Arbuscular mycorrhizal fungal composition in semi-arid soils of Western Ghats, southern India

Plants growing in arid and semi-arid soils like deserts, grasslands and scrublands are commonly associated with arbuscular mycorrhizal (AM) fungi. Despite the prevalence of AM association in these soils, studies on composition and structure of AM fungal communities are limited. In India, studies on AM in arid and semi-arid regions have been mainly concentrated on mycorrhizal status of plants rather than AM fungal communities. A few studies however, have reported AM fungal species from these soils, which indicates limited species distribution. The Western Ghats, a valuable repository for biodiversity after the Himalayas, is one of the 25 mega diversity hot spots of the world. It contains ~ 4000 (27%) of the country’s plant species, of which 38% (1500 species) are endemic. The high biodiversity of the Western Ghats can be attributed to its varied habitat types ranging from semi-arid grasslands to tropical rainforests. We recently reported mycorrhizal association in several plant species from different habitat types from the Western Ghats region, southern India. However, the species composition in AM fungal communities from different habitats of the Western Ghats is largely unknown.

The only method to assess species composition of AM fungal assemblages is enumeration and identification of spores in the soil. Repeated field collections or establishment of successive trap cultures greatly improve the assessment of species composition in natural ecosytems. One of the major limitations of direct field assessment is the low level of spores that can be collected. Further, spores of some species can be absent at the time of sampling, even though they may be present within roots, resulting in low species richness. Alternatively, establishment of trap cultures greatly improves the assessment of species composition in an ecosystem. All reports on AM fungal communities in arid and semi-arid Indian soils are those of single or repeated field collections. The aim of this study was to record and find whether similarity exists among AM fungal communities in different vegetation types in semi-arid soils and also to test the influence of the methods used to determine AM fungal species on AM fungal community composition. To assess these, we examined AM fungal species composition in a semi-arid grassland and scrubland in the Western Ghats, by isolating AM fungal spores directly from the soil and also using successive pot culture to induce sporulation.

The study was conducted at Maruthamalai Hills (11°04’N and 76°93’E, altitude 426.7–550 m asl) an offshoot of the Western Ghats in southern India. The climate is monsoon and total annual rainfall ranges between 500 and 700 mm. Average monthly maximum and minimum temperature ranges from 27 to 37°C and 15 to 24°C, respectively. The vegetation is Cymbopogon caesius Stapf., dominated ungrazed grasslands at the foothills and scrub jungles at slopes dominated by Acacia species.

Fifty soil samples were collected from the rhizosphere of Accacia species and C. caesius between October 2000 and January 2001. Approximately 2 kg of rhizosphere soil was collected up to a depth of 25 cm from each point and placed in polythene bags for transport. Soil samples were air-dried and stored at room temperature before use. To establish successive pot cultures (three cycles), 500 g field soil was mixed with autoclaved sand (1:1, v:v) and seeded with sorghum and cowpea as hosts, according to the protocol of Strutz and Morton. Spores of AM fungi in field and trap culture soils were extracted by wet-sieving and decanting technique and enumerated. Spore density was expressed per 100 g dry soil. Sporocarps and spore clusters were taken as one unit to count. Spores of each morphotype were mounted in polyvinyl alcohol–lactic acid–glycerol (PVLG) and PVLG mixed with Melzer’s reagent (1:1, v:v). Whole and broken spores were examined using a compound microscope. Taxonomic identification of spores to species level was based on spore size, colour, ornamentation and wall characteristics by matching original descriptions and those provided by the International Collection of Vascular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu). Total spore population data were ln(x + 1) transformed prior to statistical analysis. Paired t test was performed using SPSS to compare individual and total AM fungal spore densities among vegetation types.

The soils at the study sites were an Alfisol and soil chemical analysis indicated low nutrient availability, especially phosphorus (0.58–0.72 mg kg⁻¹ soil). Low spore populations were observed in most soil samples with high variance and the vegetation effect on total spore count was significant (t₀ = 2.561; P < 0.014). Total spore counts averaged around 75.26 ± 3.87 and 62.84 ± 2.98 (mean ± SE) spores 100 g⁻¹ soil for grassland and scrubland, respectively. In contrast, high spore populations have been reported by Mukerji and Kapoor (140–280 spores 100 g⁻¹ soil), Singh and Varma (257–1399 spores 15 g⁻¹ soil), and Neeraj et al. (50–710 spores 50 g⁻¹ soil) from Indian semi-arid soils. Plant senescence or inactivity, either natural or induced is known to trigger AM fungal sporulation. Consequently, seasonal grassland and scrubland studied here is expected to have large spore populations than aseasonal vegetation, but the low spore populations observed are rather surprising. However, the low spore counts need not imply a low inoculum potential of these soils, as important propagules of AM fungi also include mycorrhizal roots, organic materials occupied by AM fungal structures and a network of hyphae in the soil.

In the present study two methods were used for characterizing the AM fungal community and many of the identified AM fungi are shown in Figures 1 and 2. Assessment of field soils directly yielded a total of 10 and 8 species respectively, in grasslands and scrublands. However, a large proportion of these spores were devoid of contents and/or parasitized, indicating that they were dead or merely spore cases. Spores
Figure 1.  

(a–l) Photomicrographs of arbuscular mycorrhizal fungi in semi-arid soils; 

(a) Spore of Acaulospora scrobiculata (SP) attached to sporiferous sacculus (SS); 

(b) Fractured spore of A. scrobiculata in Melzer’s reagent. Note the purple reaction of the innermost wall (arrow); 

(c) Surface pitting of A. scrobiculata spore; 

(d) Auxiliary cells of Gigaspora gigantea; 

(e) Spores of Glomus aggregatum; 

(f) Spores of Glomus geosporum; 

(g) Funnel-shaped subtending hyphae and curved septum of Glomus mosseae; 

(h) Spores of Glomus sp. 1 within root; and 

(i) Spore of Glomus intraradices. (Scale bar: a–c, i = 50 μm; d–f, h = 100 μm; g = 20 μm.)
Figure 2. a–h, Photomicrographs of more arbuscular mycorrhizal fungi in semi-arid soils; a, Part of Glomus taiwanensis sporocarp; b, Crashed sporocarp of Glomus sinuosum. Note the peridium (P); c, Spores of Glomus viscosum; d, Spore of Scutellospora calospora; e, Fractured spore of S. calospora in Melzer’s reagent. Numbers indicate wall layers. Note the purple reaction of wall 4; f, Spore of Scutellospora heterogama; g, Surface ornamentation in S. heterogama spore; and h, Auxillarv cells of S. heterogama. (Scale bar: a, g = 20 μm; b, d, e, h = 50 μm; c, f = 100 μm.)
of AM fungi retrieved directly from the field soils included one species in Acacia (Acacia sp., Glomus sp., and Gigaspora sp.) and two species in Gigasporaceae. Walker & Sanders, and S. heterogama (Nicol. & Gerd.) Walker & Sanders) and eight species in Glomaceae (Glomus aggregatum Schenck & Smith emend Koske, G. geosporum (Nicol. & Gerd.) Walker, G. intraradices Schenck & Smith, G. microaggregatum Gemma, Koske & Olexia, G. mosseae (Nicol. & Gerd.) Gerd. & Trappe, G. sinuosum (Gerd. & Bakshi) Almeida & Schenck, G. taiwanaensis (Wu & Chen) Almeida & Schenck and G. vicosum Nicolson.

The successive trap-culture technique used for isolation of AM fungi successfully yielded three additional species (Acacia sp., Glomus sp., and Gigaspora sp.) and two additional species (G. etunicatum Becker & Gerd. and Glomus sp.) from the soil samples. This finding is pertinent to studies reporting the reappearance of additional fungal species in trap cultures started from field soils. However, the successive trap-culture technique was not successful for certain species like Glomus sinuosum and G. taiwanaensis, as we did not find any new sporecarps of these species in trap-culture soils nor was there any increase in their frequency of occurrence. The biology of Glomus species that once constituted the genus Sclerotocystis is little known and pot cultures of these fungi have often failed (http://invam.caf.wvu.edu).

The generic diversity of AM fungi in semi-arid soils studied here appears to be more elaborate than those reported from Indian arid and semi-arid soils, where species are restricted to Glomaceae and Gigasporaceae, with species in Glomaceae dominating. In contrast, this study indicates the frequent occurrence of A. sclerocaulata and an Acacia sp. in addition to members of Glomaceae and Gigasporaceae. However, members of Glomaceae recorded the highest species diversity. The total species richness recorded per vegetation type using direct field assessment and trap cultures (10 and 13 species) is higher than those reported from Indian arid and semi-arid soils and six or less species reported by Allen et al. across 64 semi-arid sites in North America. However, Stutz et al. reported 6 to 12 species in arid soils of southwestern America and Namibia, Africa. Available evidence indicates that community structure of AM fungi in arid environments is influenced by local ecological forces. Seven species (A. sclerocaulata, G. aggregatum, G. geosporum, G. mosseae, G. sinuosum, G. vicosum and S. calospora) were detected in both vegetation types which corroborate reports of Johnson and Wedin who also found high similarity in AM fungal species composition in a Costa Rican grassland and an adjacent forest. The high degree of overlap between species composition found in the two vegetation types surveyed suggests that similar selection pressure factors operate in these adjacent habitats.

The isolates of AM fungi vary greatly in spor production; some isolates produce copious spores while others sporulate sparsely. So interspecific comparison of spore populations is generally not useful, whereas intraspecific comparison (across vegetation) for isolates may be meaningful. Such a comparison revealed that among the seven common AM fungi occurring at both vegetation types, spore populations of three species (G. geosporum, G. mosseae, G. vicosum) were significantly affected by vegetation type. Though AM fungi are assumed to be non-host specific and determine plant community composition, recent studies, however, do indicate the existence of host preference by AM fungi, substantiating the vegetational effect observed.

The frequent occurrence of A. sclerocaulata spores in trap cultures from both vegetation types reflects its high competitiveness over other species under the culture conditions. Fungi that sporulate rapidly in pot cultures may be more aggressive colonizers of root, and/or adjust faster to changes in soil conditions than other fungi. Average spore numbers of members of Gigasporaceae across both sites were lower (3.68 ± 100 g⁻¹ soil) compared to average spore numbers of many members of Aculosporaceae (9.66 ± 100 g⁻¹ soil) and Glomaceae (10.32 ± 100 g⁻¹ soil). AM fungi differ in their requirements for carbon, or alternatively other small-spored species more efficiently utilize the scarce carbon resource. In addition, the energetic expenditure required to produce large spores occurs at a greater cost to the mycobiont than for small spores, although such cost for reproduction has yet to be determined experimentally for mycorrhizas.

In conclusion, a combined use of different isolation methods, as used in this study may give much valuable information about AM fungal community rather than individual methods, since each method reflects only a part of the fungal flora. Furthermore, results of this study also provide an evidence for high AM fungal species diversity in semi-arid soils similar to mesic habitats and the influence of vegetation on the abundance of AM fungal species. However, further studies on characterization of AM fungal species in these semi-arid vegetation types could indicate whether ecotypic differences (e.g. symbiotic effectiveness, etc.) do exist within species and enlighten the effects of physiological or life history attributes that may be of interest in these soils.

Effect of S-allyl cysteine sulphone on lipid metabolism and free radical scavengers in alcohol-fed rats

Alcohol consumption is increasing steadily in humans all over the world, and it has become one of the serious health hazards. Several harmful effects of alcohol are known, but liver is the major organ that is most susceptible to the toxic effects of ethanol\(^1\). Ethanol can also alter the normal function and composition of membranes, which may ultimately lead to serious cellular impairment. Ethanol administration causes alcoholic fatty liver and hyperlipidemia\(^2\). Hepatic cirrhosis is a major cause of death in young and middle-aged individuals who are chronic alcoholics. Partial protection against alcoholic fatty liver has been observed on administration of antioxidants\(^3\).

Garlic (*Allium sativum* Linn) and compounds derived from it are found to have many therapeutic effects\(^4\). There are several reports on the effect of garlic extract on lipid metabolism, lipid peroxidation and antioxidant enzymes\(^5\)-\(^7\). Members of *Allium* family are reported to have antimutagenic properties and therapeutic values and possess diuretic properties, aid in digestion, act as a heart stimulant and alleviate rheumatic problems\(^8\). These properties of garlic can be attributed to the presence of sulphur compounds present in it. S-allyl cysteine sulphoxide (SACS), commonly called allin, is an amino acid isolated from garlic\(^9\) and is found to have a major effect on lipid metabolism.

Garlic was purchased from the local market. SACS was extracted from fresh garlic according to the method of Itohara *et al.*\(^10\) with some modifications, using ion exchange resins washed with 1N HCl, 1N NaOH or deionized water. Fresh garlic was boiled in water to inactivate the enzyme allinase. It was then ground and extracted with 80% methanol, filtered and passed through a column of amberlite IR-120 (strong cation exchanger) so as to absorb the amino acids. The column was washed with deionized water to remove the impurities and the amino acids were eluted with NH\(_2\)OH (2N). Ammonia was removed by concentration of the eluates in a rotary evaporator at 40–43°C and the concentrate was loaded onto a column of amberlite CG-120 (strong cation exchanger). The column was washed with deionized water and the amino acids eluted with 0.1N NH\(_2\)OH. Fractions of the effluent were tested for the presence of SACS by thin layer chromatography (TLC) as described below. The SACS containing fractions were pooled, concentrated and passed through a column of amberlite IRA-45 (weakly basic anion) so that unwanted amino acids and ammonia were adsorbed onto the resin and removed. The effluent was collected and concentrated. Pure SACS was obtained from it after three recrystallization steps from 80% ethanol. It had a melting point of 164°C. The yield of SACS was 1.06 g/kg garlic. In TLC on silica gel G, using butanol: acetic acid : water (12 : 3 : 5) pure SACS gave an *R*\(_f\) value of 0.24, similar to that of an authentic sample obtained from the Biochemical Institute, Helsinki.

Male albino rats of Sprague Dawley strain weighing 100–120 g were used for the study. They were maintained under environmentally controlled conditions with free access to standard food (Lipton, India) and water. They were divided into three groups of six rats each. A dose of 18% alcohol was administered orally for a period of 50 days. The animals were grouped as follows for the experiment: (1) Group I: Normal diet, ad lib; (2) Group II: Normal diet ad lib and 18% alcohol (4 g alcohol/kg body weight/day) orally; (3) Group III: Normal diet ad lib, 18% alcohol (4 g alcohol/kg body weight/day) along with SACS (500 mg/kg body weight/day) orally.

The rats were maintained on respective diet for a period of 50 days. The normal control group showed a gradual increase in body weight during the experimental period. The alcohol-treated group showed an increase in body weight during the initial period of 2 weeks and later on showed a gradual decrease in body weight up to the end of the experimental period. On the other hand, the SACS-treated group showed a regular increase in body weight during the entire experimental period. At the