Mycosphere 6 (2): 150–158 (2015)
www.mycosphere.org

Article
ISSN 2077 7019
Mycosphere
Online Edition
Doi 10.5943/mycosphere/6/2/5

Diversity and species composition of arbuscular mycorrhizal fungi in *Clerodendrum* species

Songachan LS*, Kayang H and Moinao P

Microbial Ecology Laboratory, Department of Botany, North Eastern Hill University, Shillong-793 022, India.

Songachan LS, Kayang H, Moinao P 2015 – Diversity and species composition of arbuscular mycorrhizal fungi in *Clerodendrum* species. Mycosphere 6(2), 150–158, Doi 10.5943/mycosphere/6/2/5

Abstract

Two *Clerodendrum* species viz., *Clerodendrum colebrookianum* and *Clerodendrum buchananii* were studied for diversity of arbuscular mycorrhizal fungi (AMF). A rhizosphere soil was found to be acidic and soil phosphorus was low for both the plant species. AMF colonization in the form of arbuscules, vesicles and hyphae were observed. Hyphal colonization was high compared to vesicles and arbuscules. The percent of AMF colonization in *C. colebrookianum* was 29.77 %, whereas in *C. buchananii* it was 25.83 %. However, AMF colonization in trap culture from *C. buchananii* derived inoculum source was found to be higher (18.07 %) as compared to *C. colebrookianum* (16.31 %). On the basis of morphological characteristics, a total of 20 AMF species belonging to two genera viz., *Acaulospora* and *Glomus* were isolated from the two studied plant species, whereas from trap culture, 14 AMF species were isolated. This study gives the gist of AMF status of two *Clerodendrum* species and it revealed that the AMF composition and diversity varies in the two *Clerodendrum* species.

Key words – colonization – diversity – mycorrhiza – rhizosphere soils

Introduction

Arbuscular mycorrhizas are symbiotic relationships between the plant roots and soil fungi belonging to phylum Glomeromycota (Schussler et al. 2001). The ubiquity of arbuscular mycorrhizal fungi (AMF) at the interface between soil and plant roots makes them a key functional group of soil biota. AMF are known to benefit plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition, water relations, disease resistance and improving soil quality (Smith & Read 2008).

Clerodendrum species comprised of small trees, shrubs and herbs that belong to the family Verbenaceae (Jadeja et al. 2012). Different species of *Clerodendrum* are present in different parts of India, although it is mostly confined to the Northeast region, with two commonly found species *Clerodendrum colebrookianum* Walp. and *Clerodendrum buchananii*

Submitted 21 October 2014, Accepted 28 February 2015, Published online 27 March 2015
Corresponding Author: Songachan LS – e-mail – rayskybl@yahoo.co.in

Roxb. These species contains saponins, flavonoids, alkaloids, tannins, glycosides and reducing sugars (Adeneye et al. 2008). Traditionally, the crude extracts of the leaves of these plants are used for treatment of high blood pressure, hypertension and for controlling rheumatism by the native people of this region. It was also reported that aqueous leaf extract of *C. glandulosum* Coleb. is traditionally used to alleviate symptoms of diabetes, obesity and hypertension by people of Northeast India (Jadeja et al. 2011). *Clerodendrum inerme* L. is a medicinal shrub reported to be used in treatment of various ailments (Somasundram & Sadique 1986, Nakata 1999, Chae et al. 2004). Several classes of secondary metabolites present in the plant extracts are responsible for various biological activities. Thus, *Clerodendrum* species have been used from time immemorial for its various medicinal properties.

Although several *Clerodendrum* species has been found to be one of the most well known medicinal plant species having a high demand, AMF association in *Clerodendrum* species has not been reported so far. One reason which is worrisome is that an increasing demand for plant products may endanger many traditionally used and pharmaceutically important plant species and their habitats (Fuchs & Haselwandter 2008). Considering the influence of AMF on plants, it is crucial that more attention should be paid to the monitoring of soil and mycorrhizal development during the process of their growth. Moreover, the importance of mycorrhiza for many plant species and the possibilities of its practical application strengthen the need for identification and cultivation of mycorrhizal fungi present in roots of naturally occurring plants (Ryszka et al. 2010). Therefore, the aim of this investigation is to study AMF diversity of *C. colebrookianum* and *C. buchananii*.

Materials & Methods

Study Site and Sampling

The study was conducted at North Eastern Hill University Campus, Meghalaya, India, located at 25°36'400"N, 091°53'57"E with an altitude of 1,424 m above sea level. Sampling was done during September to December, 2012. The roots and rhizosphere soils of *C. colebrookianum* and *C. buchananii* (ten replicates of each plant species) were collected in sterilized plastic bags and transported to the laboratory for analysis.

Estimation of AMF colonization

The root samples were washed thoroughly in tap water, cut it to approximately 1 cm and cleared in 10 % KOH for 1 h at 90 °C, acidified with 1 % HCL and stained with trypan blue (Philips & Hayman 1970). The stained root samples were mounted on microscope slides in lactoglycerol and examined for AMF colonization under light microscope. Root lengths with mycorrhizal colonization in the form of arbuscules, vesicles and hyphae in 100 root segments from each plant species were estimated using the magnified intersection method of McGonigle et al (1990).

AMF spore isolation, enumeration and identification

AMF spores were extracted by wet sieving and decanting method of Uma et al (2010). Suspension of 25 g rhizosphere soil sample in water was decanted through a series of 710 to 37 µm sieves. The residues on the sieves were washed into beaker with water and filtered through filter papers. Each filter paper was spread on petri dish and spores were counted using a dissection microscope at 40 × magnification. Sporocarps and spore clusters were considered as

one unit. AMF spores were picked up using a needle, mounted in polyvinyl alcohol-lactoglycerol with Meltzer's reagent. AMF spores were identified based on morphological characteristics such as shape, size, colour, wall ornamentation, etc. using identification keys of International culture collection of vesicular and arbuscular mycorrhizal fungi, i.e. INVAM (http://www.invam.caf.wvu.edu) and AMF phylogeny (www.amf-phylogeny.com). Spore density and species richness were expressed as number of AMF spores and numbers of AMF species in 25 g soil sample.

Trap culture

The methods of AMF trap culture were followed from INVAM (http://invam.caf.wvu.edu). Trap cultures were established using *Trifolium repens* L. as a host plant. Rhizosphere soils and roots of *C. colebrookianum* and *C. buchananii* were collected in a separate plastic bag. Roots were chopped into small fragments and mixed thoroughly with the associated soil that serves as inoculum. This chopped blend is mixed 1:1 (v/v) with autoclaved coarse sand. Seeds of *T. repens* were evenly sown on 25 cm diameter plastic pots containing the AMF inoculum and monitored in green house for five months. It was watered whenever required. After five months, the roots of the trap plants were evaluated for AMF colonization and spores were isolated and analyzed as described above.

Soil Physico-Chemical Analysis

Soil pH was determined using a digital pH meter. Soil moisture was determined by drying 10 g fresh soil at 105°C for 24 h in a hot-air oven. Organic carbon was analyzed by colorimetric method (Anderson & Ingram 1993) and available phosphorus by molybdenum blue method (Allen et al. 1974). The soil physico-chemical properties are presented in Table 1.

Table 1 Soil physico-chemical properties of *Clerodendrum* rhizosphere soils.

Plant species	pH	Moisture Content	Organic Carbon	Phosphorus
C. colebrookianum	6.80 ± 0.02	7.50 ± 0.02	1.72 ± 0.12	0.01 ± 0.01
C. buchananii	7.83 ± 0.04	7.20 ± 0.12	1.79 ± 0.25	0.001 ± 0.00

Results

AMF association

Arbuscular mycorrhizal colonization were observed in the roots of both *C. colebrookianum* and *C. buchananii* (Table 2). Total AMF colonization was higher in *C. colebrookianum* (29.77 %) as compared to *C. buchananii* (25.83 %). AMF colonization in trap culture for *C. buchananii* was found to be higher (18.07 %) as compared to *C. colebrookianum* (16.31%) as shown in Table 3. The AMF structures found in the two plant species such as hyphae, arbuscules and vesicles are given in Fig. 1.

Table 2 AMF colonization in two *Clerodendrum* species.

Plant species	Mycorrhizal structure			Total Colonization (%)
	Arbuscules	Vesicles	Hyphae	
C. colebrookianum	8.32 ± 0.02	4.23 ± 0.01	17.23 ± 0.02	29.77
C. buchananii	8.75 ± 0.01	4.21 ± 0.01	12.87 ± 0.01	25.83

Table 3 AMF colonization in trap plants (*Trifolium repens*) with inoculum source from two *Clerodendrum* species.

Plant species	Mycorrhizal structure			Total Colonization (%)
	Arbuscules	Vesicles	Hyphae	
Trifolium repens (C.c)	4.23 ± 0.01	3.13 ± 0.01	8.95 ± 0.01	16.31
Trifolium repens (C.b)	1.96 ± 0.01	2.61 ± 0.00	13.50 ± 0.02	18.07

Note: C.c = *Clerodendrum colebrookianum,* C.b = *Clerodendrum bachananii*

Statistical analysis

Relative abundance, isolation frequency, Shannon-Wiener index of diversity (H') and Simpson index of dominance were calculated (Dandan & Zhiwei 2007).

Fig. 1 – Mycorrhizal structural colonization in the roots of *Clerodendrum* species; (a) Arbuscules (b) vesicles and (c) Hyphae.

AMF composition and diversity

A total of 20 AMF species belonging to two genera viz., *Acaulospora* and *Glomus* (15 AMF species from *C. colebrookianum* and 13 AMF species from *C. buchananii*) were isolated and identified on the basis of morphological characteristics (Table 4). Fourteen AMF species were isolated from the trap cultures belonging to two *Acaulospora* and *Glomus* species (Table 5). The AMF spore density in 25 g dry soil sample each was 643 in *C. colebrookianum* and 460 in *C. buchananii* (Fig. 2), whereas in trap culture it was 75 in *C. colebrookianum* derived trap culture and 58 in *C. buchananii* derived trap culture (Fig. 3). Micrographs of some of the isolated AMF species from two *Clerodendrum* species are given in Fig. 4.

Shannon-Weiner index of AMF diversity was found to be 2.29 in *C. buchananii* and 2.54 in *C. colebrookianum*, and Simpson's index of AMF was 0.09 in *C. colebrookianum* and 0.14 in *C. buchananii*. In the trap culture Shannon's index of AMF *C. colebrookianum* was found to be 2.00 and Simpson's index was 0.14 and for *C. buchananii* Shannon's index of AMF was found to be 2.18 and Simpson's index was 2.12.

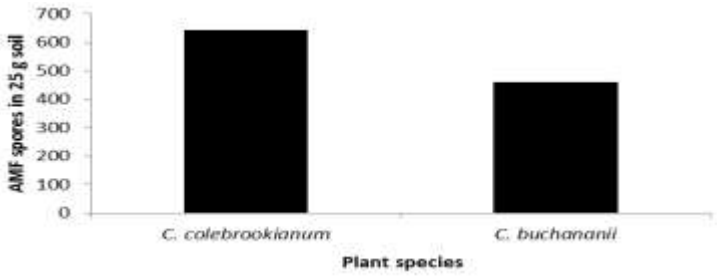

Fig. 2 – AMF spore density in the rhizosphere soil of *Clerodendrum* species.

Table 4 AMF species isolated from two *Clerodendrum* species.

Sl. No.	AMF species	Relative abundance (%)		IF (%)
		C. c	C. b	
1.	*Acaulospora capsiculata* Blaszk.	—	5.71	50
2.	*Acaulospora delicata* Walker, Pfeiffer & Bloss	—	2.86	50
3.	*Acaulospora denticulata* Sieverding & Toro	7.89	—	50
4.	*Acaulospora lacunosa* Morton	—	2.86	50
5.	*Acaulospora tuberculata* Janos & Trappe	5.26	2.86	100
6.	*Glomus clarum* Nicolson & Schenck	—	2.86	50
7.	*Glomus clavisporum* (Trappe) Almeida & Schenck	7.89	5.71	100
8.	*Glomus eburneum* Kenn., Stutz & Morton	2.63	—	50
9.	*Glomus etunicatum* Becker & Gerdemann	7.89	25.71	100
10.	*Glomus geosporum* (Nicol. & Gerd.) Walker	2.63	8.57	100
11.	*Glomus lamellosum* Dalpe, Koske & Tews	2.63	—	50
12.	*Glomus luteum Kenn.,* Stutz & Morton	10.53	14.29	100
13.	*Glomus macrocarpum* Tul. & Tul.	13.16	8.57	100
14.	*Glomus melanosporus* Gerd. & Trappe	2.63	—	50
15.	*Glomus microcarpum* Tul. & Tul.	5.26	5.71	100
16.	*Glomus spurcum Pfeiff.,* Walker & Bloss	2.63	—	50
17.	*Glomus tortuosum* Schenck & Smith	7.89	—	50
18.	*Glomus trimurales* Koske & Halvorson	—	2.86	50
19.	*Glomus verruculosum* Blaszkowski & Tadych	15.79	11.43	100
20.	*Glomus versiforme* (Karsten) Berch	5.26	—	50

Note: C.c = *Clerodendrum colebrookianum,* C.b = *Clerodendrum bachananii,* IF = Isolation frequency and '—' = absence of species.

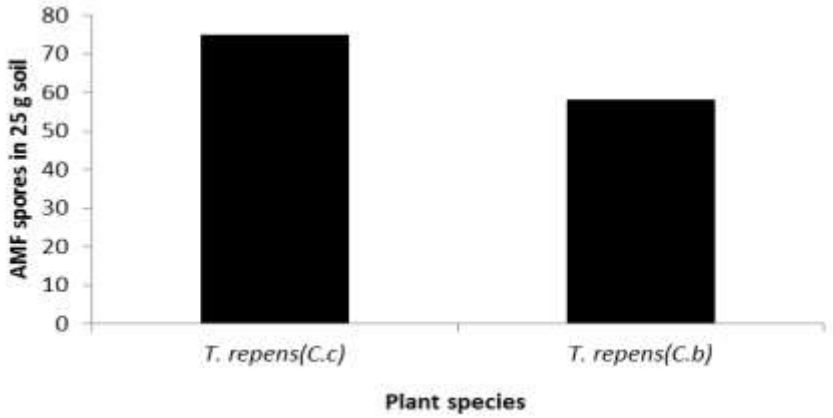

Fig. 3 – AMF spore density in the trap culture of *Clerodendrum* species.
Note: *C.c = C. colebrookianum, C.b = C. bachananii*

Table 5 AMF species isolated from trap plants (*Trifolium repens*) with inoculum source from two *Clerodendrum* species.

Sl. No.	AMF species	Relative abundance (%)		IF (%)
		T.r (C.c)	T.r (C.b)	
1.	*Acaulospora delicata* Walker, Pfeiffer & Bloss	—	6.25	50
2.	*Acaulospora denticulata* Sieverding & Toro	7.14	—	50
3.	*Glomus badium* sp. nov. Oehl, Redecker & Sieverd.	7.14	6.25	100
4.	*Glomus brasilianum* Spain & Miranda	—	6.25	50
5.	*Glomus clarum* Nicolson & Schenck	14.29	—	50
6.	*Glomus eburneum* Kenn., Stutz & Morton	7.14	—	50
7.	*Glomus etunicatum* Becker & Gerdemann	14.29	18.75	100
8.	*Glomus fistulosum* Skuo and Jakobsen	—	6.25	50
9.	*Glomus geosporum* (Nicol. & Gerd.) Walker	14.29	12.5	100
10.	*Glomus intraradices* Schenck & Smith	—	18.75	50
11.	*Glomus luteum* Kenn., Stutz & Morton	—	6.25	50
12.	*Glomus macrocarpum* Tul. & Tul.	21.43	—	50
13.	*Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe	—	6.25	50
14.	*Glomus verruculosum* Blaszkowski & Tadych	14.29	12.5	100

Note: T.r = *Trifolium repens*, C.c = *Clerodendrum colebrookianum*, C.b = *Clerodendrum bachananii*, IF = Isolation frequency and '—' = absence of species.

Discussion

Low AMF colonization rates were observed in *Clerodendrum colebrookianum* and *C. buchananii*. AMF colonization in this study was lower than those reported in other plant species from this region (Das & Kayang 2010; Songachan & Kayang 2011). The variation in AMF colonization associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics of rhizosphere under host species, mycorrhizal dependency, host plant-mediated alteration of the soil microenvironment (Wu et al (2009), AMF diversity and species composition (Gange et al. 1990), or seasonal and ontogenetic variations (Jakobsen et al. 2002) and nutrient demands of the host (Muthukumar & Udaiyan 2002). All together, 20 AMF species (15 from *C. colebrookianum* and 13 from *C. buchananii*) belonging to *Acaulospora* and *Glomus* species were isolated and identified. *Glomus* were the most common species followed by *Acaulospora*, in both plants, which is consistent with the study of Li et al (2007) and Charoenpakdee et al (2010) in different ecosystems. In fact, *Glomus* is the most widely distributed AMF species and it is considered as cosmopolitan in many ecosystems (Sýkorová et al. 2007). Tchabi et al (2009) reported that *G. etunicatum* which is one of the dominant species in this study tend to be a dominant species in soil samples from the yam field sites.

Other AMF species such as those belonging to Gigasporaceae are capable of propagation only with viable spores or from an intact mycelium whereas Glomeraceae are capable of colonizing even with fragments of mycelium (Biermann & Linderman 1983). Moreover, large spores AMF species require a longer period to develop than the small spore species (Hepper 1984) and as sampling was done just once, it is possible that in our study we could not detect

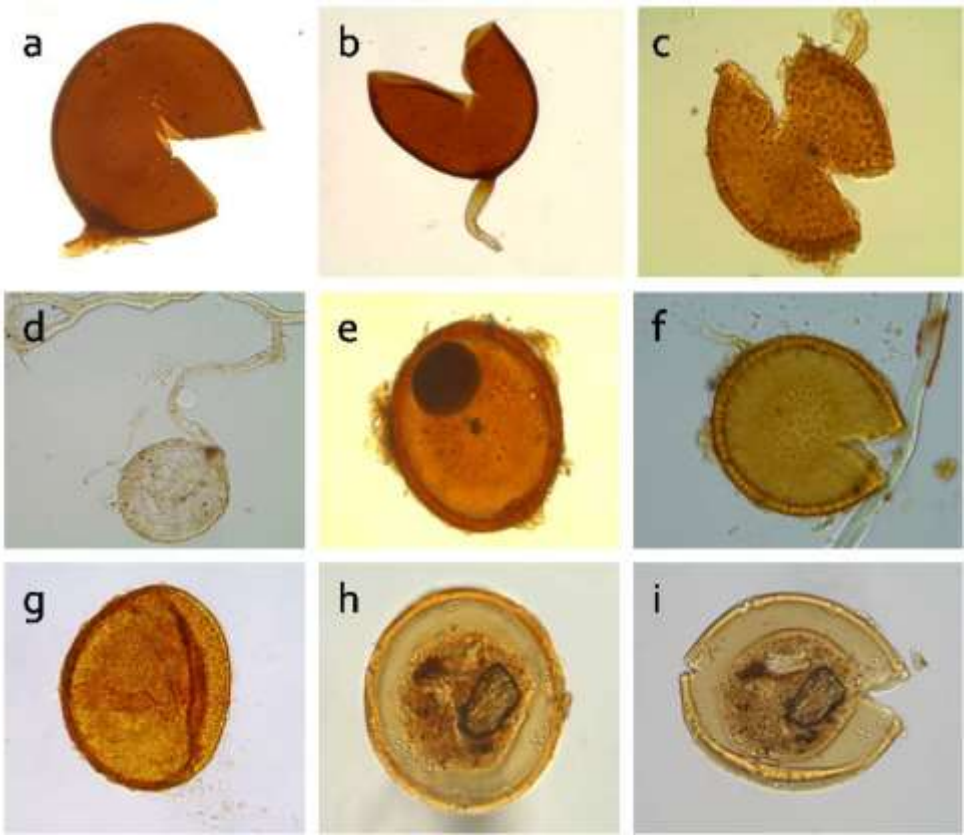

Fig. 4 – Some of the isolated AMF spores from *Clerodendrum* rhizosphere soils. a-f: *Glomus* species (*G. etunicatum, G. geosporum, G. lamellosum, G. viscosum, G. macrocarpum* and *G. clarum*; g-i: *Acaulospora* species (*A. denticulate*, h & i *A. delicata*).

other genus of AMF. Individual AMF species compete for resources through a combination of strategies resulting in the maintenance of a diverse AMF community (Koske 1987) and thus, AMF species having high competitive interaction and adaptability likely dominates in the rhizosphere of the *Clerodendrum* species.

AMF colonization was higher in trap culture set up with inoculum source from *C. buchananii* (18.07 %) as compared to that set up with *C. colebrookianum* (16.31 %). However, spore density was higher in *C. colebrookianum* derived inoculum source than those with *C. buchananii* derived inoculum source. 14 AMF species were recovered from trap cultures (8 species from *C. colebrookianum* and 10 species from *C. buchananii*). Additional AMF species i.e., two species in *C. colebrookianum* trap culture (*Glomus badium* and *G. clarum*) and five species in *C. buchananii* (*G. badium, G. brasilianum, G. fistulosum, G. intraradices* and *G. mosseae*) were obtained which otherwise were not recovered in the original field soils. Similarly, high proportions of additional species appearing exclusively in the trap cultures and not in the original field soil were reported by Oehl et al (2004). Occurrence of additional AMF species in the traps is a well documented phenomenon, justifying the use of trap cultures for more complete AMF surveys than direct isolation of spores from the field soils (Oehl et al. 2004). Therefore, this approach should be incorporated in the analysis of AMF species diversity as it revealed additional AMF species (Stutz & Morton 1996) and produce healthy spores that can be use for inoculum conservation.

AMF are ecologically important root symbionts of most terrestrial plants, and its benefits are increasingly acknowledged. The present study provides basic information on the status of AMF colonization and diversity in *Clerodendrum* species. Many plant species are in high demand for their medicinal properties and other various purposes. Therefore, recognition of mycorrhizal status, and application of mycorrhizal technology could be of particular value in different field.

Acknowledgement

The authors thank the Head, Department of Botany, North Eastern Hill University, Shillong, Meghalaya, India for providing laboratory facilities.

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