Journal of Applied Biology & Biotechnology Vol. 7(05), pp. 25-30, Sep-Oct, 2019
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2019.70504

Root-fungal associations in plants from home gardens of Tripura, Northeast India

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ARTICLE INFO

Article history:
Received on: February 10, 2019
Accepted on: April 20, 2019
Available online: September 10, 2019

Key words:
Arbuscular mycorrhizal fungi, dark septate endophyte, colonization, terrestrial ecosystems

ABSTRACT

Mycorrhizal association is an integral part of terrestrial ecosystems. The present work was focused to examine arbuscular mycorrhiza (AM), dark septate endophyte (DSE) fungal colonization, and the composition of AM fungi from two home gardens of Tripura in Northeast India. The results reveal eight plants commonly occurring in two sites belonging to seven families. Of the eight plants, dual colonization of AM and DSE fungi was observed in seven plants from two sites. A total of 18 AM fungal species were recovered from both the sites. The study reveals a robust composition of AM fungi in the home garden ecosystem. AM fungi isolated from these ecosystems confirm their occurrence and these fungi may be beneficial in improving the cultivation practices in the home garden systems of the region.

1. INTRODUCTION

Arbuscular mycorrhiza (AM) have a symbiotic association between soil fungi of the phylum Glomeromycota [1] and plant roots, which is ubiquitous in the terrestrial ecosystem [2]. It is generally accepted that AM fungi can help in the uptake of plant nutrient like phosphate [3], defend plants against various types of stress [4,5], and decrease the damage caused by root pathogens [6].

There are a group of fungi belonging to ascomycetes called the dark septate endophyte (DSE) fungi that colonize root tissues intracellularly and intercellularly [7] and characterized by microsclerotia and septate melanized hyphae [8]. The common occurrence and are likely to function as mycorrhizal fungi suggest that these endophytes are vital components of natural ecosystems that co-colonized with AM fungi in the same host plants [9,10].

Home gardens are considered as one of the oldest subsistence farming systems practiced by rural communities in many parts of the world, consisting of multilayer systems of trees, shrubs, and herbs around homesteads [11,12]. Home gardens are generally multifunctional and play key roles in providing goods and ecosystem services and also provide numerous benefits for sustaining the livelihood of local inhabitants [13,14].

Mycorrhizal fungi have been studied from forest ecosystems in relation to its ecology and diversity [15–17]. Mycorrhizal associations regarding nutrient status, colonization, and diversity have been studied from plantations and agricultural soils [10,18–23].

The cultivation of fruits, vegetables and ornamental plants in home gardens has a long tradition in Northeast India, especially among the people residing in the states of Assam, Manipur, Nagaland, Meghalaya, and Tripura. The diversity of AM fungi in home garden along with different land use system in Arunachal Pradesh has been reported recently [24]. The study of mycorrhizal associations in home garden has not been solely concentrated. Moreover, the colonization status by AM and DSE fungi of plants in the home garden is scarce [25]. Therefore, mycorrhizal colonization of commonly occurring plants and the composition of AM fungi from two home gardens was examined.

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2. MATERIALS AND METHODS

2.1. Study sites

The root of the plants and soil samples were collected from two home gardens of Tripura, Northeast India. The sites considered for this study were Khowai (24°0′52.18″N; 91°36′48.48″E; 23 masl) and Amtali (23°46′14.23″N; 91°15′46.98″E; 22 masl). The sampling period of roots of plants and soil was during March–June 2016.

2.2. Collection of root and soil samples

The commonly occurring plants were selected for the assessment of AM and DSE symbiosis from both the sites. To assess the colonization in root samples, root from two to three plants of each species was collected and brought to the laboratory. Care was taken in sampling of root samples of plant species that the roots were traced to the target plants. The rhizospheric soil samples were collected at 0–20 cm depth around each species and approximately 500 g soil per plant was collected. All the soil samples from each location were combined and collected in polythene bags, tagged, and brought to the laboratory for further analysis.

2.3. Analysis of soil properties

Soil moisture was determined by drying 10 g fresh soil at 100°C for 24 hours in a hot-air oven. For pH and electrical conductivity, 10 g of soil was dissolved in 50 ml distilled water and stirred for 20 minutes and kept it for overnight. Measurement of the soil pH and electrical conductivity was done by using a digital pH and electrical conductivity meter. Soil texture was determined by the soil hydrometric method [26]. Soil organic carbon (%) was determined [27]. Available phosphorus was estimated [28]. Soil organic carbon and available phosphorus were estimated by spectrophotometer (UV-VIS Biospectrometer, Eppendorf). There is no known history of fertilizer application in these home gardens. However, biodegradable wastes from the kitchen are used sometimes.

2.4. Preparation of roots and assessment of AM and DSE fungal colonization

The root samples brought from the home garden were thoroughly washed in tap water and cut into small pieces of approximately 1 cm in size. Then the root pieces were processed and stained for observation of mycorrhizal colonization [29]. Root segments were then mounted with lactoglycerol on the slide and examined under a compound microscope (Olympus) for various AM and DSE fungal structures. The mycorrhizal structures were also photographed under Olympus CX21i fitted with camera and software, SImage in computer. The quantification of AM fungal colonization was done by the magnified intersection method and DSE fungi were measured together for microsclerotia and septate hyphae. One plant was assessed only for vesicles and aseptate hyphae with the same method [30].

2.5. Extraction and identification of AM fungi

Debris was removed from the soil brought from the field taking utmost care so that the soil attached to the litter and debris was not lost by this process. Fifty grams of soil was placed in the sieves of size 2 mm–35 μ and processed with tap water using the wet sieving and decanting method [31]. Then the spores were extracted from each sieve to the filter paper by filtering out the water. The spores on the filter paper laid on the 13.5 cm Petri dish and were counted under the microscope at 100× magnification. The spores were then picked up with a needle and mounted in polyvinyl alcohol-lactoglycerol on the slide [32]. Then spores were examined using a compound microscope. The identification of AM fungi was done based on morphological characteristics by matching with original descriptions and e-resources available on the website (www.amf-phylogeny.com).

2.6. Data analysis

For evaluation of AM fungi from the home gardens, spore density and species richness were measured. Student *t*-test was performed to assess the significance of means for soil chemical properties occurring at two sites. The colonization data were subjected to analysis of variance and the means were separated by Duncan test ($p < 0.05$). All the data were analyzed using the software, Statistica 9.0.

3. RESULTS

The list of commonly occurring plants in two sampled sites is provided in Table 1. Eight plants were found growing in both the sites, of which four were fruit plants and four were vegetables belonging to seven families.

The soil pH was acidic in both the sites and soil from Khowai exhibiting the lower pH. Electrical conductivity, organic carbon, and available phosphorus were significantly ($p < 0.05$) higher in soil from Khowai than Amtali. Soil texture reveals a high amount of sand in Amtali. The texture indicates soil to be loamy sand of both the sites. The soil properties are presented in Table 2.

Table 1: Family and their uses of commonly occurring plants from two home gardens of Tripura.

Plants	Family	Habit	Uses	Flowering time	Fruiting time
A. tricolor L.	Amaranthaceae	Shrub	Vegetable	Throughout the year	Throughout the year
A. comosus (L.) Merr.	Bromeliaceae	Shrub	Fruit	April–May	June–July
A. squamosa L.	Annonaceae	Tree	Fruit	April–May	August–November
C. annuum L.	Solanaceae	Herb	Vegetable	July–September	August–October
C. pepo L.	Cucurbitaceae	Climber	Vegetable	July–September	August–October
S. melongena L.	Solanaceae	Herb	Vegetable	July–September	August–October
S. pinnata (L. f.) Kurz	Anacardiaceae	Tree	Fruit	March–April	June–December
S. cumini (L.) Skeels.	Myrtaceae	Tree	Fruit	February–April	May–June

AM fungal structures, *viz.,* aseptate intracellular hyphae, intercellular hyphae, vesicles, and arbuscules were observed in the roots of plants from home garden of two different sites (Fig. 1). DSE fungal colonization was characterized by melanized septate hyphae, microsclerotia and vesicles-like body were observed in the roots of plants from the home garden of two different sites (Fig. 2). The extent of AM and DSE fungal colonization in the studied plants is presented in Table 3. Dual (AM and DSE fungi) colonization was observed in seven plants. The roots of *Amaranthus tricolor* were attached with the spore-like structure of AM fungi and extraradical aseptate hyphae (Fig. 2h and i). However, arbuscule was absent in *A. tricolor* in both the sites. AM fungal colonization was maximum in *Capsicum annuum* and lowest in *Ananas comosus*. DSE fungal colonization was maximum in *A. tricolor* in both the sites and minimum was recorded in *Solanum melongena*. *C. annuum* showed the highest percentage of arbuscule and *A. comosus* showed the lowest number of arbuscule. Root length percentage of a vesicle was maximum in *C. annuum* and minimum in *Spondias pinnata*.

The significantly ($p < 0.05$) higher spore density was observed in Khowai than Amtali. Out of 18 morphotypes, 14 and 13 were isolated from Khowai and Amtali, respectively. There were four species from *Acaulospora*, one from *Clarideoglomus*, two from *Funneliformis*, one from *Gigaspora*, seven from *Glomus*, two from *Rhizophagus*, and one from *Sclerocystis*. Ten species of AM fungi were commonly found in both the sites (Table 4).

4. DISCUSSION

The study involves mycorrhizal colonization status in plants from home gardens. Dual colonization was reported earlier in other ecosystem from this region [33]. AM fungal colonization was higher than DSE fungal colonization which is in agreement with an earlier report [33]. The colonization between the sites

Table 2: Soil physicochemical characteristics of soils of two home gardens of Tripura.

Site	Texture (%)			pH	EC (cS cm^{-1})	Organic carbon (%)	Available phosphorus (%)
	Sand	Silt	Clay				
Khowai	73.64	12.15	14.19	5.14 ± 0.01	148.00 ± 1.15	0.99 ± 0.003	3.68 ± 0.05
Amtali	78.03	14.36	7.61	5.58 ± 0.01	128.67 ± 4.37	0.73 ± 0.002	1.36 ± 0.02
t value	-	-	-	890.274	128.171	33.953	70.726
$p <$	-	-	-	0.001	0.001	0.01	0.01

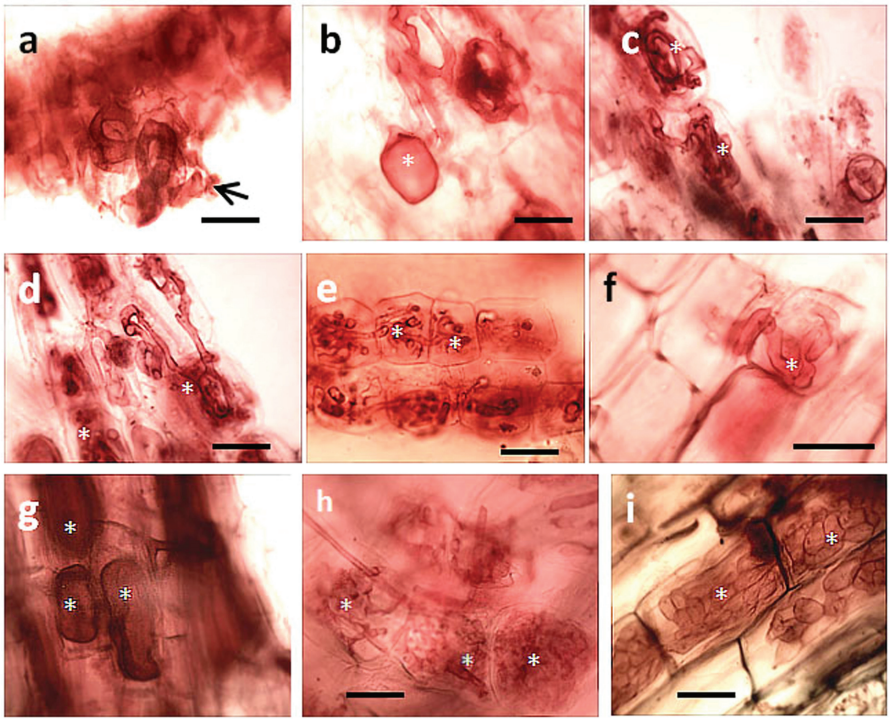

Figure 1: Arbuscular mycorrhizal fungal colonization of plants from the home garden. (a) hyphal appressorium entering the epidermal layer of *A. comosus*, (b) vesicles in the root segment of *A. comosus*, (c) cell-to-cell hyphal coiling in root of *Annona squamosa*, (d) arbusculate coils in the root of *A. squamosa*, (e) arbusculate coils in root portion of *A. squamosa*, (f) hyphal coil in root portion of *S. melongena*, (g) vesicles in the root portion of *S. cumini*, (h) arbusculate coils in the root segment of *S. cumini*, and (i) hyphal coils in the root cells of *C. annuum* (Scale bar: a, c, d, e, and g = 200 µm; b, f, h and i = 100 µm).

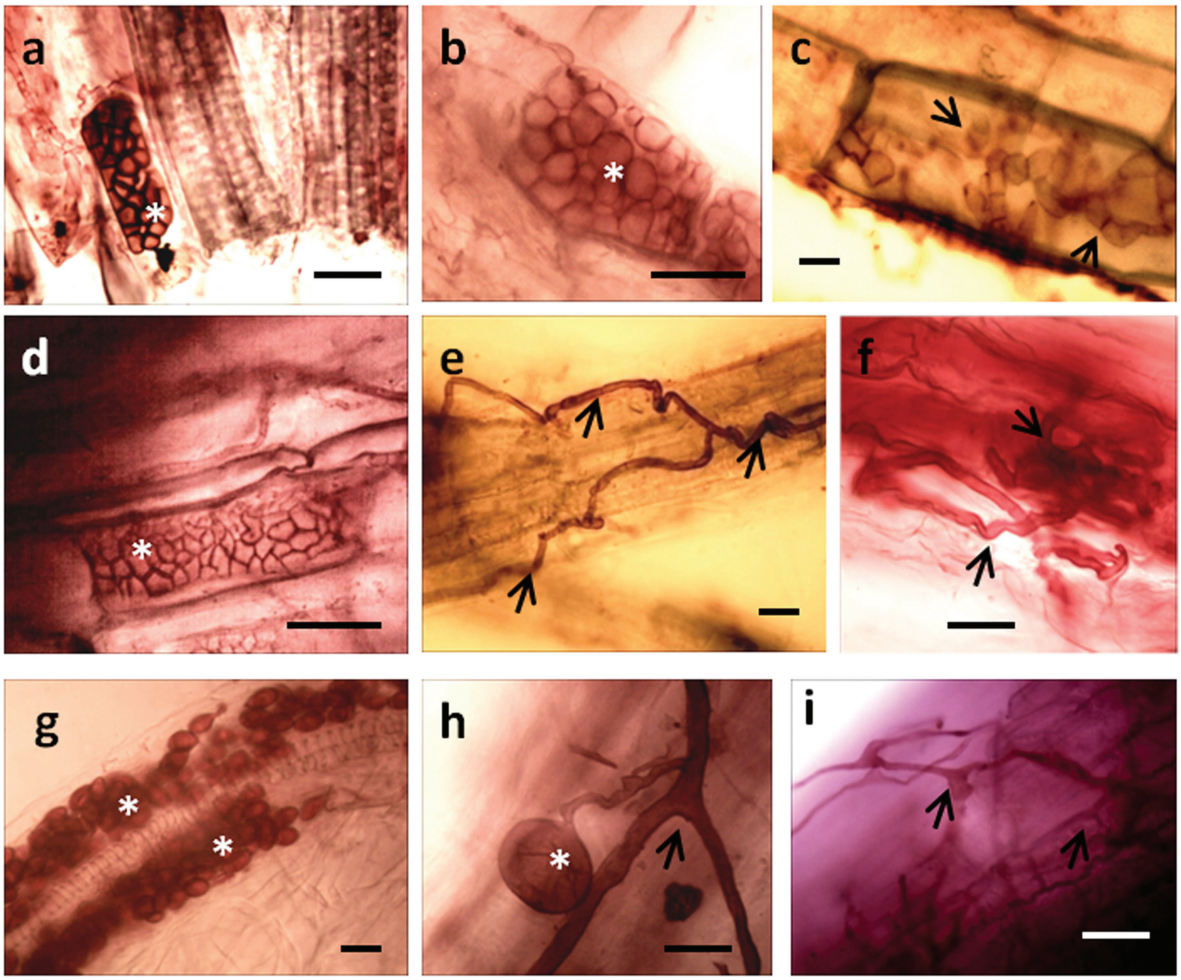

Figure 2: Endophytic fungal association in plants of the home garden. (a) Microsclerotia in the root of *S. pinnata*, (b) microsclerotia in the root segment of *A. comosus*, (c) intracellular septate hyphae in root of *S. melongena*, (d) root segment of *C. pepo* showing microsclerotia, (e) septate hyphae entering the root of *C. pepo*, (f) intercellular septate hyphae in the root of *C. annuum*, (g) microsclerotia in the root of *A. tricolor*, (h) spore-like (asterisk) and aseptate hyphae (arrow) attached to the root of *A. tricolor*, and (i) extraradical aseptate hyphae attached to the root of *A. tricolor* (Scale bar: b = 50 µm; a, e, f, and i = 100 µm; d and h= 150 µm; c and g = 200 µm).

of most of the plants exhibited no significant differences. This may be due to the same climate both the places share although there are significant differences between the soil properties. *Amaranthus tricolor* was found to be colonized by endophyte as there is no record of the presence of arbuscules in most members of Amaranthaceae which is in accord with the previous study [33].

The AM fungal colonization in roots of *A. comosus* was much higher than the earlier study [34]. Sarwade et al. [35] recorded a higher percentage of AM fungal colonization in *C. annuum*. The colonization of AM fungi in *C. annuum* falls within the range of Tanwar et al. [36]. AM fungal colonization in *Cucurbita pepo* was higher than recorded earlier [37]. AM and DSE fungal colonization in *S. melongena* was lower than the previous study [38]. This present study of AM fungal colonization percentage in *S. melongena* was within the range of earlier study [39]. AM fungal colonization in *Syzygium cumini* was higher than the study of Kumar et al. [40].

Despite the importance of AM fungi, knowledge of the diversity and ecology of these ubiquitous and important soil fungi is limited globally [41]. Total number of AM fungal spores isolated from the rhizosphere of home garden plants indicates that the spore density and species richness were maximum in Khowai than Amtali. The community of AM fungal species in the rhizosphere may vary with host species [42]. AM fungal species composition and spore density are highly variable and influenced by plant characteristics and a number of edaphic factors such as soil pH and soil moisture content [43]. It implies that AM fungal colonization may be affected by the broad interactions of several factors, such as the factors inherent to the host plant, climatic and edaphic factors, and effects of the soil community [44].

The probable reason for the prevalence of *Glomus* may be due to *Glomus* has different pH preferences [45] and *Acaulospora* are frequently isolated from acidic soils [46]. *Gigaspora* prevails in high sand content [47]. The dominance of *Glomus* was also reported from this region [23,25,33,48].

Table 3: The extent of arbuscular mycorrhizal and dark septate endophyte fungal colonization of plants growing in home gardens of Tripura.

Plants	Khowai				Amtali			
	%RLA	%RLV	%RLH	%RLDSE	%RLA	%RLV	%RLH	%RLDSE
A. tricolor	0.00 ± 0.00	14.69 ± 2.08a	37.97 ± 1.94a	44.39 ± 2.25a	0.00 ± 0.00	9.91 ± 2.04a	32.79 ± 2.80a	38.77±2.99a
A. comosus	4.50 ± 1.57a	12.28 ± 2.10a	50.11 ± 2.61b	31.15 ± 1.73b	3.09 ± 1.37a	14.23 ± 2.03a	50.53 ± 3.03b	30.43±2.86b
A. squamosal	21.54 ± 2.71b	10.70 ± 1.47a	59.53 ± 2.46c	31.75 ± 2.39b	32.64 ± 2.37b	12.78 ± 1.81a	58.68 ± 3.24c	26.42±1.54b
C. annuum	26.12 ± 3.60b	29.71 ± 2.43b	74.00 ± 2.10d	20.59 ± 1.97d	51.10 ± 2.19c	10.06 ± 1.61a	72.94 ± 1.61d	21.64±1.11bc
C. pepo	38.74 ± 3.04c	9.07 ± 1.50c	68.89 ± 1.88d	27.94 ± 2.07b	40.95 ± 3.25d	11.30 ± 1.46a	61.80 ± 2.81c	22.16±1.87bc
S. melongena	27.59 ± 1.55b	6.56 ± 1.13d	70.82 ± 2.10d	17.44 ± 2.22d	51.03 ± 1.96c	9.27 ± 1.18a	70.84 ± 1.43d	20.35±1.94c
S. pinnata	41.03 ± 3.47c	10.27 ± 1.70a	61.53 ± 3.11c	23.97 ± 2.55d	44.60 ± 3.11d	6.04 ± 1.44b	61.32 ± 3.42c	19.15±2.35c
S. cumini	47.82 ± 2.25c	10.24 ± 1.39a	69.84 ± 1.79d	21.25 ± 1.49d	31.95 ± 1.49b	9.25 ± 1.82a	61.01 ± 1.85c	26.92±1.72b

%RLA, %RLV, % RLH, and %RLDSE percent root length with arbuscules, vesicles, hyphae, and dark septate endophyte, respectively.
Different alphabets differ significantly at $p < 0.05$.

Table 4: Composition of arbuscular mycorrhizal fungal species from home gardens**.**

AM fungal spore morphotype	Khowai	Amtali
Acaulospora cavernata Błaszk.	−	−
Acaulospora foveata Trappe & Janos	+	+
Acaulospora sp. 1	−	+
Acaulospora sp. 2	+	−
Claridеoglomus sp. 1	+	+
Funneliformis sp. 1	+	−
Funneliformis sp. 2	+	−
Gigaspora sp. 1	−	+
Glomus tortuosum Schenck & Sm.	+	−
Glomus macrocarpum Tul. & Tul.	+	+
Glomus sp. 1	+	+
Glomus sp. 2	+	+
Glomus sp. 3	+	+
Glomus sp. 4	+	+
Glomus sp. 5	+	+
Rhizophagus irregulare (Błaszk., Wubet, Renker & Buscot) Walker & Schüßler	+	+
Rhizophagus intraradices (Schenck & Sm.) Walker & Schüßler	+	+
Sclerocystis sinuosa Gerd. & Bakshi	−	+
Species richness	14.00	13.00
Spore density/50 g soil	142.67 ± 6.39	88.33 ± 4.67

+ = Present; − = Absent.

5. CONCLUSION

The study reveals the existence of a well-established relationship between plants and mycorrhizal fungi with regard to suitable colonization and robust composition of AM fungi. Further work should be directed to evaluate these essential native fungi on the growth of these plants. This study is also an effort to create awareness among the small scale farmers that the efficiency of these mycobiota which can be harnessed for long-term applications.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Department of Botany, Tripura University, for providing the laboratory facilities. KC is grateful to the Department of Science and Technology, Government of India, for the INSPIRE fellowship.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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How to cite this article:
Chakraborty K, Debnath A, Das AR, Saha AR, Das P. Root-fungal associations in plants from home gardens of Tripura, Northeast India. J Appl Biol Biotech 2019;7(05):25-30.