**RESEARCH ARTICLE**

# Arbuscular Mycorrhizal and Dark Septate Endophyte Fungal Associations in Two Dominant Ginger Species of Northeast India

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**Abstract** Studies on the prevalence of arbuscular mycorrhizal fungi (AMF) and dark septate endophytic fungi (DSEF) are limited for rhizomatous crops in subtropical ecosystems compared to other terrestrial habitats. Hence, the authors assessed the incidence of AMF and DSEF in roots and diversity of AMF in the rhizosphere of *Zingiber montanum* and *Z. officinale* collected from shifting cultivation fields of Manipur, Northeast (NE) India. Both the gingers had dual colonization of AMF and DSEF structures in different cortical cells of the same examined root segments and revealed the Intermediate type of AM morphology. Such endomycorrhizal symbiotic association is being reported for the first time in *Z. montanum*. Distribution of different AMF and DSEF structures varied significantly across the two ginger species. The total root length colonization with AMF and DSEF was highest in *Z. montanum*. The maximum spore population of AMF was recorded in *Z. officinale* soil, whereas the AMF species richness was highest in *Z. montanum* rhizosphere. Altogether, 13 spore morphotypes of AMF corresponding to eight genera, i.e., *Acaulospora*, *Claroideoglomus*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus* were isolated from the field and trap culture soils of both ginger species. Significant positive correlations were recorded between some soil properties and root-colonizing variables of AMF and DSEF. Thus, the occurrence of native AMF and DSEF associations in two important indigenous gingers cultivated in the Jhum fields of hilly terrains reveals the possibility of utilizing them in the future for sustainable agriculture.

**Keywords** AMF diversity · DSE fungi · Root colonization pattern · *Zingiber montanum* · *Z. officinale*

## Introduction

Members of Zingiberaceae, commonly known as gingers, are among the important horticultural crops mainly cultivated for its aromatic rhizomes which are used since times immemorial as spices, condiments, flavoring agents and for its potential medicinal properties being largely distributed in tropical and subtropical regions of Asia [1]. Manipur being a part of Northeast (NE) India lies within the Indo-Burma mega-biodiversity hot spot region having several indigenous wild and domesticated gingers belonging to 88 species under 19 genera [2], among which *Zingiber montanum* (J.Koenig) Link ex A.Dietr. (syn. *Z. cassumunar* Roxb.) and *Zingiber officinale* Roscoe. are most abundantly found in hilly tracts and valley regions of Manipur and is locally known as Tekhaoyaikhu and Shing, respectively. The fleshy rhizomes of naturally grown mountain ginger (*Z. montanum*) have a strong camphoraceous odor, bitter taste and are used as folk medicines to

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combat various ailments and disorders, whereas the common ginger (Z. officinale) is extensively cultivated due to its pungency and high fiber contents [2]. NE India is among the highest productivity area of different Zingiber species in the world, i.e., 5.8 t/ha against the national average of 3.7 t/ha, and considered as one of the organic ginger hubs of India [3].

Arbuscular mycorrhizal (AM) fungi (AMF, phylum Glomeromycota) are the vital components of soil microbial community forming symbiotic associations with the majority of crop plants including some tuberous and rhizomatous ones [4, 5]. AMF have been observed to increase the crop growth and productivity by enhancing the uptake of labile and non-labile mineral elements, especially in weathered field soils of tropical and subtropical areas that are characterized by low percentage of total and available P [6–8] and also improve the soil quality, water relations and plant tolerance to abiotic and biotic stresses [9]. However, only approximately 76% of monocot families are reported for their mycorrhizal status till date [6]. Therefore, Wang and Qui [10] proposed the need for examining as many plant species from different land types for their mycorrhizal status to understand the extent of root-colonizing fungi in various habitats. Recently another root colonist, the dark septate endophytic fungi (DSEF) belonging to phylum Ascomycota and characterized by the presence of septate melanized hyphae and microsclerotial structures, has gained increasing interest as they coexist with AMF and protect the plant from different stresses [11, 12]. However, information on DSEF colonization patterns in tropical and subtropical plant roots, particularly to that of NE India, is very limited compared to other climatic zones and temperate ones [13, 14].

Taber and Trappe [15] were the first who investigated the presence of AMF in rhizomes and scale-like leaves of edible ginger (Z. officinale) grown in Hawaii and Fiji, and the benefits of AM symbiosis on growth parameters of ginger species have already been observed in abroad [16–18]. Moreover, Muthukumar et al. [19] and Uma et al. [5] also reported the AM status of Z. officinale from South India. Nevertheless, the occurrence of AMF and DSEF associations in mountain ginger (Z. montanum) has not yet been reported till date. Considering the importance of AM fungi for sustainable agriculture, a survey of native AMF species richness in the subtropical habitat of this region would provide insight for their field applications in the future [13, 14]. Hence, the present study aims to determine the AM morphology and root length colonization by different structures of AMF and DSEF as well as the species diversity of AMF in the rhizosphere of two dominant gingers cultivated in the subtropical Jhum fields of Manipur, NE India.

## Material and Methods

### Study Area and Conditions of Crop Growth

The present work was carried out with two indigenous ginger species, viz., Z. montanum and Z. officinale grown in shifting cultivated fields for the previous five years and situated on the gentle slope of a hillock under the Langol forest extension of Imphal West District, Manipur (location: 24°51′45.0″ N latitude; 93°53′15.7″ E longitude), having elevation of 950 m. a.s.l. Geographically, about 90% of the total area in Manipur is covered by hill ranges being the part of Eastern Himalayas along with 1813 sq km of central valley area [20]. According to the United States Department of Agriculture Classification (USDA), the soil is Ultisol type developed from shale and sandstones and is heterogeneous [20]. The annual mean temperature of the study site varied from 15.3 to 26.8 °C and mean relative humidity between 61 and 88%, while the total rainfall was 1535 mm (ICAR Research Complex for NEH Region, Manipur Centre, Imphal). For each ginger species, three cultivated fields were selected randomly which were situated adjacently on the western side of the hill slope having uniform growth patterns and without any alternate crop grown during the off-seasons.

In NE India, gingers are mainly grown by tribal farmers following traditional practices as a rainfed crop on the raised beds in Jhum or shifting cultivation fields receiving the annual rainfall between 980 and 2050 mm and a temperature range of 12–36 °C. In hilly tracts, planting of the ginger rhizomes is conducted during March–April when the first shower starts and the harvesting is done from November and extends till January [3, 21]. No inorganic fertilizers and biocides were applied during the cultivation of gingers.

### Sample Collection

The root and soil samples were collected by digging mineral soil core (0–20 cm depth) around the root zones of five randomly selected plants (6 months old), at a distance of 4–4.5 m apart each, belonging to a ginger species during harvesting period of crop growth, i.e., October–November 2017. Approximately 1 kg of rhizospheric soil was collected from the individual plant of a selected crop species, kept in polythene bag separately, labeled and then brought to the laboratory. After air-drying in shade, one part of the soil corresponding to each plant of a ginger species was utilized for enumeration of AMF spore density and species richness, while the remaining samples were pooled and used for establishing the trap culture and analyzing the physico-chemical characteristics. The roots were gently

washed with water to make them free from soil and were fixed in FAA solution till further processing.

## Determination of Soil Properties

The texture of the soil samples was assessed by the Bouyoucos Hydrometer method as described by Allen et al. [22], whereas the soil pH and electrical conductivity (EC) were analyzed in the aqueous soil–water (1:1, v/v) solutions using the digital pH and conductivity meter. Organic carbon (OC) of the soil was determined by the rapid titration method [23]. Total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) of the soil samples were evaluated according to Jackson [24]. All the soil properties were assessed in triplicate for each ginger species.

## Assessment of Ginger Root Colonization by AMF and DSEF

After washing with distilled water, 15 root segments (1 cm length) of each ginger species were cut, processed for clearing with KOH (2.5%) at 90 °C for 90–120 min in water bath, acidified with HCl (5 N) for 15 min [25], stained in Trypan blue-Lactoglycerol (0.05%) overnight, then mounted in Lactoglycerol on glass slides and examined for the AMF and DSEF structures in stereoscopic compound microscope (Nikon Eclipse Ni-U, Japan) for estimation of their root length colonization (%) which was assessed by magnified intersection method as described by McGonigle et al. [26]. The morphology of AM was categorized as *Arum*, *Paris* or Intermediate type depending on the presence of inter- or intracellular nature of AMF structures (arbuscules, hyphae, vesicles, hyphal or arbusculate coils) within the root cortex [27]. The authors could not differentiate the Intermediate-subtypes of AM morphologies as only the squashed root pieces were examined. Moreover, when the parallel running hyphae were intracellular, then the morphology was considered as Intermediate type [28].

## Isolation and Identification of AMF Spores

The AMF spores were isolated by wet sieving and decanting technique [29], in which 100 g of soil sample (in triplicate) belonging to each plant of a ginger species was dispensed into 1L of water and the suspension was decanted through 710–37 μm sieve's series. After washing into beakers, the residues were filtered through girdled filter papers, then spread on glass plates and observed under the stereoscopic microscope (40× magnifications). All the AMF spores were counted, transferred to an individual glass slide by wet needle and then mounted in polyvinyl alcohol-Lacto glycerol (PVLG) either with or without Melzer's reagent [30]. The non-collapsed AMF spores and sporocarps were identified on the morphological basis, and the sub-cellular features were compared with the culture database established by INVAM (http://invam.cag.wvu.edu/) and Schüssler's web site (http://www.lrz-muenchen.de/~schuessler/amphylo/amphylo_species.html). The AMF spore population and species richness were indicated as the total number of spores and the number of species in 100 g air-dried soil samples, respectively. The relative abundance (%RA) and isolation frequency (%IF) of each AMF species were calculated according to Dandan and Zhiwei [31].

## Establishment of Trap Culture

The composite soil samples along with root fragments corresponding to each ginger species were mixed with autoclaved (120 °C, 15 psi for 30 min. on three consecutive days) coarse sand (1:1 v/v) and filled in triplicate earthen pots (2 kg/pot). Each pot was sown with 10 maize (*Zea mays* L.) seeds, placed in greenhouse condition (temperature range 20–26 °C, relative humidity between 77 and 90% along with natural daylight and photoperiod) and was watered every alternate day. After 120 days of culture initiation, the developed AMF spores were extracted and identified as stated above.

## Statistical Analysis

A paired *t* test was performed to assess the differences in soil parameters, AMF spore density and root-colonizing fungal structures between two ginger species (SPSS version 20, SPSS Inc., Chicago, Illinois). Pearson's correlation was applied to determine the relationship between soil characters and endorrhizal colonization levels.

## Results and Discussion

To our knowledge, the present finding reveals the first report on AM morphology, AMF and DSEF colonization patterns in the roots and species diversity of AMF in the rhizosphere of *Z. motanum* under Jhum cultivation system of NE India, whereas *Z. officinale* have earlier been reported to be associated with AMF [15, 19, 32] and DSEF [5]. The soils of both gingers were sandy loam in texture and slightly acidic (Table 1). Maximum concentrations of EC, total N and available P were found in *Z. officinale* soil, whereas that of OC and exchangeable K was highest in *Z. montanum* rhizosphere. No significant difference could be observed between the soil properties (except EC) of the two ginger species (Table 1).

**Table 1** Physico-chemical properties in the upper 20 cm soil profiles of two studied *Zingiber* species

| Ginger species | Soil variables[a] | | | | | |
|---|---|---|---|---|---|---|
| | pH | EC (d Sm$^{-1}$) | OC (%) | N (kg ha$^{-1}$) | P (kg ha$^{-1}$) | K (kg ha$^{-1}$) |
| *Z. montanum* | 5.80 ± 0.29 | 0.23 ± 0.02 | 2.10 ± 0.15 | 105.3 ± 4.16 | 77.4 ± 4.80 | 210.5 ± 5.91 |
| *Z. officinale* | 6.12 ± 0.19 | 0.27 ± 0.02 | 1.83 ± 0.17 | 111.8 ± 3.64 | 81.6 ± 2.44 | 204.8 ± 7.13 |
| *t*-test | 1.637 ns | 1.986* | 1.677 ns | 1.685 ns | 1.283 ns | 0.986 ns |

*ns* Non significant

*Significant at $P < 0.05$

[a] Means ± SE

All examined root fragments of both the gingers had dual colonization of AMF and DSEF structures present in different lengths of root cortical cells (Table 2, Fig. 1), and revealed the Intermediate type of AM morphology which is similar to the findings of Uma et al. [5] who observed that 80% of the ginger and spiral ginger species have Intermediate-type AM. When roots are colonized by more than one AMF, as possible in the field condition, the formation of Intermediate-AM morphology, having the features of both *Arum* and *Paris* types, might be greater [33].

The entry of AMF into roots of *Z. officinale* was characterized by appressorium (Fig. 1a) and other structures such as arbuscules, intercellular hyphae and intracellular hyphal coils (Fig. 1b–e), whereas in *Z. montanum* intracellular hyphal coils, inter- and intracellular hyphae and vesicles were observed (Fig. 1f–j). In this study, the root length with total AM colonization (%RLTC) was lower in *Z. officinale* (56.2%) (Table 2), when compared to those reported for the same plant species from South India by Muthukumar et al. [19], Muthukumar and Tamilselvi [32] and Uma et al. [5] with mean values of 74%, 91% and

63%, respectively. This indicates the variations in mycorrhizal dependency of specific plants in different ecosystems having P-deficient soils [34]. Moreover, at higher altitudes, low temperatures and heavy rainfall followed by soil erosion may also affect the AMF sporulation and subsequently reduce the root colonization levels. Differences in AMF communities and their root-colonizing patterns have been reported among plants, ecosystems, locations and seasons, and therefore, it seems inherent that the coexisting AMF have distinct functional abilities [35].

Soil properties are known to affect AMF functioning and colonization levels [6]. In this study, the soil EC negatively affected the root length colonized by arbuscules (%RLA), although the measured EC concentrations were below to that reported to influence the AMF colonization frequency [36]. Füzy et al. [37] also observed that arbuscule richness in *Plantago maritime* was negatively correlated with soil EC, which shows that the soil salinity may affect the AMF spore germination, rate of hyphal growth and colonization percentage [38].

**Table 2** Arbuscular mycorrhizal fungal (AMF) and dark septate endophytic fungal (DSEF) colonizations in the roots of two studied *Zingiber* species

| Ginger species | AMF[a] | | | | | | DSEF[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Colonization[c] | | | | | SPN[c] | Colonization[c] | | |
| | %RLH | %RLA | %RLHC | %RLV | %RLTC | | %RLDH | %RLMO/MS | %RLTDC |
| *Z. montanum* | 45.6 ± 1.9 | 0.0 ± 0.0 | 17.9 ± 0.8 | 5.7 ± 0.6 | 69.2 ± 2.3 | 16.7 ± 2.6 | 3.5 ± 1.5 | 2.4 ± 1.4 | 5.9 ± 2.1 |
| *Z. officinale* | 32.6 ± 1.4 | 6.8 ± 0.6 | 16.8 ± 0.7 | 0.0 ± 0.0 | 56.2 ± 1.2 | 47.8 ± 8.3 | 1.3 ± 0.4 | 1.7 ± 0.4 | 3.0 ± 0.6 |
| *t*-test | 7.073*** | 11.697*** | 0.721 ns | 16.486*** | 6.872*** | 3.617** | 1.920* | 0.791 ns | 1.898* |

*ns* Non significant

*, ** and ***Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$
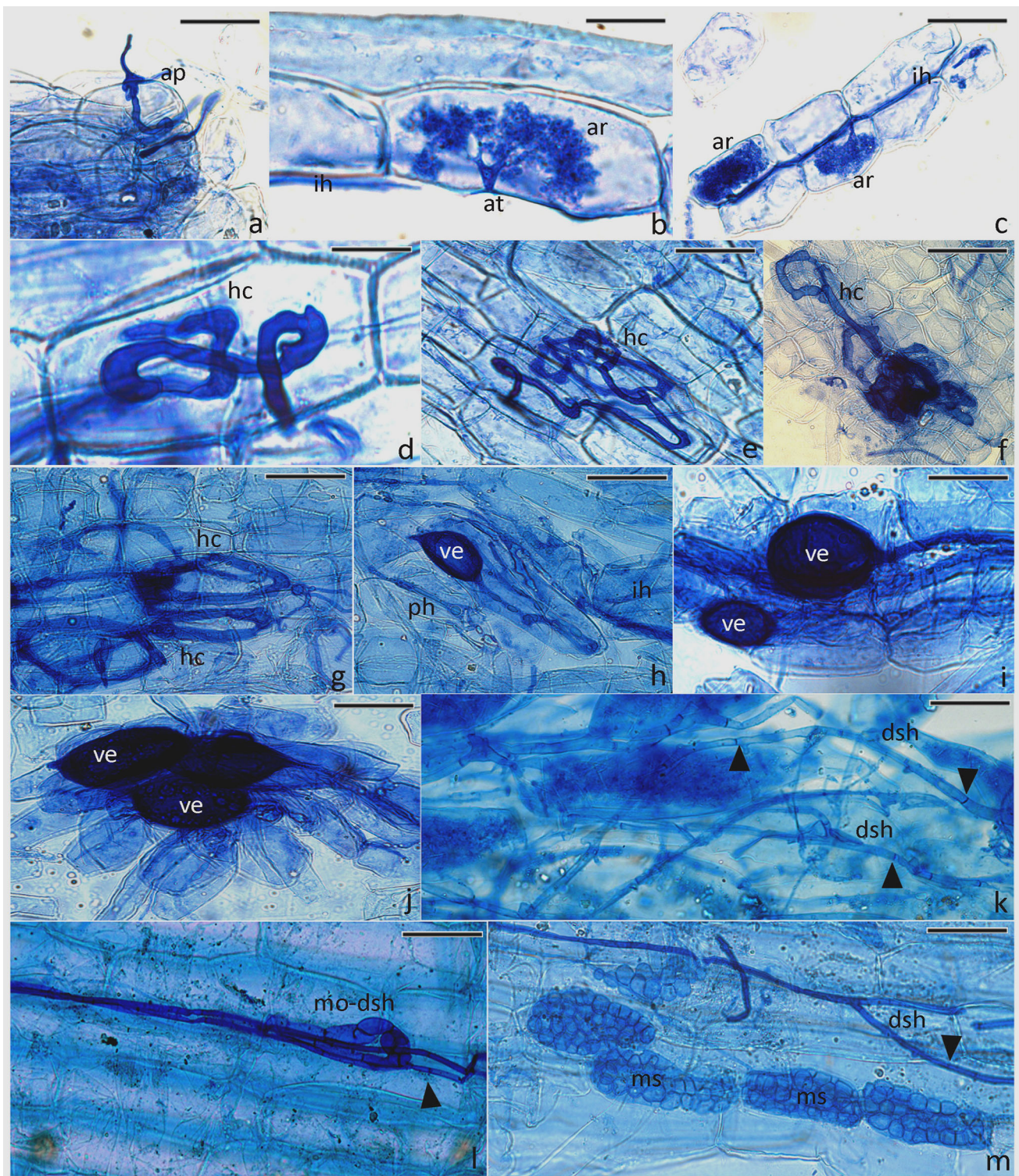
[a] %RLH, %RLA, %RLHC, %RLV, %RLTC and SPN indicate percentage root length with hyphae, arbuscules, hyphal coils, vesicles, total AM colonization and spore number (per 100 g soil), respectively

[b] % RLDH, %RLMO/MS and %RLDTC indicate percentage root length with dark septate hyphae, moniliform/microsclerotial structures and total DSE colonization, respectively

[c] Means ± SE

**Fig. 1** Arbuscular mycorrhizal (**a–j**) and dark septate endophyte (**k–m**) fungal colonization in roots of *Zingiber officinale* (**a–e**, **k**) and *Zingiber montanum* (**f–j**, **l**, **m**). **a** Appressorium formation in *Z. officinale*, **b**, **c** intercellular hyphae and arbuscules in *Z. officinale*, **d**, **e** intracellular hyphal coils in *Z. officinale*, **f**, **g** intracellular hyphal coil in *Z. montanum*, **h–j** vesicles in *Z. montanum* (ve), **k** DSE hyphae in *Z. officinale*, **l**, **m** moniliform-DSE hyphae and microsclerotia in *Z. montanum*. ap, appressorium; ih, intercellular hyphae; ar, arbuscules; at, arbuscular trunk; hc, hyphal coils; ph, intracellular hyphae; ve, vesicle; dsh, dark septate endophyte fungal hyphae (arrow heads); mo-dsh, moniliform-DSE hyphae; ms, microsclerotia. Scale bars = 40 μm

**Table 3** Pearson's correlation between arbuscular mycorrhizal fungi (AMF), dark septate endophyte fungi (DSEF) and soil variables ($n = 10$)

| Variables | | AMF[a] | | | | | | DSEF[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | SPN | %RLH | %RLA | %RLHC | %RLV | %RLTC | %RLDH | %RLMO/MS | %RLDTC |
| Soil | pH | 0.397 | 0.169 | − 0.234 | 0.204 | 0.402 | 0.275 | 0.329 | 0.404 | 0.435 |
| | EC | 0.744* | 0.268 | − 0.560* | 0.444 | 0.465 | 0.348 | 0.462 | − 0.024 | 0.504 |
| | %OC | − 0.649* | − 0.225 | 0.351 | − 0.445 | − 0.446 | − 0.351 | − 0.446 | 0.005 | − 0.350 |
| | N | 0.449 | − 0.027 | − 0.382 | 0.410 | 0.475 | 0.078 | 0.525 | − 0.072 | 0.462 |
| | P | − 0.579* | − 0.382 | 0.264 | − 0.345 | − 0.267 | − 0.461 | − 0.224 | − 0.044 | − 0.196 |
| | K | 0.339 | − 0.012 | − 0.324 | − 0.038 | 0.354 | − 0.034 | 0.496 | 0.488 | 0.504 |
| AMF | SPN | | 0.420 | − 0.596* | 0.133 | 0.616* | 0.432 | 0.733* | 0.472 | 0.785** |
| | %RLH | | | − 0.821** | 0.010 | 0.794** | 0.971*** | 0.405 | 0.578* | 0.572 |
| | %RLA | | | | − 0.346 | − 0.951*** | − 0.842** | − 0.823** | − 0.442 | − 0.843** |
| | %RLHC | | | | | 0.314 | 0.210 | 0.523 | − 0.422 | 0.230 |
| | %RLV | | | | | | 0.849** | 0.827** | 0.444 | 0.846** |
| | %RLTC | | | | | | | 0.486 | 0.469 | 0.588* |
| DSEF | %RLDH | | | | | | | | 0.272 | 0.909*** |
| | %RLMO/MS | | | | | | | | | 0.649* |

*, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

[a]SPN, %RLH, %RLA, %RLHC, %RLV and %RLTC indicate AMF spore number, percentage root length with hyphae, arbuscules, hyphal coils, vesicles and total AM colonization, respectively

[b]%RLDH, %RLMO/MS and %RLDTC indicate percentage root length with dark septate hyphae, moniliform/microsclerotial structures and total DSE colonization, respectively

The roots of studied ginger species were colonized by different DSEF structures, viz., melanized septate hyphae and moniliform or microsclerotia (Fig. 1k–m), though their hyphae and sclerotia occurred in low percentages (Table 2). The extent of total root length colonization by DSEF, i.e., %RLTDC, was highest in *Z. montanum* (5.9%) compared to that of *Z. officinale* (3%) which is similar to the findings of Uma et al. [5] who reported 0–3.9% total root length colonization by DSEF in three different ginger species. In this study, a significant positive correlation was observed between total root length colonized by AMF and DSEF which suggests that these fungal types could coexist together in the same plant roots as also reported by Muthukumar et al. [28]. DSEF are frequently present in the roots of many plant taxa belonging to cold and nutrient-stressed or degraded ecosystems where the colonization rate of AM fungi is lower [11, 39]. Like AMF symbiosis, the host responses to DSEF are variable and usually differ from mutualism to parasitism, depending on the plant and fungal genotype and environmental conditions [40]. Therefore, the mechanism of interactions between mycorrhizal and DSE fungi is inevitable and some strains of DSEF are beneficial to the plant growth and performances [12]. In contrast to a wide knowledge of AMF functioning, the role of DSEF in the ecosystem and the nature of coexistence among two fungal groups are largely unknown. Earlier work on the effect of an ectomycorrhizal and DSE fungal strains on conifer plants has shown a reduction in the DSEF colonization level, and the influence was concluded as the result of competition for nutrients and space and the antagonistic interactions between these two fungal groups [41]. However, such findings are still lacking in the case of AMF and DSEF interactions till date.

The AMF spore density observed in the air-dried rhizosphere soils of *Z. montanum* (17 spores per 100 g) and *Z. officinale* (48 spores per 100 g) are comparatively higher to that reported in *Z. officinale* and other spiral gingers (11–22 spores per 100 g soil) grown in natural and agricultural fields of South India [5, 19]. As reported by Aguilar–Fernández et al. [42], the present findings revealed that crop cultivation by slash-and-burn practices in hilly terrains does not affect the AMF sporulation and community composition adversely for a longer duration. Soil properties are known to affect the AMF population. In the present study, both ginger soils were slightly acidic which might be a factor for low-to-moderate AMF spore population. Rajeshkumar et al. [43] reported that the pH of the soil can influence the status of AMF spores in crop rhizosphere, and slightly acidic soils comparatively harbor a higher number of AM propagules than that of acidic ones. The AMF spore density was significantly and negatively correlated with %OC of studied ginger soils (Table 3). Singh et al. [44] also observed a similar relationship among these variables from the soil of Jhum fallow in NE India. However, the

variations in density and diversity of AMF in different crop field soils were found to be influenced by an array of environmental, host and fungal growth factors [6].

A total of 13 and 9 AMF spore morphotypes corresponding to 8 genera, i.e., *Acaulospora*, *Claroideoglomus*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus*, were isolated from the field and trap culture soils, respectively, of the selected ginger species (Table 4, Fig. 2). However, Uma et al. [5] isolated a higher number of AMF spore types (18 species) from the root-zone soils of other Zingiberaceae members cultivated in Kerala, India. Such differences in the diversity of AMF species have been previously reported in a variety of habitats and agroecosystems [13, 14, 45]. In this study, the maximum AMF species were recorded in the field cultivated soils of Z. *montanum*. Three different species of *Funneliformis* were isolated, whereas the genera such as *Acaulospora*, *Glomus* and *Sclerocystis* were represented with two species each. Spores morphotypes of *Claroideoglomus etunicatum*, *Funneliformis geosporum* and *Septoglomus constrictum* were recovered from the field soils of both ginger species. *Funneliformis monosporus*, *Rhizophagus intraradices* and *Sclerocystis rubiformis* were exclusively recorded in the root-zone soil of Z. *montanum*, while *Glomus macrocarpum* was specifically found in Z. *officinale* soil. Hence, the present findings revealed that the majority of the isolated AMF species belonging to order Glomerales and the genera, i.e., *Funneliformis*, *Glomus*, *Claroideoglomus*, *Septoglomus*, *Rhizophagus* and *Sclerocystis*, contributed higher species richness than the order Diversiporales including *Acaulospora* and *Scutellospora*. Uma et al. [5] also recorded 14 AMF species under the order Glomerales and only six from other AM taxonomic groups in gingers and spiral gingers rhizosphere. *Glomus* has been reported as the most widely distributed genus from different geographical regions around the globe probably due to their high adaptability and smallest sized spores among other AMF taxa which enable them to sporulate in numbers within a limited period and to disperse easily [13, 46].

The spores of *Acaulospora* sp.1, *Funneliformis mosseae* and *Glomus multicaule* were specifically isolated from trap pot soil of Z. *montanum*, while *Acaulospora spinosa*, *Sclerocystis taiwanensis* and *Scutellospora* sp.1 were

**Table 4** Relative abundance (%RA) and isolation frequency (%IF) of arbuscular mycorrhizal fungi (AMF) in field and trap pot soils of two examined *Zingiber* species

| AMF species | Relative abundance (%) | | | | Isolation frequency (%) |
|---|---|---|---|---|---|
| | Isolated from field soils of ginger species | | Isolated from trap culture soils of ginger species | | |
| | Z. montanum | Z. officinale | Z. montanum | Z. officinale | |
| *Acaulospora spinosa* C. Walker & Trappe | – | – | – | 12.9 | 16.7 |
| *Acaulospora* sp.1 | – | – | 31.8 | – | 25.0 |
| *Claroideoglomus etunicatum* C. Walker & Schuessler | 28.3 | 23.3 | 39.8 | 20.8 | 83.3 |
| *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & Schuessler | 19.5 | 35.0 | 28.4 | 36.6 | 91.7 |
| *Funneliformis monosporus* (Gerd. & Trappe) Oehl, G.A. Silva & Sieverd | 17.7 | – | 11.4 | – | 25.0 |
| *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & Schuessler | – | – | 11.4 | 11.9 | 33.3 |
| *Glomus macrocarpum* Tul. & C. Tul. | – | 16.7 | – | – | 33.3 |
| *Glomus multicaule* Gerd. & B.K. Bakshi | – | – | 9.1 | – | 8.3 |
| *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & Schuessler | 18.6 | – | – | – | 8.3 |
| *Sclerocystis rubiformis* (Gerd. & Trappe) | 9.7 | – | – | – | 8.3 |
| *Sclerocystis taiwanensis* C.G. Wu & Z.C. Chen | – | – | – | 6.9 | 25.0 |
| *Scutellospora* sp.1 | – | – | – | 10.9 | 16.7 |
| *Septoglomus constrictum* Sieverd. G.A. Silva & Oehl | 6.2 | 25.0 | – | – | 33.3 |
| | 100 | 100 | 100 | 100 | |
| Species richness | 6 | 4 | 6 | 6 | |

– Indicates the absence of AMF species
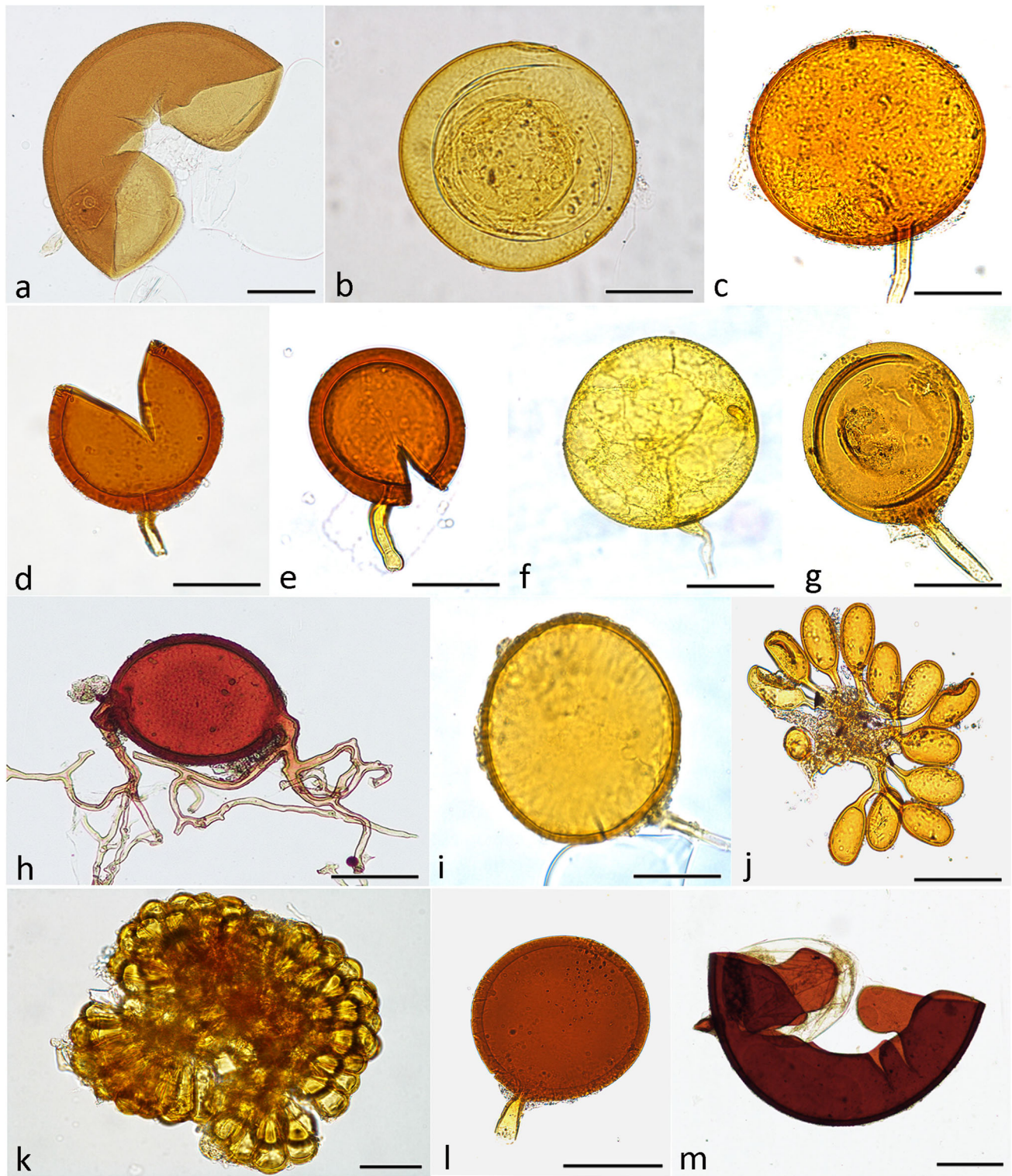
**Fig. 2** Arbuscular mycorrhizal fungal spores isolated from field and trap culture soils of studied *Zingiber* species. **a** *Acaulospora spinosa*, **b** *Acaulospora* sp., **c** *Claroideoglomus etunicatum*, **d** *Funneliformis geosporum*, **e** *Funneliformis monosporus*, **f** *Funneliformis mosseae*, **g** *Glomus macrocarpum*, **h** *Glomus multicaule*, **i** *Rhizophagus intraradices*, **j** Spores of *Sclerocystis rubiformis*, **k** Sporocarp of *Sclerocystis taiwanensis*, **l** *Septoglomus constrictum*, and (**m**) *Scutellospora* sp. Scale bars: (**a**, **k**, **m**) = 20 μm and (**b**, **c**, **d**, **e**, **f**, **g**, **h**, **i**, **j**, **l**) = 40 μm

exclusively recorded from Z. *officinale* trap culture soil (Table 4). The occurrence of AMF species in field and trap pot soils of two gingers varied significantly. Bever et al. [46] reported that AMF species isolated from natural field soil may not be always recovered from the trap cultures, because sporulation of a specific AMF may be influenced by the host species through their effects at the time of propagule activation and extent of hyphal development, whereas the trap culture can develop the sporulation of cryptic AMF species that might be unable to regenerate during the sampling period in field. Isolation of AMF spores from the natural field and trap culture soils generally provides a complete understanding of AMF species richness with associated host plant rhizosphere.

## Conclusion

The occurrence of both AMF and DSEF in the examined gingers indicates the dynamic nature of diverse root-colonizing fungal communities in shifting cultivation systems. It has been proposed by different workers that the DSEF association generally increases the root functions of native plants growing in multifactorial stressed ecosystems and also emphasizes their potential to function as mutualists along with the mycorrhizal fungi [11, 12, 32]. Further investigations would focus on determining whether compositional variations and root colonization patterns among AMF and DSEF community have a functional role in the growth and productivity of the gingers cultivated in different soil types and climatic conditions.

**Compliance with Ethical Standards**

**Conflict of interest** The authors declare that they have no competing interests to publish this manuscript.

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