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Research Article

Arbuscular mycorrhiza and dark septate endophyte fungal associations of *Oryza sativa* L. under field condition: colonization features and their occurrence

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Abstract

The present study was aimed to study monthly colonization of arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations in rice. The presence of mycorrhizal structures in the roots confirms the colonization by AM fungi. The pattern of hyphae and arbuscules denotes *Arum* type of AM fungal morphology. The presence of dark coloured septate hyphae running frequently on the epidermal layer and in root cortex and the occurrence of microsclerotia marks the colonization by DSE fungi. The co-occurrence of both AM and DSE fungi ensure dual colonization by two distinct fungal groups. There was significant increase in arbuscules, vesicles and hyphal percentages from first to third month in both the samples collected from two sites. In the third month, AM colonization significantly higher in both the sites. DSE colonization percentages do not differ significantly in first to third month. A total of nine AM fungal species were recovered from two sites. This study is an effort to make aware the local farmers about the usefulness of these native AM mycobiota which can be a preferable choice over chemical fertilizers leading to ecofriendly organic farming.

Keywords: Rice; AM fungi; DSE; *Arum* type; AM fungal species

Citation
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Introduction

Mycorrhizal association between beneficial soil fungi and plant roots are ubiquitous in terrestrial plant communities (1). The colonization of crop plants by arbuscular mycorrhizal (AM) fungi can improve their nutrients uptake predominantly phosphorus and are linked with crop yields (2,3). Based on the occurrence of AM fungal structures within plant roots, AM morphology has been classified as *Arum*, *Paris* or intermediate-types (4).

Roots of crop plants are also colonized by a group of melanized, septate fungi known as dark

septate fungal endophytes (DSE) (5,6). DSE facilitates nutrient uptake of the plant, helps in water uptake and increases stress tolerance (7).

Rice (*Oryza sativa* L.) is one of the major staple food of Northeast India and flourishly grown throughout India. The rice straw is also used as cattle feed and for making straw boards, mats and papers. Rice bran oil is used for making soap and cosmetics (8). Certain studies revealed that AM fungi root colonization has seasonal dynamics (9,10). Rice plants readily form mycorrhizal associations under upland conditions and limited colonization occurs under submerged conditions due to the anoxic environment (11). Application of mycorrhizal inoculum increased the soil nutrients and root colonization in rice plants (12).

There is no report related to monthly AM and DSE fungal colonization of rice in relation to structural features within the root. In this connection, the present study was focused on the evaluation of AM and DSE fungal colonization and AM fungal composition associated with rice growing in two areas of Tripura, Northeast India.

Materials and methods

Study sites

For assessing the AM, DSE fungal colonization and AM fungal composition, root and soil samples were collected regularly at an interval of one month from two selected study sites of Suryamaninagar and Teliamura, Tripura, Northeast India. The study sites of rice field were Suryamaninagar (N 23º 46.0' E 91º 16.4', 22 masl) and Teliamura (N 23º 56.361' E 91 º47.341', 136 masl) is depicted in Fig. 1. Pooja variety of rice was planted in the rice fields of Suryamaninagar and Teliamura. It is also capable of tolerating water stagnation and suitable for late transplanting with aged seedlings. Urea 10-15 kg per hectare and DAP 5 kg per hectare were applied once to the fields during first month after transplanting. The sampling period of roots of the plant and soil was during August to November 2016. This rice variety was collected from the local Block offices by the local farmers. The rice fields were irrigated by canal water. The selected study sites experiences humid tropical climate with 22.68ºC, 78.57 %, and 18.57 mm of average temperature, relative humidity and rainfall during the sampling period, respectively.

Root and soil sampling

The fine root samples of ten randomly selected healthy and disease free paddy plants were harvested from each of the three selected paddy field. The roots were sampled four times i.e, 1^{st} - 4^{th} months after transplanting of seedlings in the field. Root samples were collected by uprooting the plants. The root samples were washed gently to remove the adhering soil and fixed in formalin-acetic acid (FAA) solution and brought to the laboratory for further processing. Soil adhering to the roots and next to plants were collected and mixed to form a composite soil mixture after the fourth month. This composite soil mixture was brought to the laboratory for the assessment of composition and density of AM fungi inhabiting in the rhizosphere of paddy fields.

Assessment of AM and DSE fungal colonization

Fixed root samples were washed several times in running tap water and cut into approximately 1 cm in size. Then root segments were cleaned with 10% NaOH at 90°C. The duration of heating depends on the clearance of the root samples. The cleaned roots were again washed with tap water for several times. Then 2-3 drops of hydrogen peroxide (H_2O_2) were given to the root segments and slightly heated for additional cleaning. After this the root segments were again washed with tap water, the root segments were stained (13). Then the root segments were mounted on lactoglycerol and observed under bright field microscope (Olympus CX21i) for various AM and DSE fungal structures. Aseptate linear and coiled inter or intracellular fungal hyphae accompanied with arbuscules were considered for AM fungal colonization whereas regularly or irregularly septate melanized fungal hyphae along with microsclerotia were taken in account for DSE colonization. The estimation of AM fungi and DSE colonization was done by magnified intersection method (14).

Isolation and identification of AM fungal spores

The soil brought from the field was cleaned by removing leaf litter and other debris taking utmost care so that the soil attached to the litter and debris were not lost by this process and the AM fungal spores were isolated by Wet Sieving and decanting method of (15). Spores were recovered by filtering the sieved fraction onto a filter paper. The filter paper was then spread over a large petridish (13.5 cm) and intact spores were counted according to morphologically distinct types and recorded as total per sample in 50 g soil as spore density under a dissecting microscope. The collection of intact spore types from the petri dish and slides were prepared and pinpointing features were recorded for the identification of AM fungi. The spores were then mounted on glass slides in polyvinyl-lactic acid and carefully crushed under a dissecting microscope (16). Taxonomic identification of these spores was done on the basis of spore morphology, subcellular characters and ornamentation in the spore wall. Spore characters were compared with the original descriptions (www.amf-phylogeny.com). The field-collected spores are generally unreliable due to the lack of fine taxonomic characters or presence of inadequate number of spores. Therefore, the identification of AM fungal spores was restricted

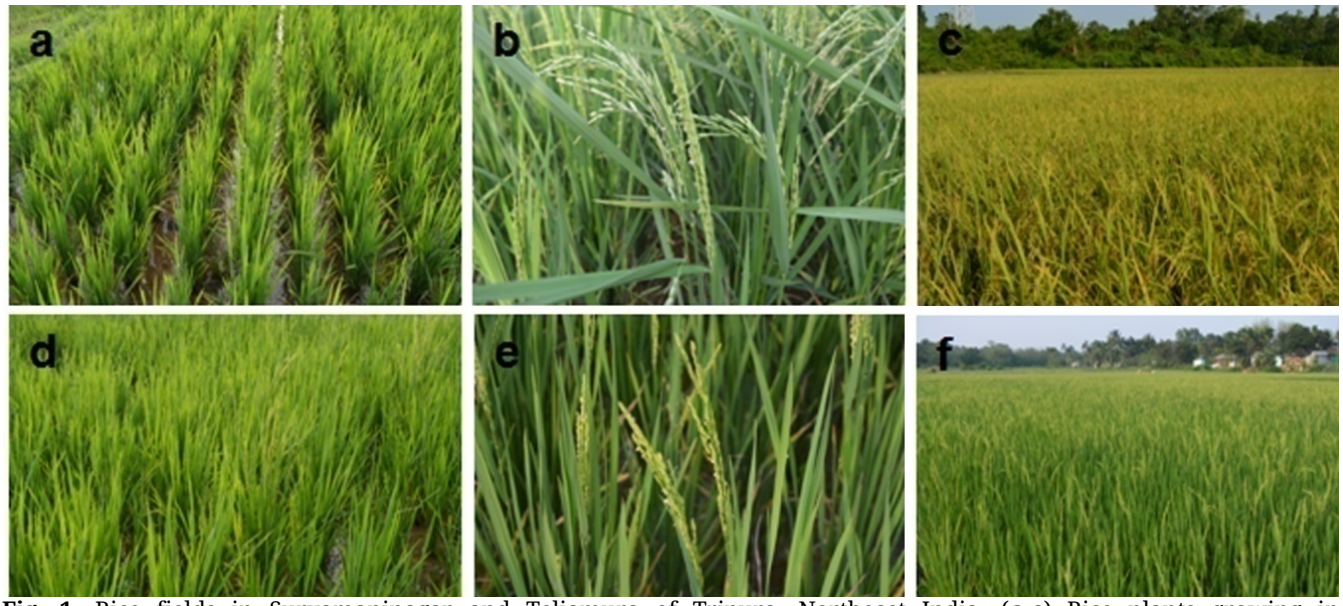

Fig. 1. Rice fields in Suryamaninagar and Teliamura of Tripura, Northeast India. (**a-c**) Rice plants growing in Suryamaninagar, (**d-f**) Rice fields and plants in Teliamura.

Table 1. AM fungi and DSE colonization (%) in rice collected from two areas of Tripura

Month	Suryamaninagar				Teliamura			
	RLA	RLV	RLH	RDSE	RLA	RLV	RLH	RDSE
1	0.00±0.00a	8.44±1.43a	31.74±3.12a	15.30±2.22a	0.00±0.00a	1.26±0.61a	27.11±4.71a	17.18±3.75a
2	16.54±3.01b	13.71±3.40b	44.66±4.57b	21.20±2.90a	14.00±2.04b	9.39±1.62b	39.14±2.87b	22.27±1.63a
3	31.62±0.92c	19.72±0.88c	74.49±1.34c	20.67±1.03a	25.38±0.97c	21.42±1.17c	70.08±1.17c	24.40±0.99a
4	31.37±1.58c	18.58±1.08b	62.27±1.83d	29.55±1.61b	23.06±3.06c	18.21±2.74c	53.54±4.97d	24.36±3.2a

Different alphabets differ significantly at *P<0.05*.

RLA= root length with arbuscules; RLV= root length with vesicles; RLH= root length with hyphae/hyphal coils; RDSE= root length with dark septate endophytes.

to the genus level and morphologically distinct types within each genus were designated as morphotypes.

Data analysis

Spore density (SD) was calculated in terms of number of spores in 50 g soil samples. Mean and standard error were calculated. The colonization data were subjected to analysis of variance (ANOVA) and the means were separated by Duncan test (P<0.05) using the software, Statistica 9.0.

Results

Mycorrhizal structural features

After staining of roots of rice plant from the first month sample showed the presence of vesicles, aseptate hyphae. Very few of them forming appressoria (Fig. 2 b & c) on the epidermal layer of root which are connected to the entangled mass of extraradical hyphae (Fig. 2 a). Also some of the vesicles (Fig. 2 h & i) and intracellular aseptate hyphae (Fig. 2 f & g) within the root cortex were observed in the first month. However, no arbuscules were observed in the first month in the stained root samples. From second month onwards all the mycorrhizal structures such as hyphae, intracellular hyphae, vesicles and arbuscules (Fig. 2 l & m) were observed. The presence of these structures confirms the colonization by AM fungi. The longitudinal running of the hyphae (Fig. 2 d & e) in the intercellular cells of the cortex and from these hyphae certain hyphae protrudes and enters the cells more or less perpendicularly to forms arbuscules (Fig. 2 j, k & n) which denotes the presence of *Arum* type of AM fungal morphology. Finally, young spores developed in the fourth month associated with the roots (Fig. 2 o).

The presence of septate hyphae running in frequent sections on the epidermal layer (Fig. 3 a-e) and the occurrence of microsclerotia (Fig. 3 f) marks the colonization by DSE fungi. The co-occurrence of both AM and DSE fungi ensures dual colonization by two different fungal groups.

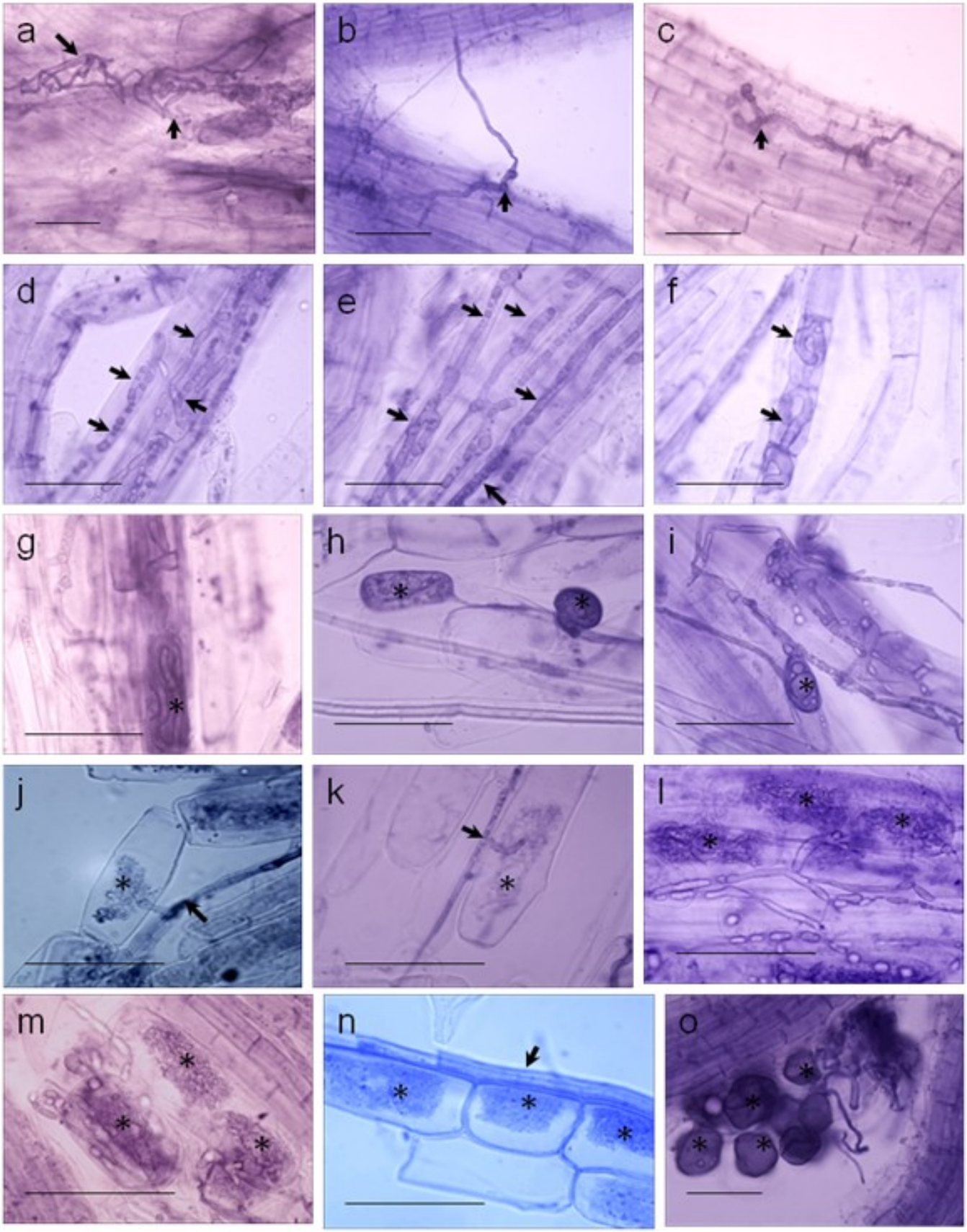

Fig. 2. Light microscopic images of Arbuscular mycorrhizal fungal colonization in the roots of rice. (**a**) Extraradical hyphae of AM attached with the root of rice. (**b & c**) Appressoria in the epidermal layer of root. (**d & e**) Intercellular hyphae in the root cortex of rice. (**f & g**) Intracellular hyphal coil of AM. (**h & i**) Vesicles in the cortical layers. (**J, k & n**) *Arum* type of morphology. (**l & m**) Arbuscules with hyphae. (**o**) Young spores of AM fungi attached with roots. Scale bars: a=300µm; b & c=200µm; d & e=150µm; f-i= 100µm; j-n=50µm; o=200µm.

Mycorrhizal colonization

The degree of AM and DSE fungal colonization is depicted in (Table 1). Significant increase was observed in arbuscule, vesicles and hyphal percentages of AM from first to third month in both the samples collected from two sites. DSE

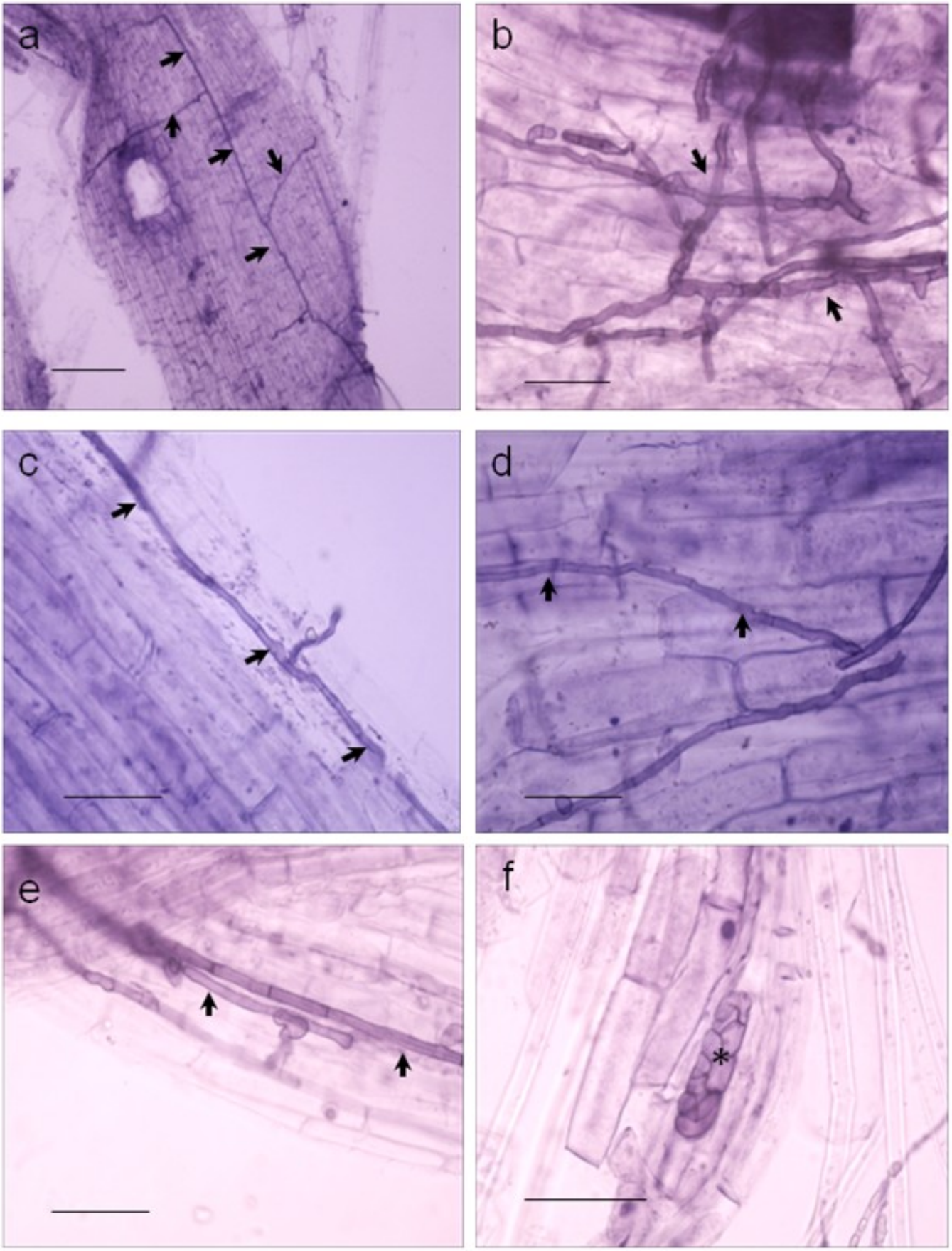

Fig. 3. Light microscopic images of Dark Septate Endophyte colonization in the roots of rice. (**a-e**) DSE hyphae in the root epidermal layer of rice. (**f**) Microsclerotia in the root epidermis. Scale bars: a=200µm; b-e=100µm; f=50 µm.

hyphal percentages do not differ significantly in first to third month. However, there was significant increase in the last month of root samples collected from Suryamaninagar. There was no significant increase in DSE percentages in the root samples of Teliamura. In the third month, AM colonization significantly enhanced in both the

Table 2. AM fungi isolated from the rice fields of Tripura

AM fungi	Suryamaninagar	Teliamura
Gigaspora sp. 1	+	-
Gigaspora sp. 2	+	-
Glomus macrocarpum	+	+
Glomus sp. 1	+	-
Glomus sp. 2	+	-
Glomus sp. 3	+	-
Glomus sp. 4	-	+
Glomus sp. 5	-	+
Rhizophagus diaphanus	+	+
Total number of species	07	04
Spore density/ 50g of soil	204±17.17	307±40.73

sites. AM hyphal colonization significantly differ in all the months between two sites (Fig. 3).

AM fungal composition

AM fungi extracted from two sites are depicted in Table 2. Spore density/50 g was higher in Teliamura than Suryamaninagar. A total of 9 AM morphotypes were recovered from two sites, of which, seven from Suryamaninagar and four from Teliamura.

Discussion

Most of the ecosystems harbour AM fungi as one of the main constituent of soil microbiota **(**17) and form symbiotic association with roots of most terrestrial plants including many agricultural crops (14). Colonization by native AM fungi in rice plant has been reported earlier (18). Partial dependency of upland rice on native AM fungi for phosphorus acquisition has also been reported (19). The occurrence of AM fungi at varying stages of growth of rice plants has been studied (20). In this present assessment the extent of mycorrhizal colonization varies in rice plants of two studied sites of Tripura. Dual colonization was observed during the different growth phases of rice plants. AM and DSE fungal colonization exhibited increasing trend upto the three month of rice plants from their planting time and then start declining. The possible reason for this may be the plants attained maturity after the fruiting phase and require a lesser amount of nutrients compared to the vegetative and reproductive phases of growth.

The fungal colonization percentage was found to be higher than the range given earlier (21) for contaminated and non-contaminated soils of rice plants. Ruiz-Sunchez *et al.* (22) observed growth pattern of rice plants inoculated with *Azospirillum* and AM fungi and proclaimed that dual colonization of AM and bacteria promote growth of rice plants. Watanarojanaporn *et al.* (23) studied AM fungal colonization pattern in different growth phases of rice plants which is in accordance with this present observation. There is an increasing trend in AM fungal colonization till the harvesting stage i.e, upto four month old rice plants which is in contrast to the result obtained in earlier study (24). The difference in AM colonization between two sites in the present study may be due to their high response to physico-chemical and biological conditions of the soil. For understanding the plant-fungus-soil interaction assessing the distribution and quantity of AM fungi is important (25). The colonization reported earlier (26) is lower than the present study.

In spite of vast significance of AM fungi, information on diversity and biology of these important soil fungi is very inadequate globally (27). AM fungal spore density of this present study is within the range specified (26) in various crop soils of Pakistan while Trindade *et al.* (28) postulated lower range of AM fungal spores in papaya plantations of Brazil. AM fungal inhabited in the rhizosphere may vary with host plant species (29). AM fungal taxa *Glomus* was highly abundant in the rhizosphere of rice plants and are in agreement with the results obtained earlier (30). The dominancy of *Glomus* species have also been reported by AM fungi occurring throughout the world (31,32,33).Earlier workers also marked out that *Glomus* is the dominant genus occurring in Indian soil (34,35) and may be attributed with their high adaptability to varied soil and temperature conditions and persist to exist in both acidic and alkaline soils (36). The copiousness of

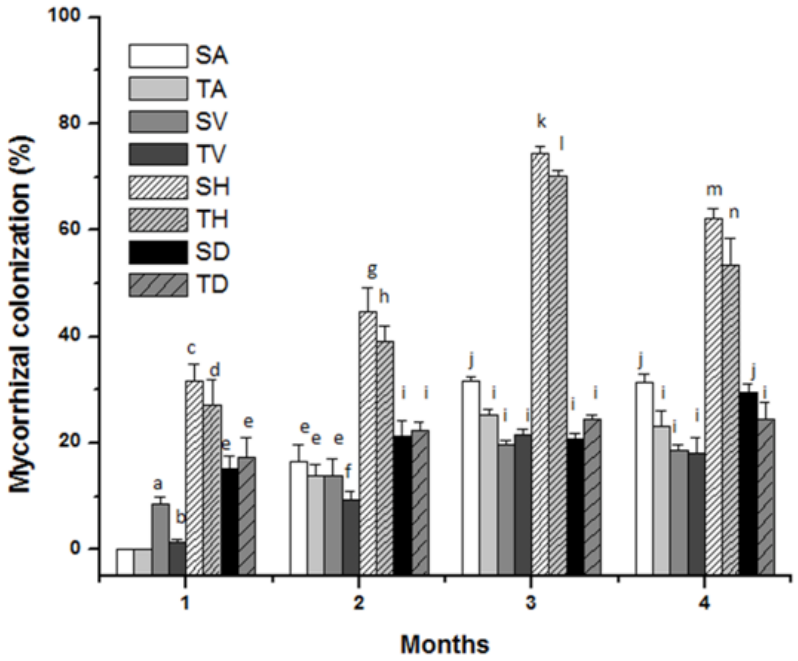

Fig. 4. Mycorrhizal structural colonization in rice from Suryamaninagar and Teliamura, Tripura, Northeast India.

SA - arbuscules from the roots of rice in Suryamaninagar; TA - arbuscules from the roots of rice in Teliamura; SV - vesicles from the roots of rice in Suryamaninagar; TV - vesicles from the roots of rice in Teliamura; SH - hyphae from the roots of rice in Suryamaninagar; TH - hyphae from the roots of rice in Teliamura; SD - DSE from the roots of rice in Suryamaninagar; TD - DSE from the roots of rice in Teliamura. Different alphabets differ significantly at $P<0.05$.

Glomus was also reported from these areas (37,38,39,40).

Conclusions

The growth and yield of rice plants under field condition is greatly influenced by the native AM fungal composition. The degree of AM fungal colonization is showing an increasing trends upto the harvesting periods. However, an extensive study on AM fungi associated with rice plants need to be carried out under experimental conditions to underpin the exact role of AM fungi on the growth of rice plants. This study is an effort to make aware the local village farmers about the efficacy of these native AM mycobiota which can be harnessed for long term applications and preferable over chemical fertilizers leading to eco friendly organic farming. The present occurrence of these fungi from rice fields also indicates that they may have contributed to healthy production of rice which supports local livelihoods.

Conflict of interest

The authors declared that they have no conflict of interest.

Author's Contribution

PD and AKS designed the objectives and plan of work. SB, KC, AD and ARD carried out the work. PD and KC analysed the data. PD, KC and AKS wrote the manuscript.

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