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FULL-LENGTH RESEARCH ARTICLE

Diversity and Distribution of Arbuscular Mycorrhizal Fungi in *Solanum* Species Growing in Natural Condition

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Abstract Species composition and diversity of arbuscular mycorrhizal fungi (AMF) in three *Solanum* species (*Solanum khasianum*, *Solanum sisymbriifolium*, and *Solanum torvum*) were investigated. The extent of AMF colonization was highest in *S. sisymbriifolium* and lowest in *S. torvum*, whereas, AMF spore density in the rhizosphere soil was lowest in *S. sisymbriifolium* and highest in *S. torvum.* Low percentage of dark septate endophyte (DSE) was also observed in all the species. A total of 24 AMF species belonging to four genera (*Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora*) were isolated and identified from the rhizosphere soils. *S. khasianum* harbored 12 AMF species, while *S. sisymbriifolium* and *S. torvum* contained 11 AMF species each. *Glomus* and *Acaulospora* were the most frequently encountered AMF species. These findings indicate that *Solanum* species are rich in AM fungal diversity, and selection and inoculation of appropriate microbial strains could be of great value in improving the quality and quantity of plant material.

Keywords Arbuscular mycorrhizal fungi (AMF) · Colonization · Diversity · *Solanum* species

Introduction

Arbuscular mycorrhiza are symbiotic relationships between the roots of plants and soil fungi from the phylum Glomeromycota [31]. Mycorrhiza improves plant growth, mineral nutrient status, and resistance to stress of associated plants [25]. They are considered essential for ecosystem functioning [21] because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities [17]. AMF is a ubiquitous symbiosis in any ecosystem, probably occurring in over two-thirds of vascular plant species [21]. In addition to AMF associations, plants are also associated with DSE fungi, which are characterized by melanized septate hyphae and microsclerotia [27] that colonize root tissues intracellularly and intercellularly. The widespread occurrence and abundance of DSE suggest not only ubiquitous presence and lack of host specificity but also a role of importance in natural ecosystems [20].

Solanum species (Solanaceae) are herbaceous plants commonly found throughout the Indian subcontinent; some of its species have been reported to have medicinal properties. *Solanum khasianum* Clarke have anti-inflammatory and anti-helmintic properties [15]. Its berries also have anti-fertility and -filarial properties. *Solanum torvum* Sw. has been reported to have anti-bacterial and -fungal activity [4]. It is also used as a sedative, diuretic, and digestive. Apart from medicinal properties, some *Solanum* species are used as trap crops for cyst nematodes. Timmermans [34] reported nematode reduction when *Solanum sisymbriifolium* Lam. was introduced as a trap crop for potato cyst nematode.

There are few reports of AMF association in the rhizosphere of *Solanum* species [13, 14]. An increasing demand for herbal products may endanger many traditionally used and pharmaceutically important plant species and their habitats [16]. Considering the influence of AMF on medicinal plants, it seems crucial that more attention should be paid to the monitoring of soil and mycorrhizal

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development during the process of their growth. Moreover, the importance of mycorrhiza for many medicinal 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 [30]. Although, it is generally assumed that most terrestrial plants have an association with AMF, only a small percentage of plant species have been actually examined for their mycorrhizal status [35]. The objective of this study was to analyze AM fungal diversity and colonization, and DSE colonization of three *Solanum* species (*S. khasianum*, *S. sisymbriifolium*, and *S. torvum*) commonly growing in the North-East India.

Materials and Methods

Study Site and Sampling

The study was conducted at North Eastern Hill University Campus, Meghalaya, India, located at 25°36′40″N, 091°53′57″E with an altitude of 1,424 m above sea level. Sampling was done during September to December, 2010. The roots and rhizosphere soils of three naturally growing *Solanum* species (ten replicates of each plant species) were collected in sterilized plastic bags and transported to the laboratory for analysis.

Estimation of AMF and DSE Colonization

Roots were cut into approximately 1 cm segments. It was cleared with 10 % KOH at 90 °C for 1 h, washed in tap water and stained with Trypan blue [28]. The stained root samples were mounted on microscope slides and examined for AM fungal structure under light microscope. Root lengths with mycorrhizal colonization in the form of arbuscules (RLA), vesicles (RLV), hyphae (RLH), and dark septate endophytes (RLDSE) in 100 root segments from each plant species were estimated using the magnified intersection method of McGonigle et al. [24] and converted to percentage richness.

AMF Spore Isolation, Enumeration, and Identification

AMF spore isolation and enumeration was done following the method of Uma et al. [35]. Suspension of 25 g soil sample in water was passed 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 (http://www.invam.caf.wvu.edu) and AMF phylogeny (www.amf-phylogeny.com). Spore density and species richness were expressed as number of AM fungal spores and numbers of AM fungal species in 25 g soil sample.

Soil Physico-Chemical Analysis

Soil moisture was determined by drying 10 g fresh soil at 105 °C for 24 h in a hot-air oven. Soil pH was determined using a digital pH meter. Organic carbon was analyzed by colorimetric method [3] and available phosphorus by molybdenum blue method [2]. Soil texture was determined using the bouyoucos method of Allen et al. [2]. The soil physico-chemical properties are presented in Table 1.

Statistical Analysis

Relative abundance, isolation frequency, Shannon-Wiener index of diversity (H'), Simpson index of dominance (D), Evenness (E), and Sorenson's coefficient (C_s) were calculated [11]. Relationships between AMF, DSE colonization, spore density, and soil physico-chemical properties were computed using Pearson's correlation coefficient. Data were statistically analyzed using one-way ANOVA. Standard errors of means were calculated.

Table 1 Physico-chemical properties of the soil planted to *Solanum* spp.

Plant species	Texture	pH	MC (%)	P (%)	OC (%)
Solanum khasianum	Silty sand	5.83 ± 0.06	22.99 ± 0.22	0.13 ± 0.01	0.39 ± 0.01
Solanum sisymbriifolium	Silty sand	5.87 ± 0.09	24.08 ± 0.35	0.03 ± 0.00	0.46 ± 0.01
Solanum torvum	Silty sand	6.27 ± 0.03	8.37 ± 0.19	0.03 ± 0.00	1.06 ± 0.03

MC moisture content, *P* phosphorus, and *OC* organic carbon

Result

AMF and DSE Colonization

AMF and DSE colonization were observed in all three *Solanum* species. The mean percentage of AMF colonization levels in three *Solanum* species was 39 %. AMF colonization level in *S. khasianum*, *S. sisymbriifolium*, and *S. torvum* was 39, 42, and 36 %, respectively. AMF colonization in the form of arbuscules, vesicles, and hyphae were observed in all the plant species. The mean DSE colonization in three *Solanum* species was 0.79 %. The root length colonization of AMF and DSE are given in Table 2. ANOVA shows a significant variation in percentage root length of arbuscules ($F = 16.30; p = 0.007$), vesicles ($F = 41.97; p = 0.0006$), hyphae ($F = 161.96; p = 0.0001$), and total AMF colonization ($F = 164.34; p = 0.0001$) among three *Solanum* species. DSE does not show significant variation among plant species ($F = 2.83; p = 0.1444$).

AMF Diversity

AMF spore density in 25 g soil sample was highest in *S. torvum* (740 spores), followed by *S. khasianum* (681 spores) and *S. sisymbriifolium* (498 spores). Species richness and composition of AMF are given in Table 3. Based on morphological characteristics, 24 AMF species were isolated and identified, of which two species, i.e., *Acaulospora* sp.1 and *Glomus verruculosum* were common to all three plant species. *Acaulospora koskei, A. laevis*, *Gigaspora rosea*, *Glomus claroideum*, *G. etunicatum*, and *Scutellospora heterogama* were restricted to rhizosphere soil of *S. khasianum*. AMF species restricted to *S. sisymbriifolium* rhizosphere were *Acaulospora denticulata*, *A. morrowiae*, *A. rehmii*, and *Glomus lamellosum*. AMF species restricted only in *S. torvum* were *Acaulospora scrobiculata, Glomus badium, G. coronatum, G. fistulosum, G. luteum,* and *Glomus* sp.1. The dominant AMF species was *Acaulospora lacunosa* and *Glomus verruculosum* in *S. khasianum* rhizosphere soil, *Acaulospora denticulata* in *S. sisymbriifolium* rhizosphere soil, while *Acaulospora delitata* and *Glomus verruculosum* was dominant in *S. torvum* rhizosphere soil. Isolated AMF species with their isolation frequency and relative abundance are presented in Table 4 and some of the species are shown in Fig. 1.

The diversity indices namely H', D, and E of AMF do not show much difference among three *Solanum* species (Table 5). C_s values of AMF species was 0.36 for *S. sisymbriifolium* and *S. torvum*, 0.35 for *S. khasianum* and *S. sisymbriifolium* and 0.17 for *S. khasianum* and *S. torvum*.

Discussion

AMF association in *S. khasianum*, *S. sisymbriifolium*, and *S. torvum* was investigated for the first time, although, AMF association in some other *Solanum* species has been reported earlier [1, 26]. Mean AMF spore density with a range of 498–740 in 25 g rhizosphere soils were observed in *Solanum* species which was very much higher than reported by Akond et al. [1] who reported 118–136 spores from 100 g soil in three cultivated *Solanum* species. The difference might be due to the sampling which was done from undisturbed natural environment during dry season (November–January) when the highest spore density could be expected [18]. In a cultivated plant species, soil disturbance in the form of tillage and use of machineries disturbs the hyphal development of mycorrhiza, and thus reduces the production of its spore, which further supported the view that spore density in undisturbed soil was higher than that in cropped soil [23].

Table 2 Mycorrhizal colonization (%) in *Solanum* spp.

Plant species	RLA	RLV	RLH	RLDSE	Total AMF colonization
Solanum khasianum	8.06 ± 1.32	2.05 ± 0.04	28.69 ± 1.72	0.67 ± 0.00	38.80 ± 2.03
Solanum sisymbriifolium	10.17 ± 1.19	1.66 ± 0.09	29.92 ± 2.67	1.47 ± 0.11	41.74 ± 1.8
Solanum torvum	11.22 ± 1.10	3.33 ± 0.59	21.55 ± 1.23	0.22 ± 0.06	36.10 ± 1.6

RLA root length with arbuscules, *RLV* root length with vesicles, *RLH* root length with hyphae, and *RLDSE* root length with dark septate endophytes; Mean ± SE

Table 3 Species richness (SR) and species composition of AMF in *Solanum* spp.

Plant species	SR	*Acaulospora* sp.	*Gigaspora* sp.	*Glomus* sp.	*Scutellospora* sp.
Solanum khasianum	12	6	1	3	1
Solanum sisymbriifolium	11	7	–	4	–
Solanum torvum	11	4	–	7	–

– indicates the absence of species

Table 4 AMF species isolated from rhizosphere soils of *Solanum* spp. with their isolation frequency (IF) and relative abundance (RA)

AMF species	IF (%)	RA (%)		
		Sk	Ss	St
Acaulospora delitata Morton	66.67	–	11.76	15.38
Acaulospora denticulata Sieverding & Toro	33.33	–	17.65	–
Acaulospora koskei Blaszk	33.33	7.14	–	–
Acaulospora lacunosa Morton	66.67	14.29	5.88	–
Acaulospora laevis Gerdemann & Trappe	33.33	7.14	–	–
Acaulospora morrowiae Spain & Schenck	33.33	–	5.88	–
Acaulospora rehmii Sieverding & Toro	33.33	–	5.88	–
Acaulospora scrobiculata Trappe	33.33	–	–	7.69
Acaulospora spinosa Walker & Trappe	66.67	7.14	5.88	–
Acaulospora sp.1	100.00	7.14	5.88	7.69
Acaulospora tuberculata Janos and Trappe	66.67	7.14	–	7.69
Gigaspora rosea Nicolson & Schenck	33.33	7.14	–	–
Glomus badium sp. nov. Oehl, Redecker & Sieverd.	33.33	–	–	7.69
Glomus claroideum (Schenck & Smith emend. Walker & Vestberg)	33.33	7.14	–	–
Glomus coronatum Giovann.	33.33	–	–	7.69
Glomus etunicatum Becker & Gerdemann	33.33	7.14	–	–
Glomus fistulosum Skuo and Jakobsen	33.33	–	–	7.69
Glomus intraradices Schenck & Smith	66.67	–	11.76	7.69
Glomus lamellosum Dalpe, Koske & Tews	33.33	–	11.76	–
Glomus luteum Kenn., Stutz & Morton	33.33	–	–	7.69
Glomus rubiforme Gerdemann & Trappe	66.67	7.14	5.88	–
Glomus sp.1	33.33	–	–	7.69
Glomus verruculosum Blaszkowski & Tadych	100.00	14.29	11.76	15.38
Scutellospora heterogama(Nicolson & Gerd.) Walker & Sanders	33.33	7.14	–	–

Sk *Solanum khasianum*; Ss *Solanum sisymbriifolium*; St *Solanum torvum*; – indicates the absence of a species

Percentage of mycorrhizal colonization was low to moderate and ranged from 36 to 42 %. This finding was in accordance with Akond et al. [1] and Nzanza et al. [26] who suggested that *Solanum* species has a low AMF colonization.

One-way ANOVA showed that AMF structures varied significantly ($p < 0.05$) among the different plant species. Pearson's correlation analysis showed that AMF colonization was positively correlated with DSE colonization ($r = 0.99$, $p < 0.001$), suggesting that these endophytes influence each other within roots. However, this observation contradicts that of Chaudhry et al. [9] who found an inverse relationship between AMF and DSE colonization levels. Negative correlation was obtained between AMF colonization and spore density ($r = -0.96$, $p < 0.001$). Clearly, spore populations do not exactly reflect the AMF community that is actually colonizing the plant roots because of the possible existence of some non-sporulating AMF species [12]. In this study, AMF colonization shows a negative correlation with soil pH ($r = -0.80$, $p < 0.001$) and a positive correlation with soil moisture content ($r = 0.88$, $p < 0.001$). He et al. [19] also reported a positive correlation between AMF colonization and soil moisture content. Soil P did not show correlation with AMF colonization which was in agreement with the study of Ruotsalainen et al. [29] and Becerra et al. [5], albeit had, a significant positive correlation with species richness ($r = 1.00$, $p < 0.001$). AMF species composition and spore density are highly variable and influenced by plant characteristics and a number of environmental factors such as soil pH and soil moisture content [7].

Isolation of 24 AMF species supports the view of Singh et al. [33] that acidic to neutral soils harbor a good number of AMF species. In our present investigation, *Glomus* and *Acaulospora* were the most frequent AMF species, which is consistent with the study of Choudhury et al. [10]. As individual AMF species compete for resources through a combination of strategies resulting in the maintenance of a diverse AMF community [22], the competitive nature is probably high among *Glomus* and *Acaulospora* species. Moreover, the composition of AMF community may be strongly affected by the individual plant species through differential effects on hyphal growth and sporulation [6].

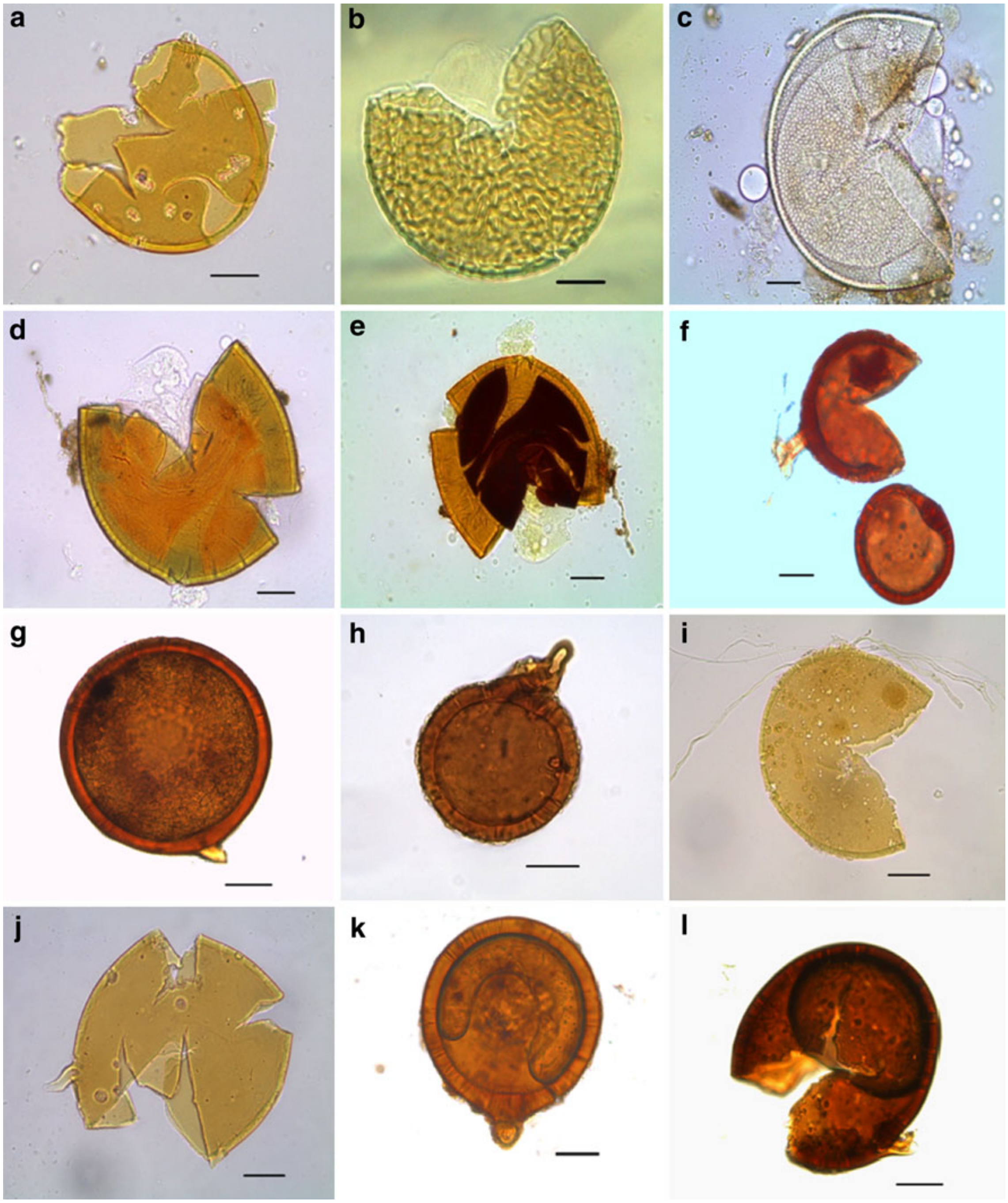

Fig. 1 AMF spores isolated from rhizosphere soil of three *Solanum* species. **a**–**e** *Acaulospora* species—*A. dilatata, A. scrobiculata, A. spinosa, A. tuberculata,* and an unidentified *Acaulospora* species. **f**–**l** *Glomus* species—*G. badium, G. etunicatum, G. indraradices, G. lamellosum, G. luteum*, an unidentified *Glomus* species, and *G. verruculosum. Scale bar* 40 µm

Solanum khasianum harboring highest number of AMF species as compared to other two *Solanum* species had higher H' value. The H' value in this study was much higher than reported by Singh et al. [32] and Charoenpakdee et al. [8]. Simpson's dominance index shows slight differences among the three *Solanum* species. The lower index of dominance for AMF in *S. khasianum* as compared to *S. sisymbrifolium* and *S. torvum* indicates higher number of shared dominance of AMF species. *E* value was same for *S. khasianum* and *S. torvum* indicating that distribution of AMF species was more uniform in these two plant species. A higher C_s value of AMF species was observed between *S. khasianum* and *S. sisymbriifolium*, and between *S. sisymbriifolium* and *S. torvum*, resulting in a higher degree of overlap in fungal species composition as compared to that between *S. khasianum* and *S. torvum*.

Table 5 AMF diversity indices in *Solanum* spp.

Diversity index	*S. khasianum*	*S. sisymbriifolium*	*S. torvum*
Shannon-Weiner diversity index	2.44	2.31	2.35
Simpson's dominant index	0.09	0.11	0.10
Evenness	0.98	0.96	0.98

AM fungi are ecologically important root symbionts of most terrestrial plants, and their benefits are being increasingly acknowledged. This study provides information on the status of AMF colonization and diversity in the three *Solanum* species. Many plant species are in high demand for their medicinal properties and various other purposes. Therefore, recognition of mycorrhizal status and selection of appropriate microbial strains to inoculate medicinal plants could be of particular value to improve the quality and quantity of plant material.

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