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Assessment of arbuscular mycorrhizal and dark septate endophytic fungal symbioses in five fruit plants of Manipur, North-East India

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Rhizosphere soils and roots of five fruit plants viz., *Citrus hystrix*, *Citrus medica*, *Docynia indica*, *Prunus salicina* and *Pyrus communis*, collected from Tamenglong district of Manipur, North-East (NE) India were assessed for arbuscular mycorrhizal (AM) fungal spore density and diversity, AM morphology and the colonization pattern of AM and dark septate endophytic (DSE) fungi in their roots. A total of 25 AM fungal species corresponding to 9 genera i.e., *Acaulospora*, *Clariodeoglomus*, *Funneliformis*, *Gigaspora*, *Glomus*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus* were isolated from field and trap soils of all the studied fruit plants. Of these, *Glomus* was the most prevalent genera with 8 species. Both AM and DSE fungi colonized the roots of selected fruit plants. The roots of *C. medica*, *P. communis*, and *P. salicina* exhibited *Arum-Paris* type of AM morphology, whereas that of *C. hystrix* and *D. indica* had Intermediate-4 type morphology. The percentage root length colonization with AM fungi was highest in *C. hystrix* (93.57 %), while DSE fungi were most prevalent in *P. salicina* (13.94 %) and varied significantly (P<0.05).

Keywords: AM fungal diversity, AM morphology, DSE fungi, root colonization, trap culture, fruit plants

INTRODUCTION

Manipur is the easternmost state of the Indian sub-continent and part of North-East (NE) India that falls under the Himalayas and the Indo-Burma biodiversity hotspot regions (Hazarika and Singh, 2018). It is endowed with a wide range of fruit species among which *Citrus hystrix* DC., *Citrus medica* L., *Docynia indica* Colebr. exWall. Decne, *Prunus salicina* Lindl, and *Pyrus communis* L. locally known as Heiribob, Heijaang, Heitoop, Heikha and Naspati respectively, are abundantly found in home gardens, orchards and forest areas of Manipur. Even though these fruits have been extensively consumed, they also have been used in ethno-medicine and in producing other value-added products

The unripe fruit of *P. communis* is used as an astringent and to treat urinary issues, whereas the seeds of *C. medica* are used as stimulants, anti-inflammatories and to combat emmenagogues and hemorrhoids. In Manipur, *D. indica* is used to produce the native wine known as Atingba/Yu, while *P. salicina* fruit is used to treat asthma and constipation (Hazarika and Singh, 2018). Furthermore, the dried fruit peel of *C. hystrix* is a common spice for fish and other cuisines in Manipur.

As obligate biotrophic organism, arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) form a mutualistic symbiotic relationship with the roots of majority of land plants, including staple crops, fruits, medicinal plants etc. and they are also the vital parts of the soil biota, which connects the soil and plant community in various ecosystems (Smith and Read, 2008). This symbiosis reduces biotic and abiotic stressors and increases the host plant's absorption of mineral nutrients, especially phosphorus (P) (Morte *et al*. 2001; Hill *et al*. 2010). Furthermore, by promoting the stability of soil aggregates

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through their robust extraradical mycelial networks, AM fungi enhance the soil structure (Rillig *et al*. 2010) and in turn they obtain carbon and lipids (plant photosynthates) from the associated host plants which are essential for their growth (Smith and Read, 2008). The spread and position of AM fungal intraradical structures determine whether the root colonization patterns of AM within plant roots are *Arum*, *Paris*, or Intermediate type (Dickson, 2004).

Another varied set of fungi known as dark septate endophytic (DSE) fungi invade the plant roots and are distinguished by dematiaceous, septate hyphae that form the microsclerotia or moniliform structures (Muthukumar *et al*. 2018). In a variety of hosts and environments, they frequently coexist with different mycorrhizal fungi and comprise a polyphyletic collection of fungal genera and species with cryptic diversity within the phylum Ascomycota (Grunig *et al*. 2011). Recent research shows that DSE fungi have a positive impact on plant growth and yield through enhanced nutrient uptake and increased tolerance to abiotic and biotic stresses despite the earlier works showing a negative, neutral or positive influence on plant performance (Mandyam and Jumpponen, 2005; Zhang *et al*. 2012).

Although several authors have documented mycorrhizal status and the AM and DSE fungal association in other plants of this region, including subtropical forest trees, gingers and vegetable tree species (Pandey *et al*. 2016; Surbala and Pandey, 2020; Surendirakumar *et al*. 2021), there is less information on their association with horticultural fruit plants of this region. Hence, the present study has been conducted to analyses the AM morphology as well as AM and DSE fungal colonization in the roots of five important fruit plants and to evaluate the diversity of AM fungi in the rhizosphere soils.

MATERIALS AND METHODS

Study sites

The present study was conducted with five fruit plants: *Citrus hystrix* DC., *Citrus medica* L., *Docynia indica* Colebr. exWall. Decne, *Prunus salicina* Lindl and *Pyrus communis* L. collected from Dolang village (24°40´21" N Latitude; 93°39´59" E Longitude) in Tamenglong district of Manipur having an elevation of 1308.2 m a.s.l. The maximum and minimum temperatures of the study site were 34.23°C and 6.14°C respectively. The relative humidity (RH) ranged from 15.47% to 100%, and the total annual rainfall was 2609.8 mm (Directorate of Environment and Climate Change, Porompat, Manipur).

Sample collection

A total of 25 root and soil samples were collected from five randomly chosen individuals of the selected fruit plants. The soil cores excavation depth ranged from 0 to 20 cm. The fine roots (less than 2 g) were collected after digging the soil core of a particular fruit plant and were carefully kept in a polythene bag. After being transported to the laboratory, the roots were cleaned under running water and then preserved in FAA solution (formalin: glacial acetic acid: 70% ethyl alcohol, 5:5:90 ml v: v: v) until further processing. The rhizosphere soil around the roots of each plant (about 1 kg) was collected in a separate polythene bag, labeled and sealed before being transported to the laboratory. After air drying in the shade, half of the soil samples from each plant species were used to extract the AM fungal spores and the other half of the soil samples belonging to each fruit plants were combined to form a composite soil sample which was then used to analyses the soil characteristics and for establishing trap cultures.

Analysis of soil properties

The Bouyoucos Hydrometer method was used to determine the soil texture (Allen *et al*. 1974). The soil pH and electrical conductivity (EC) were measured at room temperature in an aqueous solution of 20 g of soil in 50 ml of water using a digital pH meter (Eutech PC 700). The organic carbon (OC) content of the soil was measured by the rapid titration method (Walkley and Black, 1934). Available phosphorous (P) was determined by following the Bray-2 method (Bray and Kurtz, 1945), whereas exchangeable potassium (K) was analyzed using the ammonium acetate method (Hanway and Heidel, 1952). The micro-Kjeldahl method was used to determine the total nitrogen (N) level of the soil (Jackson, 1971). Three separate evaluations of every soil property were conducted for each fruit plant.

Extraction, enumeration and identification of AMF spores

The AM fungal spores were extracted from soil samples by wet sieving and decanting method (Gerdeman and Nicolson, 1963). For this, 100g of soil belonging to each fruit plant was mixed in one liter of water and then passed through a series of sieves with mesh sizes ranging from 710 to 37 µm. The mixture was filtered via filter paper, spread onto the glass plates and observed under a compound microscope (Nikon Eclipse E100, Japan) at ×40 magnification. The healthy and intact AM fungal spores (non-collapsed spores with cytoplasmic content) were counted, and sporocarps and spore clusters were regarded as a single unit. The isolated AM fungal spores were mounted using polyvinyl alcohol-lactoglycerol (PVLG) (Schenck and Perez, 1990) and were identified based on the morphological and subcellular features provided on Schüssler's website (www.lrz-muenchen.de/~schuessler/amphylo/ amphylo_ species.html) and the culture database established by INVAM (http://invam.cag.wvu.edu/).

Estimation of AM and DSE fungal colonization in roots

After washing with distilled water, the fixed roots for each plant species were cut into one cm-long segments. They were then cleared in a water bath using 2.5% KOH at 90°C (Koske and Gemma, 1989), acidified with 1 N HCL for 15 minutes, and stained overnight at room temperature with Trypan blue lactoglycerol (0.05%). Stained root fragments were placed on glass slides in lactoglycerol and were observed for AM and DSE fungal structures with a Nikon Eclipse E100 compound microscope at ×40 magnification. Using the magnified intersection approach, the proportion of total root length colonized by AM and DSE fungi was calculated (McGonigle *et al*. 1990). The proportions of root length that contained specific AM and DSE fungal structures such as hyphae (%RLH), hyphal coils (%RLHC), arbusculate coils (%RLAC), arbuscules (%RLA), vesicles (%RLV), dark septate hyphae (%RLDH), and microsclerotia/moniliform structures (%RLMI/MO), were also estimated in addition to their total colonization percentages. The AM morphology was further classified as *Arum*, *Paris*, or Intermediate-type based on the intercellular nature of the fungal structures observed within the colonized root cortex of the host plant.

Establishment of Trap culture

Fifteen trap cultures (three replications for each fruit plant) were established using 2-liter black polythene bags filled with composite soil samples (including root fragments) collected for each plant and combined with coarse sand (1: 1 v/v) that had been sterilized (120°C, 15 psi for 30 minutes on three consecutive days). Two host plants, *Zea mays* and *Plectranthus scutellaroides*, were employed to trap the AM fungal spores. All polybags were then kept in a greenhouse with a temperature range of 26 to 32º C, relative humidity of 70 to 85% and natural daylight/night photoperiod. They were watered every other day. After 120 days of culture initiation, the produced AM fungal spores were extracted and identified as previously described.

Statistical analysis

The spore density (SD), species richness (SR), Simpson's index (*D*), Shannon-Wiener index (*H'*), Evenness (*E*), relative abundance (%RA), and isolation frequency (%IF) of AM fungal species were calculated as described by Dandan and Zhiwei (2007). All data related to soil properties and root colonizing fungal structures were subjected to analysis of variance (ANOVA) (SPSS version 21, SPSS Inc., Chicago, Illinois) to ascertain the significance of variance among different fruit plant species. Pearson's correlation test was applied to determine the association between soil characteristics, AM fungal density, and AM and DSE fungal root colonization levels in each plant species. Mean standard errors were also calculated.

RESULTS AND DISCUSSION

To our knowledge, this study provides the first report on species diversity of AM fungi in the rhizosphere soil and AM morphology as well as AM and DSE fungal colonization in the roots of *C. hystrix* and *D. indica*. Previously, Bhuiyan *et al*. (2017) had reported the AM spore population and their root colonization percentage in *Citrus*

Table 1. Physico-chemical properties of rhizosphere soils of studied fruit plants.

Fruit plants	Soil variables					
	pH	EC (dSm^{-1})	OC (%)	N (%)	P ($Kg\ ha^{-1}$)	K ($Kg\ ha^{-1}$)
Citrus hystrix	5.11±0.08[a]	0.67±0.02[c]	3.36±0.22[a]	0.29±0.02[a]	17.34±0.27[b]	476.28±13.4[b]
Citrus medica	4.88±0.09[a]	0.63± 0.03[bc]	3.42 ± 0.20[a]	0.37±0.04[ab]	16.32±0.53[b]	618.00±11.24[d]
Docynia indica	5.76±0.21[a]	0.39±0.06[a]	1.67±0.34[a]	0.51±0.06[b]	8.37±1.68[a]	423.00±5.84[a]
Prunus salicina	5.82±0.36[a]	0.43±0.09[ab]	2.44±0.56[a]	0.47±0.06[ab]	13.38±1.94[ab]	517.20±4.35[c]
Pyrus communis	5.57±0.10[a]	0.29±0.03[a]	3.46±0.52[a]	0.39±0.03[ab]	7.70±1.90[a]	732.60±9.54[e]
F-statistics	1.762ns	5.035*	2.030ns	2.090ns	4.614*	106.990***

pH, EC, OC, N, P and K- indicates hydrogen ion concentration, electrical conductivity, organic carbon, total nitrogen, available phosphorus and exchangeable potassium, respectively.
Means ± SE in the column followed by different superscript letters is significantly different according to Duncan's multiple range test (P > 0.05).
ns – non-significant; * Significant at $P < 0.05$ and *** Significant at $P < 0.001$.

Table 2. Relative abundance (%RA) and Isolation frequency (%IF) of AM fungi in the field soils of selected plant species.

AM fungal species	Relative abundance (%)					Isolation frequency (%)
	C. hystrix	*C. medica*	*D. indica*	*P. communis*	*P. salicina*	
Acaulospora laevis Gerd. & Trappe	-	5.38	7.64	7.49	-	53.33
Acaulospora scrobiculata Trappe	4.45	-	-	6.55	6.88	60.00
Acaulospora spinosa C. Walker & Trappe	-	4.25	1.99	-	-	33.33
Acaulospora sp.1	3.01	1.98	-	4.47	-	40.00
Acaulospora sp.2	-	-	0.77	5.50	-	20.00
Clariodeoglomus etunicatum C. Walker & Schuessler	11.74	-	11.35	10.05	9.52	80.00
Funneliformis geosporus (T.H. Nicolson & Gerd.) C. Walker & Schuessler	-	8.28	11.80	-	7.39	26.67
Funneliformis mosseae (T.H. Nicols on & Gerd.) C. Walker & Schuessler	10.64	5.83	7.29	-	8.52	53.33
Gigaspora sp.	-	1.64	-	-	3.59	80.00
Glomus glomerulatum Sieverd.	6.94	-	-	-	-	13.33
Glomus macrocarpum Tul. & C. Tul.	-	-	-	6.63	5.82	33.33
Glomus magnicaule I. R. Hall	7.89	-	4.67	-	6.90	53.33
Glomus multicaule Gerd. & B.K. Bakshi	2.50	5.88	6.22	8.84	6.96	86.67
Glomus multisubstensum Mukerji, Bhattacharje & J.P.Tewari	2.32	4.69	-	-	4.48	46.67
Glomus sp.1	2.52	8.39	-	-	-	26.67
Glomus sp.2	2.51	3.77	-	4.05	2.45	53.33
Glomus sp.3	-	-	-	5.10	3.07	26.67
Rhizophagus aggregatus (N.C. Schenck & G.S. Sm.) C. Walker	1.98	2.27	1.14	-	-	46.67
Rhizophagus fasciculatus(Thaxt.) C. Walker & Schuessler	11.15	15.93	-	10.84	8.79	73.33
Rhizophagus intraradices C. Walker & Schuessler	20.16	21.41	31.05	19.27	18.36	100.00
Sclerocystis rubiformis Gerd. and Trappe	0.94	-	2.23	3.20	1.86	73.33
Sclerocystis taiwanensis C.G. Wu & Z.C. Chen	0.19	-	0.68	0.44	0.54	53.33
*Scutellospor*a sp.	4.27	-	-	2.56	-	26.67
Septoglomus altomontanum Palenz., Oehl, Azcon - Aguilar & GA Silva	-	5.27	6.86	-	-	33.33
Septoglomus constrictum Sieverd., G. A. Silva & Oehl	6.79	5.04	6.31	5.02	4.88	100.00
Total = 25	100	100	100	100	100	
Species richness	17	15	14	15	16	

- indicate the absence of a particular AM fungal species.

Table 3. Relative abundance (%RA) and Isolation frequency (%IF) of AM fungi in trap culture soils of five studied fruit plants.

AM fungal species	Relative abundance (%)					Isolation frequency (%)
	C. hystrix	*C. medica*	*D. indica*	*P. communis*	*P. salicina*	
Acaulospora laevis Gerd. & Trappe	-	8.03	-	-	5.25	26.67
Acaulospora scrobiculata Trappe	4.43	6.80	6.86	6.62	8.84	86.67
Acaulospora spinosa C. Walker & Trappe	-	7.47	6.05	-	-	33.33
Acaulospora sp.1	2.38	3.68	-	-	-	26.67
Acaulospora sp.2	-	-	3.43	-	-	20.00
Clariodeoglomus etunicatum C. Walker & Schuessler	14.86	-	-	5.74	9.09	53.33
Funneliformis geosporus (T.H. Nicolson & Gerd.) C. Walker & Schuessler	13.91	-	8.91	-	9.34	46.67
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & Schuessler	11.10	15.72	9.23	8.08	9.84	86.67
Gigaspora sp.	2.97	-	-	7.63	-	33.33
Glomus glomerulatum Sieverd.	3.59	8.03	-	8.01	-	53.33
Glomus macrocarpum Tul. & C. Tul.	-	-	-	8.39	6.84	40.00
Glomus magnicaule I. R. Hall	1.57	11.26	10.29	7.57	8.34	93.33
Glomus multicaule Gerd. & B.K. Bakshi	1.67	-	-	-	-	13.33
Glomus multisubstensum Mukerji, Bhattacharje & J.P.Tewari	2.51	-	-	2.21	-	26.67
Glomus sp.1	-	-	-	3.53	2.84	26.67
Glomus sp.2	1.81	-	3.19	-	-	26.67
Glomus sp.3	9.18	-	-	11.92	12.34	53.33
Rhizophagus aggregatus (N.C. Schenck & G.S. Sm.) C. Walker	19.99	22.30	22.63	25.87	18.85	100.00
Rhizophagus fasciculatus(Thaxt.) C. Walker & Schuessler	1.08	-	6.78	2.52	-	46.67
Rhizophagus intraradices C. Walker & Schuessler	-	-	1.31	0.38	-	33.33
Sclerocystis rubiformis Gerd. and Trappe	-	6.35	4.66	1.51	3.50	53.33
Sclerocystis taiwanensis C.G. Wu & Z.C. Chen	-	10.37	8.42	-	-	40.00
*Scutellospor*a sp.	8.94	-	8.25	-	4.92	53.33
Total = 23	100	100	100	100	100	
Species richness	15	10	13	14	12	

- indicate the absence of a particular AM fungal species.

macroptera which is now named as the synonym for *C. hystrix*. Although, Allay *et al*. (2021) had earlier observed the presence of DSE fungi in *C. medica*, this study is the first to demonstrate their presence in *P. communis* and *P. salicina*.

Rhizosphere soil properties

The soil samples of *C. hystrix*, *C. medica*, *D. indica*, *P. salicina* and *P. communis* had a pH range of 4.88 to 5.82, an EC from 0.29 to 0.67dSm^{-1} and organic C from 1.67 to 3.46%. Total N, available P and exchangeable K varied between 0.29 to 0.51%, 7.70 to 17.34 kg ha^{-1}, and 423 to 732.60 kg ha^{-1}, respectively. Maximum EC and P were recorded in *C. hystrix*, while OC and K were highest in *P. communis*. On the other hand, maximum pH and N were recorded in *P. salicina* and *D. indica*, respectively**.** The soil physico-chemical properties such as EC, P, and K, except pH, OC, and N, varied significantly (at $P<0.05$ and $P<0.001$) among the studied plant species. The soil texture of the selected fruit plants was sandy loam and slightly acidic in nature (Table 1).

Table 4. Diversity indices of arbuscular mycorrhizal fungal species in field and trap culture soils of studied plant species.

Fruit Plants	Diversity Indices							
	Field				Trap			
	D	*H'*	*E*	SR	*D*	*H'*	*E*	SR
Citrus hystrix	0.12±0.01^{a}	1.99±0.24^{a}	0.87±0.02^{ab}	14.00±0.27^{c}	0.14±0.01^{a}	2.09±0.03^{ab}	0.84±0.01^{a}	12.2±0.42^{c}
Citrus medica	0.14±0.01^{ab}	2.21±0.05^{a}	0.89±0.02^{ab}	12.2±0.32^{ab}	0.15±0.01^{a}	1.96±0.05^{a}	0.94±0.02^{bc}	8.2±0.17^{a}
Docynia indica	0.18±0.02^{b}	2.00±0.09^{a}	0.84±0.03^{a}	10.8±0.50^{a}	0.14±0.01^{a}	2.15±0.06^{b}	0.91±0.02^{bc}	10.6±0.44^{b}
Prunus salicina	0.11±0.01^{a}	2.37±0.03^{a}	0.93±0.01^{b}	12.8±0.32^{bc}	0.13±0.01^{a}	2.11±0.04^{ab}	0.95±0.01^{c}	9.2±0.32^{ab}
Pyrus communis	0.12±0.01^{a}	2.30±0.07^{a}	0.92±0.01^{b}	12.00±0.55^{ab}	0.14±0.01^{a}	2.11±0.05^{ab}	0.90±0.01^{b}	10.6±0.65^{b}
F-statistics	3.978*	1.503ns	2.551ns	6.107**	0.868ns	1.646ns	8.093***	9.387***

D, *H'*, *E* and SR indicates Simpson's index, Shannon-Wiener index, Evenness and Species richness respectively
Means ± SE in the column followed by different superscript letters is significantly different according to Duncan's multiple range test (P > 0.05).
ns – non-significant; * Significant at *P* < 0.05; ** and *** Significant at *P* < 0.01and *P* < 0.001.

Table 5. Percentage of root length colonization by different structures of AM and DSE fungi in five examined fruit plants.

Fruit plants	AMF colonization				DSE colonization				
	%RLH	%RLHC	%RLAC	%RLA	%RLV	%RLTC	%RLDH	%RLMI/MO	%RLTDC
Citrus hystrix	28.01±1.38^{a}	25.69±1.53^{b}	30.49±1.76^{c}	-	9.38±1.96^{a}	93.57±1.45^{b}	0.18±0.17^{a}	0.47±0.30^{a}	0.65±0.30^{a}
Citrus medica	29.83±1.56^{a}	11.84±1.41^{a}	18.63±3.13^{b}	9.71±2.55^{b}	15.04±2.58^{b}	87.76±2.94^{b}	2.19±0.90^{ab}	3.95±1.57^{bc}	6.14±2.04^{b}
Docynia indica	29.53±1.36^{a}	25.80±2.07^{b}	10.64±3.06^{a}	-	14.56±1.83^{b}	80.53±2.34^{a}	5.46±1.33^{bc}	2.27±0.48^{ab}	7.73±1.25^{bc}
Prunus salicina	26.40±2.14^{a}	25.05±2.36^{b}	10.65±2.11^{a}	7.19±1.52^{b}	9.39±2.26^{a}	78.68±2.89^{a}	8.31±2.35^{c}	5.64±1.33^{c}	13.94±2.73^{d}
Pyrus communis	27.66±1.56^{a}	21.98±2.53^{b}	24.25±2.05^{b}	1.61±1.09^{a}	5.67±1.20^{a}	81.17±3.52^{a}	8.45±2.45^{c}	3.00±1.29^{b}	11.45±3.16^{cd}
F-statistics	1.569ns	20.923***	20.149***	26.191***	8.525***	9.697***	8.296***	8.337***	12.183***

%RLH, %RLHC, %RLAC, %RLA, %RLV, %RLTC, % RLDH, %RLMI/MO and %RLTDC indicates percentage root length with hyphae, hyphal coils, arbusculate coils, arbuscules, vesicles, total AM colonization, dark septate hyphae, microsclerotia/moniliform structures and total DSE colonization, respectively.
Means ± SE in the column followed by different superscript letters is significantly different according to Duncan's multiple range test (*P*>0.05).
ns – non-significant and *** Significant at *P* < 0.001.

Spore density and distribution of AM fungi

In the present study, the AM fungal spore density observed (SD) in 100 g air-dried soils of *C. medica* (252 spores), *C. hystrix* (679 spores), *D. indica* (362 spores), *P. communis* (256 spores) and *P. salicina* (247 spores) (Fig. 1) fall within the range (41-1050 spores per 100 g soil) reported by Khanam (2007) in 19 fruit plants of Bangladesh. However, Jaison *et al*. (2012) observed 6-61 spores per 25 g soil in 39 fruit crops of Southern India, while Sharma *et al*. (2018) reported 13-35 AM spores in 25 g soil of 7 fruit plants at horticultural experimental farm of Punjab Agricultural University which are comparatively lower to that recorded in this study. Moreover, there were significant differences (*P*<0.05) in the number of AM fungal spores observed in field and trap cultures soils of the different fruit species under study (Fig. 1). The result of Pearson correlation analysis showed that spore density (SD) did not exhibit any significant

Table 6. Pearson's correlation between arbuscular mycorrhizal (AM), dark septate endophytic (DSE) fungi and soil variables of studied fruit plants (n=5).

Variables		AM				DSE					
		SD	%RLH	%RLHC	%RLAC	%RLA	%RLV	%RLTC	%RLDH	%RLMI/MO	%RLTDC
Soil	pH	0.118	-0.310	0.318	-0.139	-0.310	-0.091	-0.353	0.278	0.067	0.240
	EC	0.302	0.029	-0.175	0.249	0.183	0.203	0.481*	-0.702**	-0.058	-0.562**
	%OC	0.002	0.288	-0.283	0.300	0.149	-0.201	0.223	-0.440*	0.190	-0.262
	N	-0.149	-0.012	0.068	-0.484*	0.015	0.137	-0.461*	0.448*	0.174	0.413*
	P	0.333	-0.098	-0.182	0.271	0.227	-0.002	0.345	-0.067	-0.084	-0.084
	K	-0.375	-0.069	-0.457*	0.271	0.280	-0.362	-0.098	0.243	0.242	0.283
AM	SD		-0.037	0.172	0.372	-0.453*	0.102	0.345	-0.384	-0.552**	-0.513**
	%RLH			-0.314	-0.092	0.046	0.118	0.102	-0.308	-0.033	-0.250
	%RLHC				-0.028	-0.580**	-0.311	0.012	0.114	-0.261	-0.016
	%RLAC					-0.348	-0.474*	0.651**	-0.322	-0.616**	-0.492*
	%RLA						0.258	0.019	0.010	0.624**	0.256
	%RLV							0.067	-0.360	0.045	-0.259
	%RLTC								-0.700**	-0.545**	-0.755**
DSE	%RLDH									0.411*	0.932**
	%RLMI/MO										0.714**

SD, %RLH, %RLHC, %RLAC, %RLA, %RLV, %RLTC, % RLDH, %RLMI/MO and %RLTDC indicates spore density, percentage root length with AM hyphae, hyphal coils, arbusculate coils, arbuscules, vesicles, total AM colonization, dark septate hyphae, microsclerotia/moniliform structures and total DSE colonization, respectively.
pH, EC, OC, N, P and K- indicates hydrogen ion concentration, electrical conductivity, organic carbon, total nitrogen, available phosphorus and exchangeable potassium, respectively.
**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

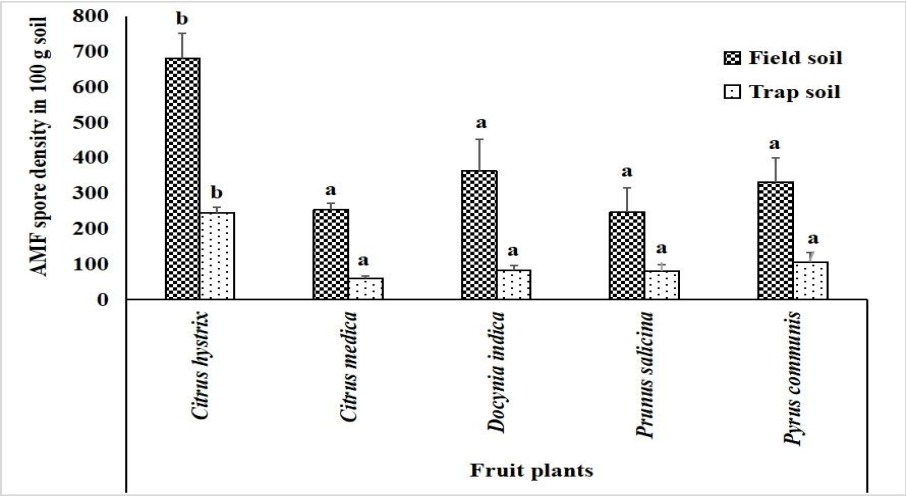

Fig. 1. Arbuscular mycorrhizal (AM) fungal spore density (per 100g soil) in field and trap soils of selected plant species. Error bars indicate ± standard error. Bars bearing same letter (s) for the fruit plants do not differ signiûcantly according to DMRT (P>0.05)

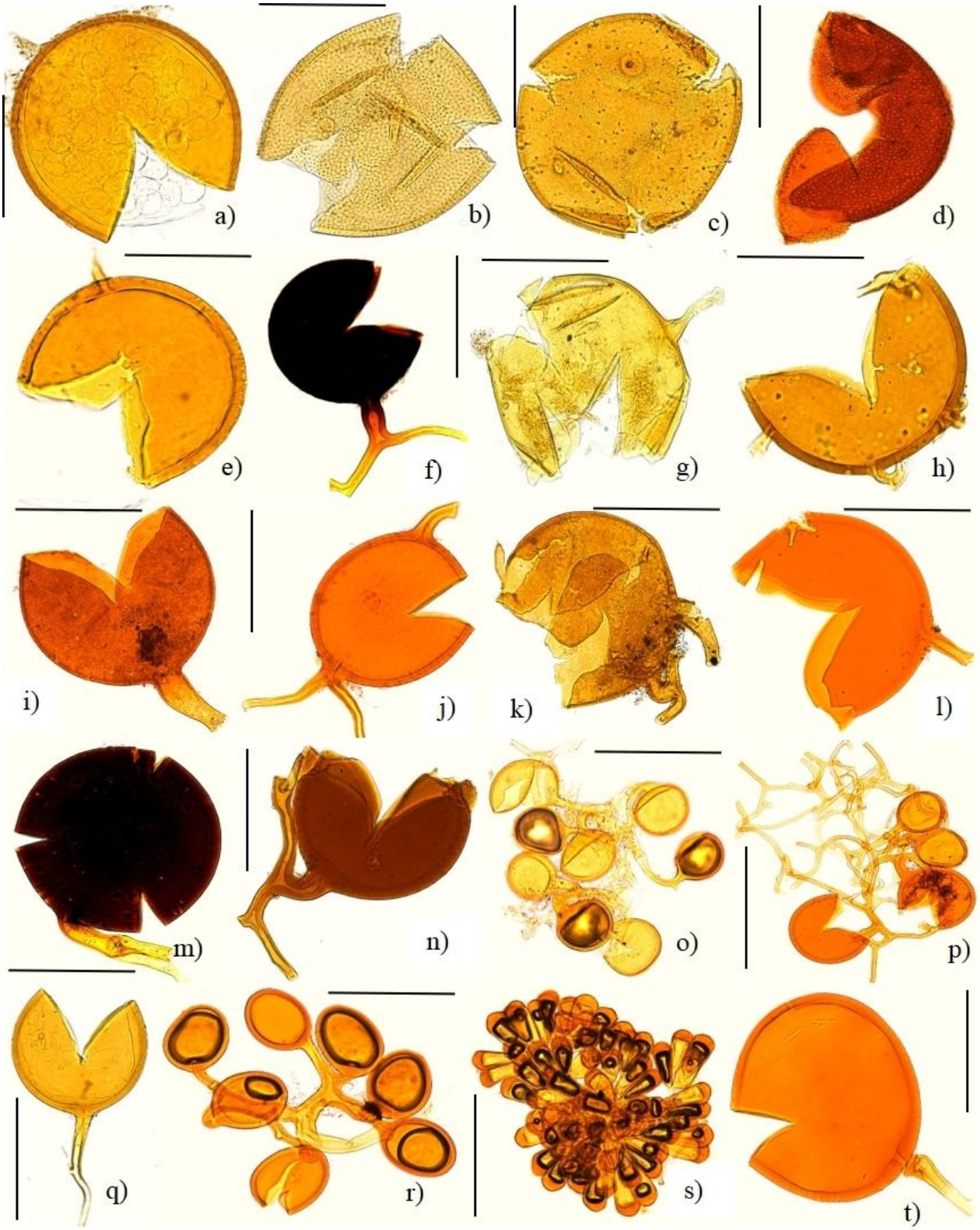

Fig. 2. AM fungal spores isolated from the field and trap soils of studied plant species. a) Spore in spore syndrome in *Acaulospora laevis*, b) *A. scrobiculata*, c) *A. spinosa*, d) *Acaulospora* sp., e) *Clariodioglomus etunicatum*, f) *Funneliformis geosporum* g) *F. mosseae*, h) *Glomus glomerulatum*, i) *G. magnicaule*, j) *G. multicaule*, k) *G. multisubstensum*, l) *Glomus* sp.1, m) *Glomus* sp.2, n) *Glomus* sp.3, o) *Rhizophagus aggregatus*, p) *R. fasciculatus* q) *R. intraradices*, r) *Sclerocystis rubiformis*, s) *S. taiwanensis*, and t) *Septoglomus constrictum*. Scale bars - d,f,g,p,q,s = 20 µm; a-c,e,h-o,r,t = 40 µm.

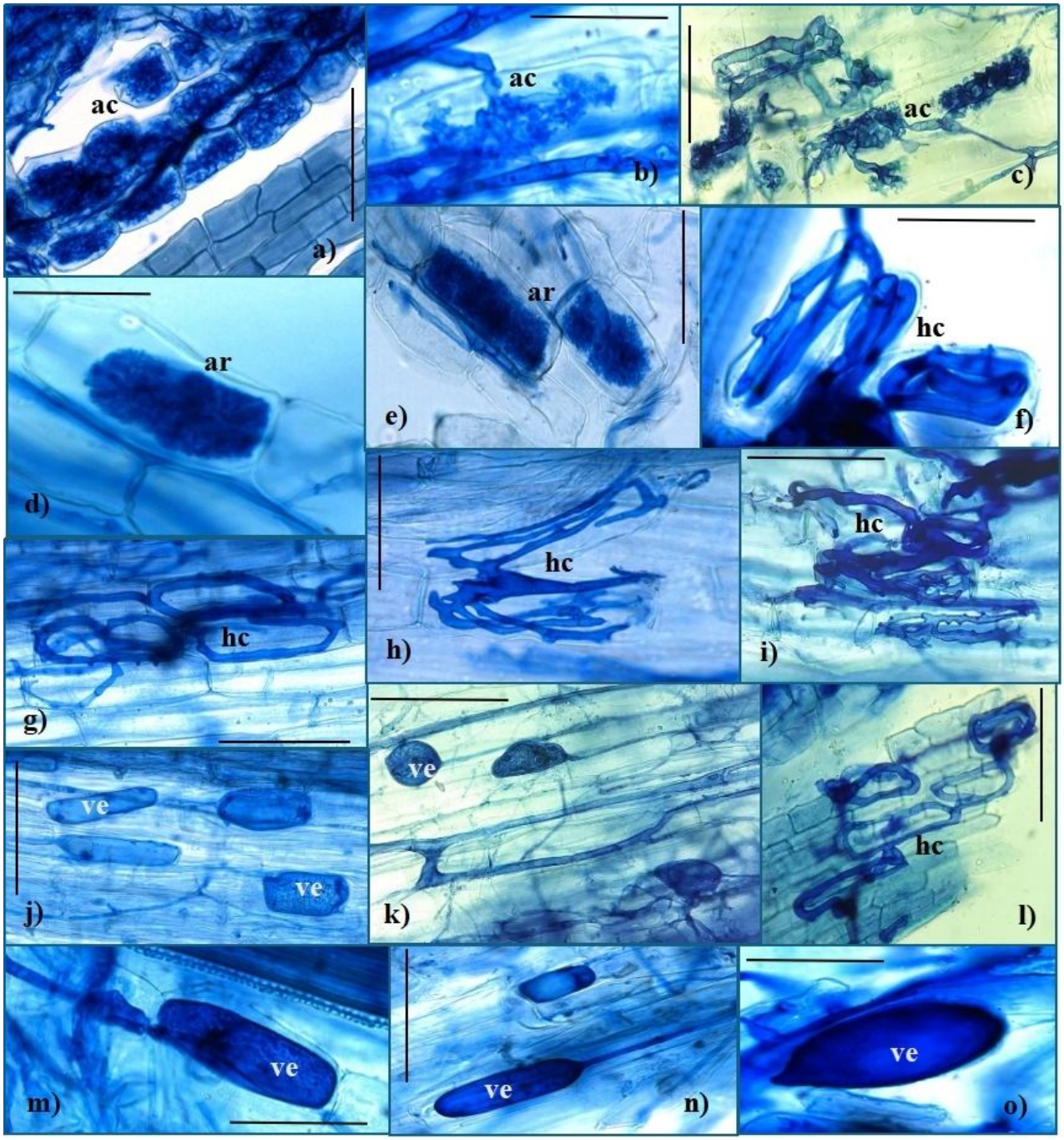

Fig. 3. AM fungal colonization in the roots of five studied fruit plants. a- arbusculate coils, f- hyphal coils, o- vesicle form in the roots of *C. hystrix*; b- arbusculate coil, i- hyphal coils, m- vesicle in *C. medica*; c- arbusculate coils, h- hyphal coils, n- vesicles in *D. indica*; d- arbuscule, l- hyphal coils, k- vesicles in *P. communis*; e- arbuscules, g- hyphal coils, j- vesicles in *P. salicina*. Scale bars: l = 20 μm; a,b,c,d,e,f,g,h,l,j,k,m,n,o = 40 μm.

correlation with the soil variables (Table 6), which is in accordance with the findings of Surendirakumar *et al*. (2021) in forest trees and *P. timoriana,* respectively growing in NE India. However, SD had a significant negative correlation with %RLA and %RLTDC. Pandey *et al*. (2020) also observed a strong negative correlation between %RLA and SD but a positive correlation between SD and % RLTDC, contrasting to the present finding.

Altogether, 25 AM fungal morphotypes belonging to 9 genera, i.e. *Acaulospora*, *Clariodeoglomus*, *Funneliformis*, *Gigaspora*, *Glomus*,

Rhizophagus, *Sclerocystis*, *Scutellospora* and *Septoglomus* were isolated from both the field and trap culture soils of studied fruit plants (Table 2 & 3, Fig. 2) which is more than the 10 AM species reported by Jaison *et al*. (2012) in 39 fruit crops. Kamareh *et al*. (2011) and Summuna *et al*. (2019) observed 9 and 21 AM species in *Pyrus glabra* and *Pyrus communis* from the Bazoft region located in Iran and Jammu and Kashmir, India, respectively. However, 12 AM fungal species were reported by Allay *et al*. (2021) from the rhizosphere of *Citrus reticulata*, *C. medica* and *C. limonia*. Fungal growth factors and the study site's microenvironmental conditions may cause differences in AM fungal diversity and density in various plant soils. Although, AM fungi and their host plants do not have a host-specific link but when the fungi are analyzed as a community, AM fungal growth rates are very host-specific and their sporulation mainly depends on particular plant species to which they are associated (Surendirakumar and Pandey, 2016). In this study, the highest relative abundance (%RA) and isolation frequency (%IF) was recorded with *Rhizophagus intraradices* from both field and trap culture soils of all studied fruit plants, indicating their wide adaptation, high sporulation rate and widespread distribution in the surrounding environment (Wang *et al*. 2019). *Glomus* was the most prevalent genera among the isolated AM morphotypes with eight species, followed by *Acaulospora* (five species) and *Rhizophagus* (three species). Several researchers have previously reported *Glomus* as a dominant genus in different geographical regions (Pandey *et al*. 2016; Wang *et al*. 2019). This may be because *Glomus* is highly adaptable and has smaller spores than other AM fungal taxa which enables them to sporulate in large numbers and to spread quickly (Wang *et al*. 2019). Two species of *Funneliformis*, *Sclerocystis* and *Septoglomus*, were found in the soils of studied plant species (Fig. 2). In contrast, only a single species was isolated in case of *Clariodeoglomus*, *Gigaspora* and *Scutellospora*. Of these, *Acaulospora scrobiculata*, *Funneliformis mosseae*, *Glomus multicaule* and *Septoglomus constrictum* were recovered from all the five examined fruit plants. While, the spores of *Acaulospra spinosa* and *Septoglomus altomontanum* were exclusively isolated from the soils of *C. medica* and *D. indica* and *Gigaspora* sp. were specific to *C. medica* and *P. salicina* soils. Moreover, *Glomus glomerulatum* was explicitly found in *C. hystrix* and *P. communis* soils, whereas the spores of *Glomus multisubstensum* were recovered from the rhizosphere and trap culture soils of *C. hystrix, C. medica*, and *P. salicina* (Tables 2 & 3). Furthermore, AM fungal species richness (SR) was found to be highest in *C. hystrix* (17 species) than the other studied plant species (Table 2). Calculated diversity indices varied significantly except for that of Shannon-Wiener index (*H'*) and Evenness (*E*) in field soils, and Simpson's index (*D*) and Shannon-Wiener index (*H'*) in case of trap soils among the selected plants. The *D* index of the AM fungal community varied from 0.11 (*P. salicina*) to 0.18 (*D. indica*), and the *H'* index ranged from 1.96 (*C. medica*) to 2.37 (*P. salicina*), whereas species evenness (*E*) varied from 0.84 (*D. indica* and *C. hystrix*) to 0.95 (*P. salicina*) in both the natural field and trap soil (Table 4).

AM morphology and extend of AM and DSE fungal root colonization

AM and DSE fungi were associated with all examined fruit plants (Table 5). *C. hystrix* and *D. indica* had Intermediate-4 (I4) type AM morphology with intra or intercellular hyphae, hyphal coils, vesicles and an abundant percentage of arbusculate coils, while the other three plants viz., *C. medica*, *P. communis* and *P. salicina* had *Arum-Paris* type morphology characterized by inter- or intracellular aseptate hyphae, hyphal coils, arbusculate coils, arbuscules and vesicles within the root cortical cells (Fig. 3). The highest percentage of total root length colonization with AM fungi was recorded in *C. hystrix* (93%) and lowest in *P. salicina* (78%). However, Allay *et al*. (2021) reported *Arum*-type morphology with hyphae, vesicles and arbuscules in *C. medica* grown in Darjeeling hills and foothills with 59-87% root colonization by both AM and DSE fungi. Additionally, Khanam (2007) and Jaison *et al*. (2012) also observed hyphae, vesicles and arbuscules in other *Citrus* species, having 23-76% and 40-80% root colonization with AM fungi, respectively. Bhuiyan *et al*. (2017) observed only AM fungal hyphae with 18-28% root colonization in *Citrus macroptera* from Rangamati hill district, Bangladesh. Dobo *et al*. (2018) reported 57%

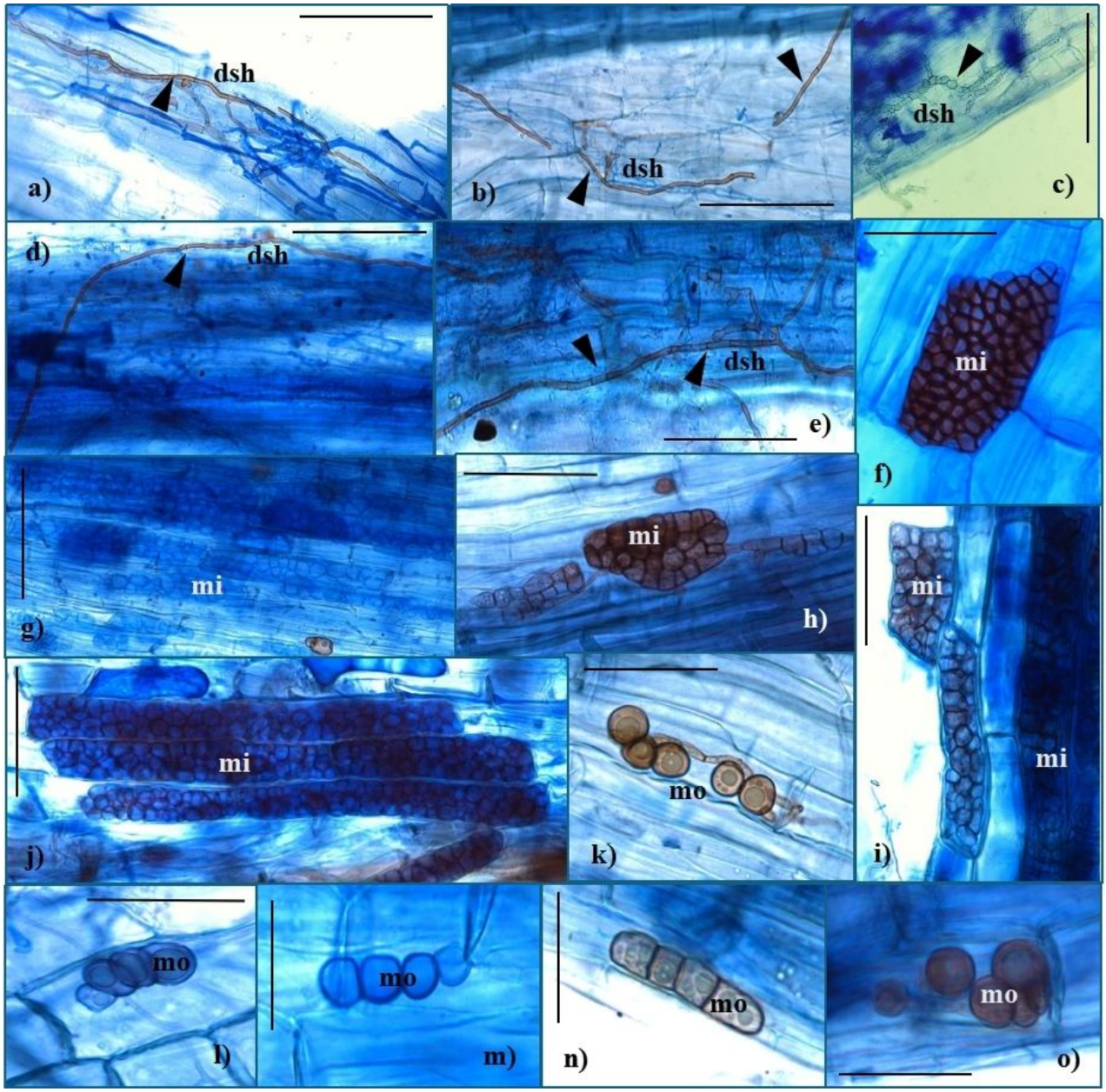

Fig. 4. DSE fungal colonization in the roots of five studied fruit plants. a- DSE fungal hyphae, j- microsclerotia, m- moniliform structures in *D. indica*; b- DSE fungal hyphae, g- microsclerotia, k- moniliform structures in *P. salicina*; c- DSE fungal hyphae, i- microsclerotia, l- moniliform structures in *P. communis*; d- DSE fungal hyphae, h- microsclerotia, n- moniliform structures in *C. hystrix*; e- DSE fungal hyphae, f- microsclerotia, o- moniliform structures in *C. medica*. Scale bars: 40 µm.

colonization having AM vesicles and arbuscules in the roots of *Prunus africana* from Sidama, Southern Ethiopia. Only 5-13.5% of root colonization was observed in *Pyrus glabra* (wild pear) from the Bazoft region of Iran (Kamareh *et al*., 2011). Different levels of root growth rates, the host plant species and the microclimatic conditions (temperature, light intensity and soil moisture) of the particular habitats might all have an impact on the ability of AM fungi to form distinct structures and colonization patterns in different species of a plant genus (Dickson, 2004). Except for %RLH, the degree of root length colonization with different AM fungal structures varied significantly (P<0.05) among the studied fruit plants (Table 5). The functioning and colonization levels of AM fungi are known to be influenced by soil characteristics (Smith and Read, 2008). In this study, the correlation analysis revealed a significant negative correlation between N and

%RLAC which is similar to the findings of Lingfei *et al*. (2005) in Southwest China grasslands. Furthermore, the results of Surbala and Pandey (2020) are consistent with the substantial negative correlation between K and %RLHC. In this study, there is a negative correlation between N and %RLTC and a positive correlation between EC and %RLTC. However, in *Asparagus* species, Muthukumar and Muthuraja (2016) found no relation between these variables.

Different DSE fungal structures such as hyaline or melanized septate hyphae, microsclerotia or moniliform were found to colonize the examined roots of all fruit plants, even though their frequency varied with each plant species. The extent of total root length colonization by DSE fungi in our investigation ranged from 0.65% in *C. hystrix* to 13.94% in *P. salicina* (Table 5), which is less than the 0.5–89% in fruit crops observed by Jaison *et al*. (2012). Such findings in differing percentage colonization by DSE fungi in various plant roots were observed because DSE fungal relationships like any plant-fungal interaction depend on the hosts and habitats variation (Newsham, 2011). The root length colonization with DSE fungal structures varied significantly (P<0.05) among the studied fruit plants (Table 5; Fig. 4). The negative correlation between soil EC and %RLDH, OC and %RLDH, and the positive correlation between soil total N and %RLTDC (Table 6) aligns with the reports of Surbala and Pandey (2020). This study revealed that %RLTDC is negatively correlated with soil EC and OC, which contradicts the finding of Surendirakumar *et al*. (2021) where no correlations were observed among them. Thus, it can be concluded that soil conditions also have an impact on DSE fungal colonization. The inverse relationship between the total percentage of root length colonization by AM (%RLTC) and DSE (%RLTDC) fungi (Table 6) in this study is in accordance with the observations of Muthukumar and Vediyappan (2010). When conditions become unfavorable for the establishment or functioning of AM symbiosis, DSE fungi serve as a backbone for the host plants (Christie and Kilpatrick, 1992).

CONCLUSION

The examined fruit plants showed strong symbiotic associations with AM and DSE fungi. Additionally, these two groups of fungi heavily colonized the roots of studied plants, indicating that they are widely distributed in this Hot Spot region. Furthermore, several soil conditions significantly influenced the spore density of AM fungi and colonization patterns of AM and DSE in selected fruit species. Given that both AM and DSE fungi have the potential to colonize horticultural plant species, more research is required to understand their diverse capacities in promoting plant growth which will make it possible to employ them in sustainable horticultural development, in future. As evident from the present study it can be assumed that many neglected fruit plants in their natural habitats still need to be investigated for their fungal associations.

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DECLARATIONS

Conflict of Interest: Authors declare no conflict of interest.

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