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Rhizosphere soil and root samples of three indigenous Rice (*Oriza sativa* L.) cultivars viz. Chakhao amubi, Chakhao white and Phourel amuba, were collected from Jhum agricultural fields of Senapati District, Manipur during harvesting period and examined for spore density of arbuscular mycorrhizal fungi (AMF), AM morphology and colonization patterns of AM and dark septate endophyte (DSE) fungi. AMF spore population was highest in soil samples of Chakhao white (173 spores 100 g^{-1} soil). A total of 11 AM fungal morphotypes belonging to different genera viz. *Acaulospora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis*, and *Scutellospora* were isolated from different soils. *Arum*- and Intermediate-types of AM morphology were observed in different Rice cultivars. Intensity of AM and DSE fungal colonization and distribution of fungal structures varied with the host cultivars. Total root length with AMF colonization ranged from 50% (Chakhao amubi) to 62% (Chakhao white). Root length with hyphae varied between 23% (Chakhao amubi) and 29% (Phourel amuba); intracellular hyphal coils between 25% (Phourel amuba) and 28% (Chakhao white) and the vesicles ranged from 6% (Chakhao white) to 7% (Phourel amuba). Extent of total root length with DSE colonization was maximum (15%) in Chakhao white, while microsclerotia varied between 5% (Chakhao amubi) to 6% (Chakhao white). Arbuscules were observed only in the roots of black scented Rice cultivar i.e. Chakhao amubi. All the AM and DSE fungal variables varied significantly ($P < 0.05$) among different cultivars.

Key words: AM fungi, DSE fungi, rice cultivars, Manipur

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are the key functional component of soil biota in agroecosystems and form symbiotic associations with majority of the crop plant roots (Smith and Read, 2008). Arbuscular mycorrhizas (AM) have been estimated to account for 5-10% of the soil microbial biomass (Fitter *et al*, 2011) and improve the plant growth and health, facilitate mineral nutrients and water uptake, protect from pathogens, prevent soil erosion by having positive impacts on soil aggregate stability and influence carbon, phosphorus and nitrogen cycles (van der Heijden *et al*, 1998; Jeffries *et al*, 2003). Many AMF species are ubiquitous in different terrestrial ecosystems (Öpik *et al*, 2006) whereas, others appear to be restricted to specific habitats, land use types, vegetation or climates (Oehl *et al*, 2010). The colonization patterns of AMF within host roots tend to vary with the plants and fungal species involved (Dickson *et al*, 2007). Ability of AMF to colonize roots and provide nutrients to the host might differ depending on their morphological types i.e. *Arum*-, *Paris*- or Intermediate (Dickson, 2004). Although AM association has been reported in many crop species their morphologies are yet to be ascertained (Muthukumar and Tamilselvi, 2010). In addition to AMF, increased attention has recently been paid to another group of root colonizing fungi, the dark septate endophytes (DSE, phylum Ascomycota) characterized by melanized septate hyphae and compactly arranged intracellular microsclerotia and moniliform structures, that also

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coexist with AMF in a wide range of hosts at different latitudes and altitudes and potentially confer benefits to the plants by mineralizing nutrients in the rhizosphere and enhancing stress tolerance to them (Mandyam and Jumpponen, 2005). Nevertheless, information on DSE fungal association in tropical crops is limited as compared to temperate crop species.

Rice (*Oryza sativa* L., family Poaceae) is the most important staple food crop for human consumption and accounts for 23% of the world's caloric intake (Khush, 2003). Manipur, being one of the eight sister states of North East (NE) India, is considered as Rice bowl and has large variability of indigenous cultivars with several locally adapted aromatic races which are cultivated in upland, lowland and wetland conditions (Medhabati *et al*., 2013; Roy *et al*, 2015). There are prominent cultivar groups within the aromatic rice gene pool among which Chakhao (Chak=rice and ahoba or hao=delicious) land races are the important ones. Upland rice is widely practiced in the hill tracts of Manipur as shifting- or Jhum- and terrace- cultivation crop (Chanu *et al*, 2010). At higher elevations, the black kernelled scented rice locally called as 'Chakhao amubi' is a novelty of the state but contributes fewer yields and thus, are limited in cultivation (Hore, 2005). In India, several upland- (Rajeshkannan *et al*, 2009), lowland- and wetland-rice varieties have been reported to develop AMF association but cultivation methods and soil types may have an impact on their spore numbers in soils and root colonization patterns. However, in NE India particularly in Manipur, very little work has been carried out on the diversity of AM fungi (Bhattacharjee and Sharma, 2011) and on the occurrence of DSE fungal associations (Das and Chaudhuri, 2009) in different rice cultivars. Knowledge on the species composition of AMF and their distribution patterns in paddy cultivars will not only increase our understanding of the diversity in upland habitats but also provide an insight on the application aspects to enhance rice yield and to promote sustainable agriculture in future. Therefore, an attempt has been made in the present investigation to determine the AMF spore density and species diversity in the rhizosphere and AM morphology as well as root colonization patterns of AM and DSE fungi in the roots of three indigenous rice cultivars grown in subtropical areas of Manipur, NE India.

MATERIALS AND METHODS

Study site

The present work was conducted in shifting cultivated rice agricultural fields located on the gentle slopes of hillocks along the Taphou Naga hill range of Senapati District (25^0 16' 43.3" N Latitude; 94^0 01' 12.1" E Longitude), having elevation between 1117-1142 m a.s.l., at a distance of 62 km north of Imphal, the capital city of Manipur. The climate of the area is subtropical humid and the year is divisible into three distinct seasons characterized by warm moist summer, wet rainy months and cool dry winter. Mean annual temperature of the site ranged from 3.4^{0}C to 34.4^{0}C, annual rainfall between 671 mm to 1454 mm and the relative humidity from 76% to 92%. Soils of the study sites were clayey loam in texture and slightly acidic in nature.

Sample collection

Fine roots and rhizosphere soil samples of three different rice cultivars i.e. Chakhao amubi, Chakhao white and Phourel amuba were collected separately by digging 0-20 cm soil depth around fifteen individuals of each during harvesting period (October-November, 2014) and were placed in individual polythene bag, labelled and brought to the laboratory. After air drying in shade, one part of the soil samples of a cultivar was bulked together and used for assessment of soil properties, while remaining soil portion of each was used for the extraction of AMF spores. Roots were gently washed and preserved in FAA solution (Formalin: Glacial Acetic acid: 70% ethyl alcohol, 5: 5: 90 ml v: v: v) until processing.

Analysis of soil properties

Soil texture was analyzed by Bouyocos hydrometer method (Allen *et al*, 1974). Soil pH and Electrical conductivity (EC) were determined at room temperature in aqueous solution of soil : water (1:1, v:v) using digital meter. Organic Carbon (OC) was assessed by Walkley and Black rapid titration method (1934). Total nitrogen (N) and available phosphorus (P) were determined according to Jackson (1971) and exchangeable potassium (K) was analyzed after extraction with ammonium acetate (Jackson, 1971).

Extraction and Identification of AMF spores

AMF spores were extracted by wet sieving and

decanting method (Gerdeman and Nicolcon, 1963). One hundred gram of each soil sample was dispersed in 1L of water and the suspension was decanted through a series of 710 to 37 µm sieves. Residues in the sieves were washed into beakers, whereas the sievates were dispersed in water and filtered through girdled filter papers. Each filter paper was then spread on Petri dish. All intact AMF spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted using a dissecting microscope (BTI Magno MS 24, India) at 40x magnification. Sporocarps and spore were considered as one unit. AMF spores were transferred onto slides using a wet needle and mounted in Polyvinyl alcohol-Lacto glycerol (PVLG) with or without Melzer's reagent for identification based on spore morphology and sub-cellular characters and were also compared to the culture database established by INVAM (http://invam.cag.wvu.edu/).

Assessment of AM and DSE fungal colonization

Fixed roots were washed with distilled water to make them free of FAA, cut into 1 cm segments and processed for clearing in 2.5% KOH at 90^{0}C for 1-2 h in water bath, depending on the degree of lignification of the roots, acidified with 5N HCL solution for at least 15-20 min and stained with 0.05% of Trypan blue-Lacto glycerol (0.05%) overnight at room temperature. Roots that remain dark after clearing were bleached in alkaline H_2O_2 (3%) prior to acidification. Stained root pieces were mounted on glass slides in Lacto glycerol and examined for AM and DSE fungal structures with compound microscope (Olympus BX 51, Japan). The percentage root length colonization was estimated according to magnified intersection method (Mc Gonigle *et al*, 1990). AM morphology were characterized based on inter- or intracellular nature of fungal structures within the roots. Since only whole or squashed root specimens were examined in this study, we could not distinguish the intermediate sub-types as described by Dickson (2004) but wherever the parallel running hyphae were seen intracellularly the morphology was designated as an intermediate-type.

Establishment of trap culture

The collected composite rhizosphere soil samples (including root fragments) were used as trap inoculum and were mixed with sterilized sand (1:1 v/v). Before transferring to the earthen pots (30 cm diam) they were surface sterilized with 90% ethanol. Trap cultures were kept in green house conditions and were seeded with Maize (*Zea mays* L.) as a trap plant host. The pots were watered on alternative days and after 120-150 days, AMF spores were extracted and identified as mentioned above.

Calculation and statistical analysis

Spore density and species richness of AMF were expressed as total number of spores and the number of species occurred in 100 g of soil sample. Relative abundance and isolation frequency of AMF species were calculated (Dandan and Zhiwei, 2007). Analysis of variance (ANOVA) was used to test the significance of variations between AM and DSE fungal variables of test Rice cultivars (SPSS version 9, SPSS Inc., Chicago, Illinois). Pearson's correlation was carried out to assess the relationship between soil physico-chemical properties and mycorrhizal colonization. Diversity indices were calculated and the statistically significant differences were determined.

RESULTS AND DISCUSSION

Soil physico-chemical properties

The rhizosphere soils of all three indigenous rice cultivars had a pH range of 5.9 to 6.03, an EC of 0.20 to 0.37 dSm^{-1}, organic C of 1.64 to 1.86%, during the harvesting period (Table 1). Total N, available P and exchangeable K varied between 87.3 to 108.6 kg ha^{-1}, 8.0 to 10.9 kg ha^{-1} and 221.5 to 238.0 kg ha^{-1} of soil, respectively. All the soil properties (except pH) examined varied significantly (ANOVA) among different Rice cultivars. Maximum concentrations of OC, N, and K were recorded in the soil of Chakhao white cultivar while P percentage was highest in Phourel amuba cultivated soil (Table 1).

AMF spores density and species distribution

Highest spore population of AM fungi was observed in root-zone soil of Chakhao white cultivar (173 spores 100 g^{-1} soil) compared to that of Chakhao amubi (139 spores 100 g^{-1} soil) (Fig. 1). However, AMF spore density did not vary significantly ($F_{2,42}$=0.281; P<0.05) among the Rice varieties and

was not significantly correlated to the AM fungal root colonizing variables (r=0.403; P<0.05).

A total of 11 AM fungal species morphotypes corresponding to 6 genera, i.e. *Acaulospora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Sclerocystis* and *Scutellospora*, were isolated from the natural field soil and trap cultures of test Rice cultivars (Table 2, Fig. 2). *Glomus* was the dominant genus that was represented by 5 different species. Out of these, *Glomus* sp. 1 and 2 were recovered from the all 3 Rice cultivar soils, while *G. aggregatum* was extracted from Chakhao amubi rhizosphere and *G. ambisporum* was found in other two Rice varieties soils. Out of all, genus *Acaulospora* was represented by 2 species, whereas in case of *Funneliformis*, *Rhizophagus*, *Sclerocystis* and *Scutellospora* a single species was recorded in each. *Funneliformis geosporum* was commonly found in all three Rice cultivars soils revealing highest percentages of Relative abundance. *Acaulospora spinosa* and *Rhizophagus fasciculatum* were exclusively isolated from Chakhao white soil, while *Glomus versiforme* and *Scutellospora* sp. were specific to Phourel amuba soil. *Acaulospora* sp. 1 and *G. aggregatum* were found only in Chakhao amubi cultivated soil. AMF community composition as assessed by Shannon-Wiener index of diversity (H), Simpson's index of dominance (D), Species evenness (E) were highest in case of Chakhao white cultivar (Table 3).

Occurrence and extent of AM and DSE fungal colonization

All the examined Rice cultivars had dual association of both AM and DSE fungi (Table 4; Fig. 3). *Arum*-type of AM morphology was observed in Chakhao amubi, while other two Rice cultivars had intermediate-type morphology. AM fungal structures within roots were characterized by the formation of an appressorium on the root surface and hyphal coils in few outer cortical cells near the point of entry (Fig. 3 c-d). Presence of inter- or intracellular hyphae, hyphal coils, arbuscules and vesicles were also observed (Fig. 3 e-h). The percentage root length with total AM colonization (%RLTC) ranged from 50% (Chakhao amubi) to 62% (Chakhao white). Percentage root length with hyphae (%RLH) varied between 23% (Chakhao amubi) and 29% (Phourel amuba); hyphal coils (%RLHC) between 25% (Phourel amuba) to 28% (Chakhao white) and the vesicles (%RLV) ranged from 6% (Chakhao white) to 7% (Phourel amuba). Arbuscules (%RLA) were observed only in the roots of Chakhao amubi (20%). Significant variations were observed in the root length colonization with AM structures i.e. RLH, RLHC, RLA and total colonization (RLTC) among different Rice cultivars, whereas in case of RLV no significant difference was recorded. Similarly, correlation analysis revealed the existence of a significant positive correlations between AMF structures i.e. %RLH (r= 0.612; P<0.05), %RLHC (r= 0.736; P<0.01), and %RLTC (r= 0.610; P<0.05) and soil EC, and between %RLV (r= 0.595; P<0.05) and soil pH. In contrast, %RLA (r= 0.780; P<0.01) was significantly negatively correlated to soil EC. Significant negative correlation was also observed between soil phosphorus and AMF spore numbers (r= 0.019; P<0.05).

All the rice cultivars were colonized with DSE fungi characterized by the presence of darkly pigmented septate hyphae and microsclerotia or moniliform structures (Table 4, Fig. 3 i-l). Extent of total root length with DSE colonization (%RLTD) was maximum (15%) in Chakhao white cultivar, while the microsclerotia/moniliform structures (%RLMI/MO) varied between 5% (Chakhao amubi) to 6% (Chakhao white). There were no significant variations between DSE fungal variables of 3 Rice culti-

Table 1 :Physico-chemical properties of three Rice cultivars rhizosphere soils

Rice cultivar	Soil properties[1#]					
	pH	EC (dSm^{-1})	OC (%)	N ($kg\ ha^{-1}$)	P ($kg\ ha^{-1}$)	K ($kg\ ha^{-1}$)
Chakhao amubi	6.3±0.11a	0.20±0.01a	1.7±0.06ab	104.2±4.37b	9.1±0.34b	221.5±5.37a
Chakhao white	5.9±0.09a	0.28±0.02b	1.9±0.14b	108.6±2.78b	7.9±0.18a	238.0±3.83b
Phourel amuba	6.0±0.15a	0.37±0.02c	1.6±0.10a	87.3±2.25a	10.9±0.26c	225.1±4.88a

[1] pH, EC, OC, N, P and K- indicates percentage hydrogen ion concentration, electrical conductivity, organic carbon, total nitrogen, available phosphorus and exchangeable potassium, respectively.
[#] Means ± SE in the column followed by same letter (s) do not differ significantly according to DMRT (P<0.05).

Table 2 : Relative abundance (%RA) and Isolation frequency (%IF) of AM fungal species in test Rice cultivars

AMF species	% RA			%IF
	Rice cultivar			
	Chakhao amubi	Chakhao white	Phourel amuba	
Acaulospora sp.1	3.1	-	-	33.3
Acaulospora spinosa Walker & Trappe	-	15.4	-	33.3
Funniliformis geosporum (T.H. Nicolson & Gerdemann) C. Walker	36.9	30.8	32.6	100.0
Glomus aggregatum N.C.Schenk & G.S. Sm. Emend. Koske	15.4	-	-	33.3
Glomus ambisporum Smith & Schenck	-	10.9	14.6	66.7
Glomus versiforme (P. Karst.) S.M. Berch	-	-	13.5	33.3
Glomus sp.1	23.1	9.8	10.1	100.0
Glomus sp.2	21.5	10.9	13.5	100.0
Rhizophagus fasciculatum (Thaxter) Gerdemann & Trappe emend. C. Walker & Koske	-	9.8	-	33.3
Sclerocystis rubiformis Gerd. & Trappe	-	12.1	6.7	66.7
Scutellospora sp.	-	-	8.9	33.3
Total	100	100	100	-

Table 3 : Diversity indices of AM fungi in three indigenous Rice cultivars

Diversity parameters	Rice cultivar		
	Chakhao amubi	Chakhao white	Phourel amuba
Shannon-Wiener index of diversity (H)	1.43	1.85	1.82
Simpson's index of dominance (D)	0.74	0.82	0.81
AMF species evenness (E)	0.84	0.91	0.88
AMF species richness (SR)	5	7	7

vars ($F_{2,42}$=0.094; P<0.05). Pearson correlation analysis revealed that %RLTD was significantly positively correlated with %RLDH (r=0.949; P<0.001) and %RLMI/MO (r=0.681; P<0.01).

This study provides the first comprehensive report on AMF diversity, AM morphology and AM and DSE fungal associations in three indigenous rice cultivars. High incidence of root colonization by AM fungi confirms the ubiquity of mycorrhizal association in tropical and subtropical agroecosystems. Total spore density of AMF in the present study ranged between 139 to 173 spores 100 g^{-1} soils of examined rice cultivars during harvesting period which is low compared to those recorded by Bhattacharjee and Sharma (2011) who observed 435-587 AMF spore population in 50 g^{-1} soil of three rice varieties during crop harvest from Barak valley, Assam, NE India. It was reported that recorded an average of 1668 VAM spores per 100 g soil of rice at harvesting time from different agroecological zones of Pakistan. Clayey soils retain a better moisture content, which is good for the rice plant growth, but are not favourable for AMF growth because their populations are generally inhibited at low oxygen levels that prevail in flooded soils of rice fields for maximum period of crop growth. However, an array of environmental, host and fungal growth factors influence the AMF population in natural soils.

We isolated 11 species of AM fungi from root-zone soils of examined Rice cultivars. *Funneliformis geosporum* and *Glomus* spp. were the most abundant species in test rhizosphere soils with maximum RA and IF values. Species richness from genus *Glomus* is earlier reported as the most widely distributed AM fungi in Rice soils of different geographical regions with varied environmental conditions; Dubey *et al*, 2008; Das and Choudhury, 2009; Bhattacharjee and Sharma, 2011). Manoharachary *et al*, (2005) reviewed the

diversity of AM fungi in India and mentioned that genus *Glomus* is ubiquitous in various ecosystems. Occurrence of several AMF species in this study i.e. *Acaulospora spinosa*, *Glomus aggregatum*, *Rhizophagus fasciculatum* (=*Glomus fasciculatum*) were also reported to be present in other rice cultivar soils (Bhattacharjee and Sharma, 2011) which show that although the association between AM fungi and host plants are not host specific but when the fungi are examined as a community AMF growth rates are highly host specific and their sporulation depends on the plant species with which they are associated.

Several Rice varieties have been previously reported to be colonized by AM fungi, but the AM morphology has not been categorically studied (Muthukumar and Tamilselvi, 2010). In this study, the two Rice cultivars i.e. Chakhao white and Phourel amuba had intermediate-type of AM morphology, while black-scented Rice e.g. Chakhao amubi revealed *Arum*-type. It is generally presumed that *Arum*-type AM morphology is often associated with cultivated crop plants which are fast growing and light-loving, and *Paris*-type morphology seems to occur in shade-loving and slow-

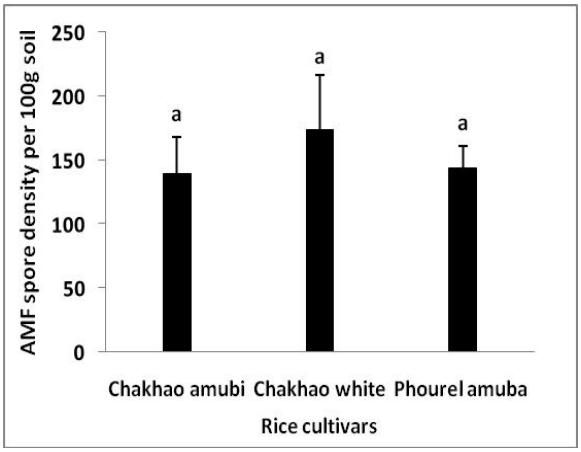

Fig. 1. : Spore density of arbuscular mycorrhizal fungi (AMF) in soils of three Rice cultivars

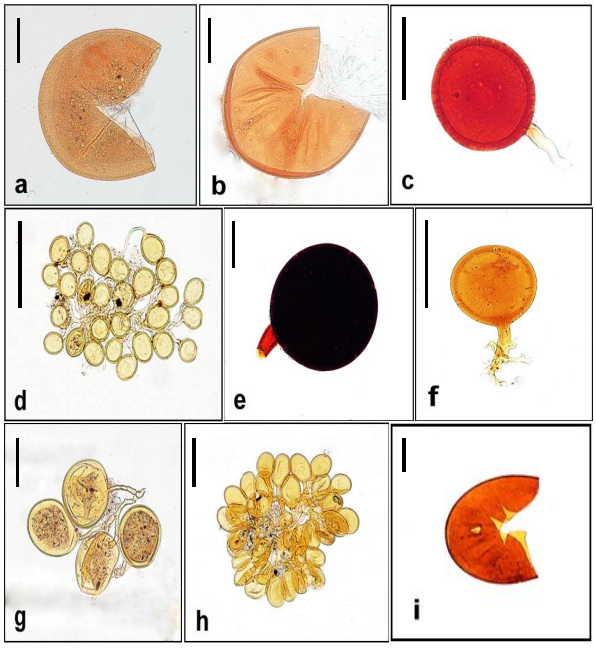

Fig. 2. : AMF spores isolated from rhizosphere soil. (a) *Acaulospora* sp., bar 20µm; (b) *Acaulospora spinosa,* bar 20µm; (c) *Funneliformis geosporum,* bar 40µm; (d) *Glomus aggregatum,* bar 40µm; (e) *G. ambisporum,* bar 20µm; (f) *G. versiforme,* bar 40µm; (g) *Rhizophagus fasciculatus,* bar 20µm; (h) *Sclerocystis rubiformis,* bar 20µm; (i) *Scutellospora* sp., bar 10µm.

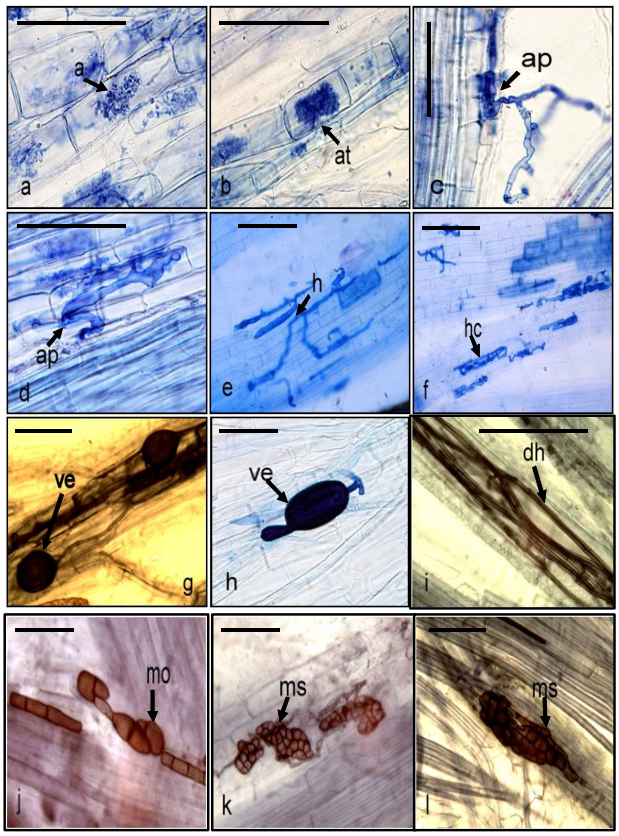

Fig. 3: Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal colonization. Arbuscule (a) and Arbuscular trunk (b) in Chakhao amubi; Appresorium (c)-(d) in Chakhao amubi and Chakhao white, respectively; Hyphae in Chakhao white (e) Hyphal coil in Phourel amubi (f); Vesicles in Chakhao amubi (g) and Chakhao white (h); DSE hyphae in Chakhao amubi (i); Moliniform structure in Chakhao amubi (j); microsclerotia in Chakhao amubi (k) and Phourel amubi (l); a- arbuscule, ap- appresorium, at- arbuscular trunk, h- hyphae, hc- hyphal coil, ve- vesicle, dh- DSE fungal hyphae, mo- moniliform, ms- microsclerotia. (Scale Bars: 40-60µm).

Table 4 : AM morphology, AMF spore density, AM and DSE fungal colonization in three Rice cultivars

Rice :ultivar	AM fungal colonization[1#]					DSE fungal colonization[2#]		
	%RLH	%RLHC	%RLA	%RLV	%RLTC	%RLDH	%RLMI/MO	%RLTD
ɲakhao ɜmubi	23.3±2.6a	0.0±0.0a	20.3±2.1b	6.5±0.7a	50.1±3.1a	9.1±1.5a	5.5±0.9a	14.7±2.1a
ɲakhao white	28.2±1.6b	27.8±1.5b	0.0±0.0a	5.8±0.7a	61.6±2.3b	9.2±1.4a	6.2±0.8a	15.4±1.8a
'hourel ɪmuba	28.6±1.3b	25.5±2.2b	0.0±0.0a	6.8±0.8a	60.9±2.1b	9.4±1.3a	5.6±0.9a	15.0±1.7a

[1] SPN- AMF spore density. [1]% RLH, %RLHC, % RLA, %RLV and %RLTC- indicates root length with hyphae, hyphal coils, arbuscules, vesicles and total colonization, respectively. [2]= % RLDH, %RLMI/MO and %RLTD- indicates root length with Dark septate hyphae, microsclerotia/moniliform structures and total root length colonization by DSE fungi, respectively.

Mean ± S.E in the column followed by same letter(s) do not differ significantly according to DMRT ($P < 0.05$).

growing plants. Whereas, intermediate-type have the characteristics of both *Arum*- and *Paris*-types of AM morphology (Dickson, 2004). Factors involved in determining the formations of different morphologies still remain unclear. However, it has been observed that differences in cell wall structure and modification produced during fungal colonization in roots, micro-environmental condition of soil and plant growth rates are the important factors in determining the AM morphology in various plant species.

Our findings revealed the presence of both AM and DSE fungal associations in roots of selected rice cultivars. Extent of total AMF colonization (50-61%) observed in the present study is partly consistent with the findings of Muthukumar and Tamilselvi (2010) and Bhattacharjee and Sharma (2011) who recorded 53% and 64-80% root colonization, respectively in different rice cultivars roots. Well developed root colonization by AMF greatly enhances the transportation of organic nutrients from the host to fungus, thereby improving hyphal extension and sporulation in the surrounding environment. It has been observed that changes in soil moisture, temperature and P availability, plant phenology, growth rate and turnover of plant roots are important factors for AMF spore production and mycorrhizal colonization in natural ecosystems. Soil EC in the present study negatively affected the percentage root length colonization with arbuscules which is in accordance with the observations of Füzy *et al*. (2010) where arbuscules richness in *Plantago maritima* was significantly negatively correlated with EC. It has been observed that increasing concentration of soil P reduces the AMF growth therefore, negative correlation between the spore population of AM fungi and P content is obvious.

Presence of DSE fungi in examined rice cultivars is expected as these endophytes are assumed to be wide spread among angiosperms . The functional relationship between DSE fungi may be comparable to that between mycorrhizal fungi and their host. Studies have shown that DSE fungal association in the plants is mutualistic rather than parasitic and the colonization by different root-associated DSE fungi reflects their potential to function as mutualists along with the AM fungi. Nevertheless, understanding the role of DSE fungi requires further studies at functional and compositional levels as the occurrence of these fungi are frequent and their function in plant survival in stressful environment could be important and beneficial in such conditions.

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