporaceae and Paraglomaceae, which were previously unrecognized groups. Despite this, AM1 is routinely used in combination with the eukaryotic primer NS31 during PCR and is the most broadly applicable primer combination suitable for field studies (Daniell *et al.* 2001, Helgason *et al.* 2002, Husband *et al.* 2002, Opik *et al.* 2003, Santos *et al.* 2006, Vallino *et al.* 2006).

The main objective of this project was to identify the AM fungal partner(s) of *Sophora*

fraseri under field conditions. Roots of *S. fraseri* were sampled across four populations in south-east Queensland and DNA was extracted, PCR amplified with AM fungal-specific SSU primers, cloned and sequenced. These methods have enabled assessment of the AM fungal taxa colonising this threatened plant species.

Materials and methods

Root collection

Three fine lateral roots were sampled from each of ten *Sophora fraseri* plants across 4 populations at Kingsthorpe, Turkey Hill, Peranga and Warwick in south-east Queensland. Roots were gently removed from the plant, placed in 1.5 mL micro-centrifuge tubes and stored until required at -80°C once back at the University of Southern Queensland, Toowoomba.

Molecular identification of *S. fraseri* mycorrhizal fungi

Roots were fragmented with a scalpel blade then ground with sterile sand, liquid nitrogen and a plastic pestle in micro-centrifuge tubes. DNA was extracted from roots with a DNeasy Plant Mini Kit (Qiagen, Doncaster, Victoria, Australia) following the manufacturers' instructions. PCR analysis of each extracted fungal DNA sample involved adding 1 µL of the extracted genomic DNA to 38 µl sterile milli-Q water, 5 µL 10 x buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100; Invitrogen, Mt Waverley, Victoria, Australia); 2.4 µL 50 mM $MgCl_2$ (Invitrogen), 1 µL 10 mM dNTP (Invitrogen), 1 µL of each of a modified AM1 primer (Helgason *et al.* 1998; 5'-GTT TCC CGT AAG GCG CCG-3') and NS31 primer (Simon *et al.* 1992; 5'-TTG GAG GGC AAG TCT GGT GCC-3') and 0.5 µL *Taq* DNA polymerase (Invitrogen). All amplification reactions were completed in duplicate sets with a negative control included. The negative control contained the master mix and 1 µL of sterilized milli-Q water. All tubes were amplified using a Thermo Hybaid-PCR Express Thermal Cylinder (Thermo Hybaid, Ashford, Middlesex, UK) with 35 cycles of 94°C and 58°C for 1 min each followed by 72°C for 2 min and a final incubation period of 10 min at 72°C. Amplicons were viewed in 2% (w/v) agarose gel containing ethidium bromide under UV light. PCR samples were purified using a DNA purification kit (Macherey-Nagel, Cheltenham, Australia) following the manufacturers' instructions. Cloning of the purified DNA samples was undertaken using the pGEM-T Easy Vector System (Promega, Annandale, NSW, Australia) following the manufacturers' instructions. A Fast Plasmid Mini Kit (Eppendorf, Nth Ryde NSW, Australia) was utilized to remove plasmid DNA from the nutrient broth solution. Plasmid DNA sizes were estimated by the use of a supercoiled DNA ladder (Invitrogen). Successfully cloned samples (determined by the size of the band after the plasmids were electrophoresed) were reamplified with PCR and SSU primers to confirm cloning of AM SSU regions.

Sequencing of fungal DNA was carried out at the Brisbane branch of the Australian Genome Research Facility (AGRF). Returned sequences were viewed with Chromas (version 2.0) to ensure sequences were not contaminated. Uncontaminated sequences were edited to remove vector sequence and to ensure correct orientation and analysed with BLAST searches through the National Centre for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) to determine closest species matches. For phylogenetic analysis AM fungal sequences were copied from the NCBI website in FASTA format. Sequences upstream from the NS31 and downstream from the AM1 primers were removed. All sequences were aligned using a ClustalX program (Thompson *et al.* 1997) with the pairwise parameters of gap opening = 10.0 and gap extension = 0.1 and multiple parameters of gap opening = 10.0 and gap extension = 0.2. The nexus file was opened in TreeView (version 1.6.6) where a neighbour-joining phylogenetic tree was created and the values were bootstrapped with 1000 replicates.

Root clearing of *S. fraseri* root remnants

S. fraseri root remnants (following DNA extraction) were cleared by boiling in 1 M KOH for 20 min. The KOH was drained from tubes and roots were rinsed with tap water followed by 0.5 M HCl and a final rise with tap water. Trypan blue (0.05% in lactoglycerol) was applied to each sample ensuring the roots were covered. Tubes were placed in a 100°C heat block for 5 min. Trypan blue was drained from the tubes and the roots were rinsed in a watch glass with a 50% glycerol solution to remove any excess stain. Segments were mounted on slides in glycerol with a cover slip applied, gently squashing the roots. Nail polish was applied to the edges of the cover slip, sealing the root sections. Each slide was observed using a compound microscope at low

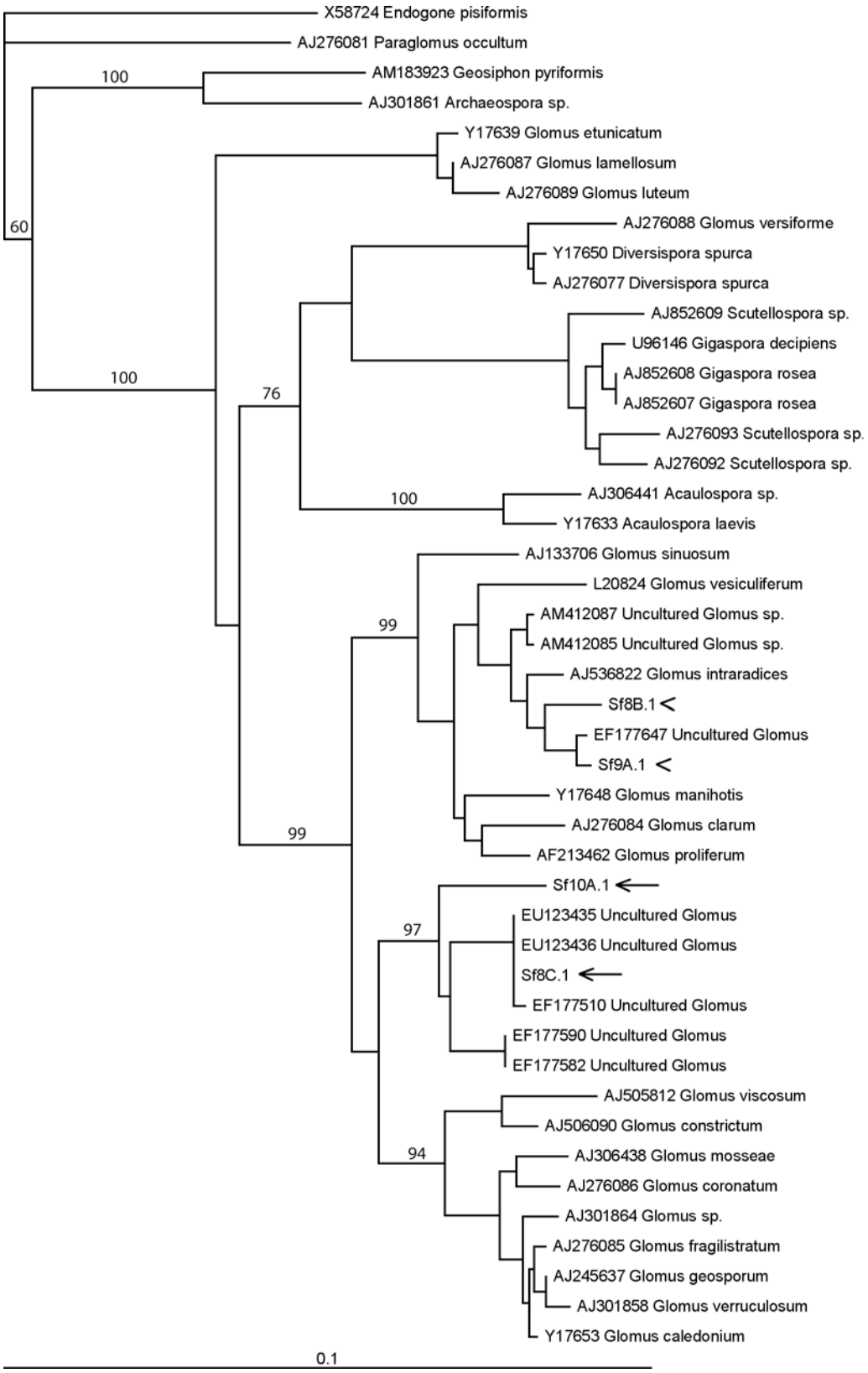

Figure 2. Neighbour-joining phylogenetic tree of fungal SSU DNA sequences obtained from *S. fraseri* from Warwick. Note there are four taxa of AM fungi isolated from these plants: roots Sf8b and Sf9a contained fungi related to *Glomus intraradices* (arrow heads) while roots Sf10A and Sf8C contained unidentified *Glomus* species (arrows). Bootstrap values are based on 1000 replicates with percentage values shown.

and high magnifications for determination of colonisation of AM fungi. Colonisation was confirmed by presence of hyphae, vesicles, or arbuscules. Pictures were taken utilizing a Micropublisher 5.0 digital camera (QImaging, Canada) on a Nikon E600 upright microscope (Nikon Corporation, Tokyo, Japan).

Results

Molecular identification of *S. fraseri* root-associated fungi

Although some root DNA extracts required a dilution of 1:10 or more (up to 1:100) to achieve an amplification product, fungal genomic DNA was successfully amplified from 20 of 30 *S. fraseri* roots. In 16 samples a single band of approximately 600 bp was amplified, however three samples contained an additional band greater than 600 bp, while one sample contained only a single band of greater than 600 bp (not shown). As no fungal DNA was amplified from the Turkey Hill plant, three more root samples were collected from this site. A PCR band of approximately 600 bp similar to the majority of initial samples was observed in two of these root samples. A total of 40 clones were ultimately obtained from the samples but following sequencing, a number of these did not contain recognisable SSU sequences, contained untransformed vector or were found to be contaminated when viewed in Chromas.

Sequencing of clones from the Warwick *S. fraseri* plants revealed a number of fungal species present. In plant 8 two taxa of the AM fungal genus *Glomus* were present (Table 1). While one of these species is related to *Glomus intraradices* (Figure 2), the other appears to be an undescribed species of *Glomus* (Figure 2). Plant 9 also contained a *Glomus* species related to *Glomus intraradices* while plant 10 contained an undescribed species of *Glomus.* Both plants 8 and 10 contained the same deuteromycete fungus, *Phoma* (Table 1). BLAST searches showed that three sequences from the Kingsthorpe plants and two sequences from the Peranga plants had no similarity to any fungal sequences in GenBank (Table 1). Plant 5 contained an *Elsinoë* species while plants 6 and 7 contained the same species of *Phoma* that was found in plants 8 and 10 from Warwick (Table 1).

AM colonisation of *S. fraseri* root remnants

Arbuscular mycorrhizal fungi were found occupying 28 of the 33 *S. fraseri* root remnant samples. Colonisation was indicated by intercellular hyphae, vesicles and arbuscules (Figure 3). In 25 of the remnant roots, non-AM hyphae and conidia were observed (not shown).

Discussion

The AM fungal taxa isolated from *S. fraseri* in this study were *Glomus* spp. This is not a surprising result as the genus is the largest within the Glomeromycota (Redecker & Raab 2006) and it dominates soil communities worldwide (Helgason *et al.* 1998, Opik *et al.* 2003). The Fabaceae appear to be readily colonised by members of this fungal genus under both natural (Sykorova *et al.* 2007, Geue & Hock 2004, Pivato *et al.* 2007) and laboratory conditions (Harrison & Dixon 1993, Hoffman *et al.* 2007, Meixner *et al.* 2007). The identification of fungi closely related to *G. intraradices* further highlights the cosmopolitan nature of this AM fungal species which has been recorded in a variety of habitats throughout the world (reviewed in Opik *et al.* 2006). Morphological examination of spores and *in planta* structures of pure cultures of the unknown *Glomus* spp. will assist with naming these taxa.

The failure to isolate identifiable AM fungal SSU sequences from the majority of roots is perplexing as microscopic assessment indicated AM colonisation in most roots. It is possible that the roots contained compounds inhibitory to the PCR process, that the DNA of contaminating fungi ie. *Phoma* and *Elsinoë* was preferentially amplified over AM-fungal DNA or that there was a failure to select AM SSU sequences from the mixture of transformed clones. Non-AM fungal amplification has been a problem in the past in molecular studies of *in planta* AM fungi when utilizing the AM1/NS31 primer combination (Douhan *et al.* 2005, Helgason *et al.* 1999, Rodriguez-Echeverria & Freitas 2006). Douhan *et al.* (2005) found a domination of ascomycota and basidiomycota sequenced DNA in *Quercus douglasii* and *Torilis arvensis*. Only 4 of 13 (30%) sequences obtained from *Q. douglasii* were AM fungal in origin and *T. arvensis* had an even lower rate of 2 of 14 (14%) (Douhan *et al.* 2005). The design of strictly AM fungal specific PCR primers is a necessity for further molecular

Table 1. Fungal SSU DNA samples from *S. fraseri* roots and the two closest GenBank matches, accession codes and sequence overlap. *Individual clones are identified by plant number first, root by letter second and clone number last.

Site	Clone*	GenBank code	GenBank closest species match	Accession codes	Sequence overlap - bp
Kingsthorpe	Sf1C.1	EU088240	No significant similarity	-	0/558 (0%)
Kingsthorpe	Sf2B.1	EU088241	No significant similarity	-	0/558 (0%)
Kingsthorpe	Sf3C.1	EU088242	No significant similarity	-	0/558 (0%)
Peranga	Sf5C.1	EU088243	*Elsinoë phaseoli*	DQ678042	520/546 (95%)
			Elsinoë centrolobi	DQ678041	520/546 (95%)
Peranga	Sf6A.1	EU088244	*Phoma* sp.	AY646226	540/546 (98%)
			Phoma medicaginis	EU167575	538/546 (98%)
Peranga	Sf6A.2	EU194872	No significant similarity	-	0/558 (0%)
Peranga	Sf6B.1	EU088245	No significant similarity	-	0/564 (0%)
Peranga	Sf6C.1	EU088246	*Phoma* sp.	AY646226	541/546 (99%)
			Phoma medicaginis	EU167575	539/546 (98%)
Peranga	Sf7A.1	EU088247	*Phoma* sp.	AY646226	540/546 (98%)
			Phoma medicaginis	EU167575	538/546 (98%)
Warwick	Sf8A.1	EU088249	*Phoma* sp.	AY646226	541/546 (99%)
			Phoma medicaginis	EU167575	539/546 (98%)
Warwick	Sf8A.2	EU194874	*Phoma* sp.	AY646226	539/546 (98%)
			Phoma medicaginis	EU167575	537/546 (98%)
Warwick	Sf8B.1	EU088250	Uncultured *Glomus*	AM412087	541/549 (98%)
			Uncultured *Glomus*	EF177647	541/549 (98%)
Warwick	Sf8C.1	EU088251	Uncultured *Glomus*	EU123436	548/548 (100%)
			Uncultured *Glomus*	EU123435	548/548 (100%)
Warwick	Sf9A.1	EU088252	Uncultured *Glomus*	EF177647	546/549 (99%)
			Uncultured *Glomus*	AM412085	541/549 (98%)
Warwick	Sf10A.1	EU088254	Uncultured *Glomus*	EF177590	535/548 (97%)
			Uncultured *Glomus*	EF177582	535/548 (97%)
Warwick	Sf10B.1	EU088255	*Phoma* sp.	AY646226	540/546 (98%)
			Phoma medicaginis	EU167575	538/546 (98%)

ecological studies of this important mycorrhizal group.

The identity of the sequences from Kingsthorpe and Peranga plant roots is not known. It is possible that these represent new genera of AM fungi but molecular and morphological analysis of pure cultures would be required to confirm this. These sequences failed to align with any other AM fungal taxa in NJ phylogenetic analysis and thus it seems likely that they are artefactual in nature i.e., during the initial PCR process the AM1 and NS31 primers misannealed and the artifact was then amplified in subsequent PCR cycles (Weiß pers. comm.). Contributing to this outcome was the modified AM1 primer used in the study, which may have decreased the specificity of the amplification.

Molecular taxonomic work on AM fungi is complicated by the multinucleate status of the hyphae and spores. Regions that are used for identification also show some variation within a

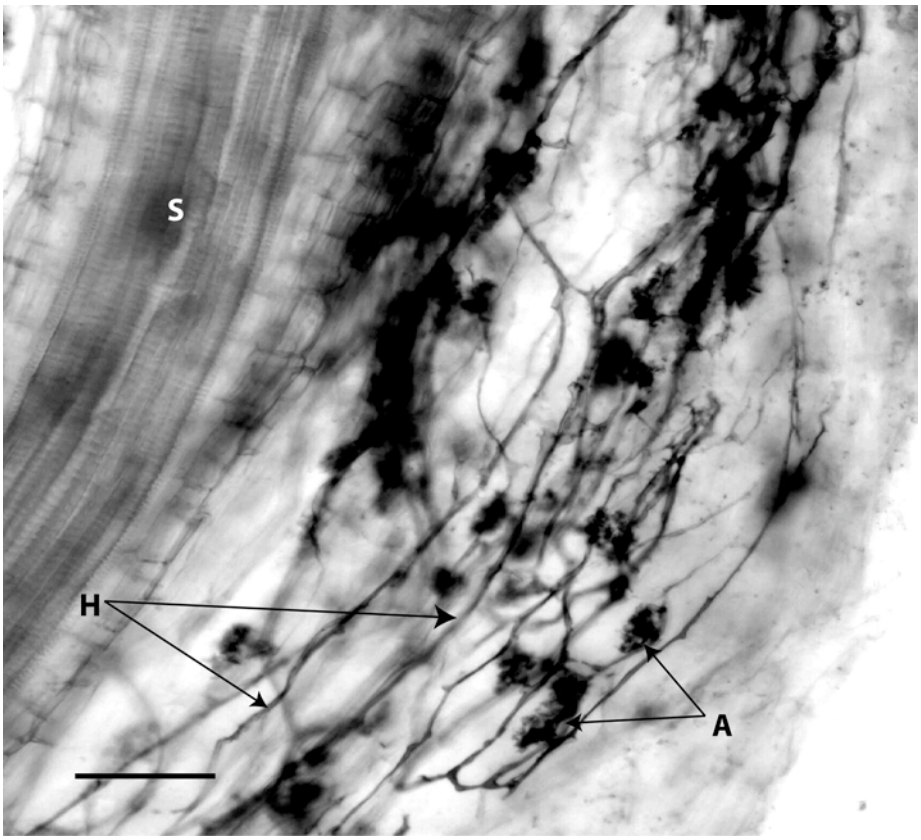

Figure 3. Arbuscules (A) and intercellular hyphae (H) in root sample Sf9A (S = stele). Scale bar = 100 µm.

single spore, causing classification problems and complicating diversity estimates (Sanders *et al.* 1995). Raab *et al.* (2005) avoided these problems by analysing sequences from an independent genetic system, the mitochondria and designed a PCR primer to amplify a fragment of the mtLSU of an AM fungus. Results of this approach indicated that AM fungal mitochondria do not have heterogenic DNA sequences (Raab *et al.* 2005). Mitochondrial LSU have the potential to distinguish different isolates of the same species (Raab *et al.* 2005), though use of the approach is restricted as the database (e.g., GenBank) of AM fungal mtLSU sequences is currently small.

In summary we have identified *Glomus* spp. in roots of *S. fraseri* using DNA extraction, PCR amplification, cloning and sequencing of fungal ribosomal SSU DNA. Further sampling of the species across a wider range and optimisation of the PCR process and cloning procedure may reveal the complete diversity of the AM fungi colonising the species. Additional investigations may involve using universal fungal primers so that other groups of AM fungi not detected with the AM1-NS31 primer pair are highlighted. Such an approach may also identify other mycorrhizal fungal types which have been shown to associate with Australian Fabaceae (Warcup 1980, Brundrett & Abbott 1991, Bellgard 1991) which may be critical to the growth of *S. fraseri*.

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References

Azcón-Aguilar, C. & Barea, J.M. (1997). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. *Mycorrhiza* **6**, 457-464.

Ahulu, E.M., Gollotte, A., Gianinazzi- Pearson, V. & Nonaka, M. (2006). Cooccurring plants forming distinct arbuscular mycorrhizal morphologies harbor similar AM fungal species. *Mycorrhiza* **17**, 37-49.

Auge, R.M. (2001). Water relations, drought and vesicular-arbucular mycorrhizal symbiosis. *Mycorrhiza* **11**, 3-42.

Bellgard, S.E. (1991). Mycorrhizal Associations of plant species in Hawkesbury sandstone vegetation. *Australian Journal of Botany* **39**, 357-364.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**, 275-304.

Brundrett, M.C. (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews* **79**, 473-495.

Brundrett, M.C. & Abbott, L.K. (1991). Roots of Jarrah Forest Plants. I. Mycorrhizal associations of shrubs and herbaceous plants. *Australian Journal of Botany* **39**, 445-457.

Cantrell, I.C. & Linderman, R.G. (2001). Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil* **233**, 269-281.

Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. (2001). Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. *FEMS Microbiology Ecology* **36,** 203-209.

Department of Environment and Climate Change, NSW (2005). *Brush Sophora - Profile*, <http://threatenedspecies.environment.nsw.gov.au/tsprofile/profile.aspx?id=10764>. Accessed 8th January 2008.

Douhan, G.W., Petersen, C., Bledsoe, C.S. & Rizzo, D.M. (2005). Contrasting root associating fungi of three common oak-woodland plant species based on molecular identification: host specificity or non-specific amplification. *Mycorrhiza* **15**, 365-372.

Geue, H. & Hock, B. (2004). Determination of *Acaulospora longula* and *Glomus* subgroup Aa in plant roots from grassland using new primers against the large subunit ribosomal DNA. *Mycological Research* **108**, 76-83.

Graham, J.H. (2000). Assessing cost of arbuscular mycorrhizal symbiosis agroecosystems fungi, in G.K. Podila, D.D. Douds Jr (eds), *Current Advances in Mycorrhizae Research,* pp 127-140, APS Press, St. Paul, USA.

Harrison, M.J. & Dixon, R.A. (1993). Isoflavonoid accumulation and expression of defense gene transcript during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula*. *MPMI* **6**, 643-654.

Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. (1998). Ploughing up the wood-wide web? *Nature* **394**, 431.

Helgason, T., Fitter, A.H. & Young, J.P.W. (1999). Molecular diversity of arbuscular mycorrhizal fungi colonizing *Hyacinthoides non-scripta* (bluebell) in semi natural woodland. *Molecular Ecology* **8**, 659-666.

Helgason, T., Merryweather, J.W., Denison, J., Wilson, P., Young, J.P.W. & Fitter, A.H. (2002). Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**, 371-384.

Hildebrandt, U., Kaldorf, M. & Bothe, H. (1999). The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* **154**, 709-717.

Hoffmann, D., Jiang, Q., Men, A., Kinkema, M. & Gresshoff, P.M. (2007). Nodulation deficiency caused by fast neutron mutagenesis of the model legume *Lotus japonicus*. *Journal of Plant Physiology* **164**, 460-469.

Husband, R., Herre, E.A., Turner, S.L., Gallery, R. & Young, J.P.W. (2002). Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**, 2669-2678.

Kjoller, R. & Rosendahl, S (2000). Detection of arbuscular mycorrhizal fungi (Glomales) in roots by nested PCR and SSCP (Single Stranded Conformation Polymorphism). *Plant and Soil* **226**, 189-196.

Meixner, C., Vegvari, G., Ludwig-Muller, J., Gagnon, H., Steinkellner, S., Staehelin, C., Gresshoff, P. & Vierheilig, H. (2007). Two defined alleles of the LRR receptor kinase GmNARK in supernodulating soybean govern differing autoregulation of mycorrhization. *Physiologia Plantarum* **130**, 261-270.

McGee, P.A. (1986). Mycorrhizal associations of plant species in a semiarid community. *Australian Journal of Botany* **34**, 585-593.

Nicholson, H. & Nicholson, N. (2000). Australian Rainforest Plants in the forest and in the garden.V. Terania Rainforest Publishing, The Channon, NSW, Australia.

Opik, M., Moora, M., Liira, J., Koljalg, 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 Phytologist* **160**, 581-593.

Opik, M., Moora, M., Liira, J. & Zobel, M. (2006). Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* **94**, 778-790.

Pivato, B., Mazurier, S., Lemanceau, P., Siblot, S., Berta, G., Mougel, C. & van Tuinen, D. (2007). *Medicago* species affect the

community composition on arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**, 197-210.

Raab, P.A., Brennwald, A. & Redecker, D. (2005). Mitochondrial large ribosomal subunit sequences are homogeneous within isolates of *Glomus* (arbuscular mycorrhizal fungi, Glomeromycota). *Mycological Research* **109**, 1315-1322.

Redecker, D. (2002). Molecular identification and phylogeny of arbuscular mycorrhizal fungi. *Plant and Soil* **244**, 67-73.

Redecker, D. & Raab, P. (2006). Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**, 885-895.

Redecker, D., Raab, P., Oehl, F., Camacho, F.J. & Courtecuisse R. (2007). A novel clade of sporocarp-forming species of glomeromycotan fungi in the *Diversisporales* lineage. *Mycological Progress* **6**, 35-44.

Renker, C., Weißhuhn, K., Kellner H. & Buscot, F. (2006). Rationalizing molecular analysis of field-collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, that is the question. *Mycorrhiza* **16**, 525-531.

Rodriguez-Echeverria, S. & Freitas, H. (2006). Diversity of AMF associated with *Ammophila arenaria* ssp. *arundinacea* in Portuguese sand dunes. *Mycorrhiza* **16**, 543-552.

Sanders, I.R., Alt, M., Groppe, K., Boller, T. & Wiemken, A. (1995). Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist* **130**, 419-427.

Santos, J.C., Finlay, R.D. & Tehler, A. (2006). Molecular analysis of arbuscular mycorrhizal fungi colonizing a semi-natural grassland along a fertilization gradient. *New Phytologist* **172**, 159-168.

Schüßler, A., Schwarzott, D. & Walker, C. (2001). A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**, 1413-1421.

Simon, L., Lalonde, M. & Bruns, T.D. (1992). Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Ecology* **58**, 291-295.

Smith, S.E. & Read, D.J. (1997). *Mycorrhizal Symbiosis*, 2nd edition, Academic Press, London.

Sykorova, Z., Ineichen, K., Wiemken, A. & Redecker, D. (2007). The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. *Mycorrhiza* **18**, 1-14.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876-4882.

Torpy, F.R., Morrison, D.A. & Bloomfield, B.J. (1999). The influence of fire frequency on arbuscular mycorrhizal colonisation in the shrub *Dillwynia retorta* (Wendland) Druce Fabaceae. *Mycorrhiza* **8**, 289-296.

Vallino, M., Massa, N., Lumini, E., Bianciotto, V., Berta, G. & Bonfante, P. (2006). Assessment of arbuscular mycorrhizal fungal diversity in roots of *Solidago gigantea* growing in a polluted soil in Northern Italy, *Environmental Microbiology* **8**, 971-983.

Warcup, J.H. (1980). Ectomycorrhizal associations of Australian indigenous plants. *New Phytologist* **85**, 531-535.