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Roots invaders: Morphology and Colonization of dark septate endophytes in invasive plants of tropical dry deciduous forest of Chhattisgarh, India

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ABSTRACT

This is the first-ever report on the occurrence of DSE in the Tropical Dry Deciduous Forest of Central India, Chhattisgarh. This study delves into the current status of root invaders (DSE) in ten invasive plants of the Tropical Forest of Central India, collected from four different study sites *i.e.* Belgahna Forest, Bilaspur Forest, Kota Forest and Ratanpur Forest. The present study emphasizes the association of DSE colonization in invasive plants under Tropical climatic conditions. More than 80% of roots of *Senna tora* was found to be colonized with the DSE, while no structure of DSE was found associated with the roots of *Alternanthera sessilis* in all four study sites. Different types of structures *i.e.* microsclerotium, hyaline hyphae, melanized hyphae, and septate mycelium were observed in the roots of all invasive plants studied except *A. sessilis*. The occurrence of DSE in invasive plants of Tropical Forest may be one of the major causes that helps them in growth and survival in extreme environmental climatic conditions. Isolation, identification, and inoculation of these DSE may be helpful for the development of healthy nursery stock for the plantation in extreme environments where no regular irrigation facility is available.

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KEY WORDS : Dark Septate Endophytes, DSE, Hyphae, Invasive plants, Microsclerotia, Tropical Forest.

Introduction

Dark septate endophytes are one of the most prevalent root endophytic fungi and have been reported to be associated with more than 600 plant species in different environments, some of which are non-mycorrhizal[1,15]. Dark septate endophytes (DSE) are members of the phylum Ascomycota[11]. The melanized hyphae of DSE fungus are often septate and invade the cortical cells and intercellular areas of roots to generate densely septate intracellular structures known as microsclerotia[11,24]. Even while interest in DSE fungi has grown steadily, very limited information about the structure, function and ecological role of DSE fungi is available. Need formatting monocotyledonous plant species than dicotyledonous ones and are frequently observed living alongside mycorrhizal fungi in these ecosystems[23]. They are likely to be significant players in plant ecophysiology. Plants that have been experimentally inoculated with DSE have been shown to have increased host resilience to contaminants such as heavy metals and drought. DSE may impact host fitness through many methods, including enhancing food absorption by colonized hosts[9,15]; and producing bioactive chemicals[13].

It is crucial to take into account these fungi in ecosystem studies along with arbuscular mycorrhizal fungi (AMF). In view of recent studies that inoculation of specific DSE species with agriculture crop under various stress condition is in progress but the occurrence and association of DSE with invasive plants of Tropical Dry Deciduous Forest has been overlooked. Present study was aimed to study the occurrence and development of DSE in invasive plants of Central India.

Materials and Methods

Study area: The study site is in the Indian state of Chhattisgarh, in the Bilaspur district. The root samples were collected from four study sites from the dominant

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TABLE-1: Occurrence of hyphae and microsclerotium of DSE in roots of invasive plants

S. No.	Name of plant	Belgahna		Bilaspur		Ratanpur		Kota	
		Hyphae	Micro-sclerotia	Hyphae	Micro-sclerotia	Hyphae	Micro-sclerotia	Hyphae	Micro-sclerotia
1.	*A. sessilis*	ND	ND	ND	ND	ND	ND	ND	ND
2.	*A. radicans*	62.15±1.52	14.64±1.20	58.16±2.33	07.33±1.45	66.00±2.08	19.26±2.08	71.33±0.57	22.15±1.85
3.	*A. conyzoides*	73.15±1.20	ND	67.12±1.52	14.33±2.33	69.50±2.33	23.02±1.45	59.05±1.45	ND
4.	*B. lacera*	65.05±2.33	13.14±1.52	71.34±2.33	17.41±1.85	67.04±1.45	9.25±1.45	74.20±1.20	21.33±1.45
5.	*C. odorata*	69.02±1.45	17.44±2.08	77.18±1.85	23.00±1.52	61.20±1.52	ND	68.05±2.33	ND
6.	*E. scaber*	71.78±1.20	25.18±1.52	62.05±1.20	ND	64.48±2.08	17.75±1.45	73.15±1.45	12.00±1.85
7.	*M. coromandelianum*	79.33±1.45	18.33±1.45	73.24±1.45	11.26±1.52	70.33±0.88	14.02±2.08	62.33±1.45	9.45±1.52
8.	*S. tora*	86.39±1.52	58.14±2.33	82.51±0.57	54.62±1.20	85.52±2.08	50.00±1.45	81.20±2.08	51.26±1.33
9.	*S. rhombifolia*	73.68±2.08	27.12±2.08	68.34±1.85	18.12±1.52	63.24±2.08	11.50±1.45	62.12±2.33	8.33±1.45
10.	*U. lobata*	57.33±1.45	9.20±1.45	63.33±1.45	24.37±1.85	68.70±1.45	12.33±1.52	69.00±1.20	25.05±1.85

ND=Not detected

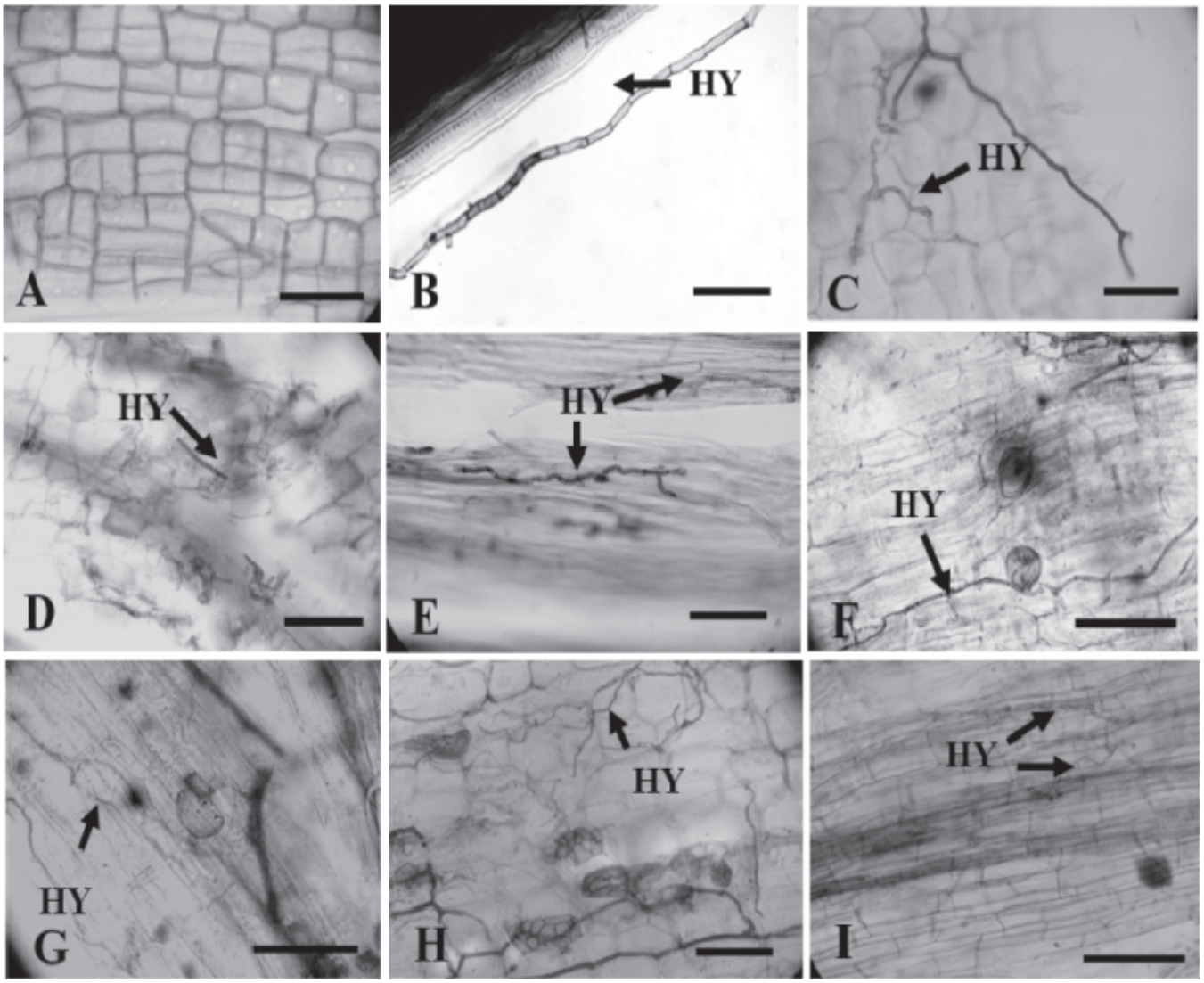

Fig. 1: (A) *A. sessilis* (DSE colonization not detected in root hence a Non-DSE host), (B) *A. radicans*- Melanized septate hyphae, (C) *A. conyzoides*- Melanized septate hyphae, (D) *B. lacera*- Melanized septate hyphae, (E) *C. odorata*- Melanized septate hyphae, (F) *E. scaber* & (G) *M. coromandelianum*- Melanized septate hyphae and Vesicles (AMF), (H) *S. tora* & (I) *U. lobata* - Co-occurrence of DSE and AMF mycelium; Scale 50µm, HY-Hyphae

Tropical dry deciduous forest area of (1) Belgahna 22°26'33.00" N, 82° 1'7.51" E, (2) Kota 22°19'26.82" N, 82° 0'43.06" E, (3) Ratanpur 22°24'28.47" N, 82° 7'35.49"E, and (4) Bilaspur 22°15'3.98 "N, 82°20'7.74 "E.

Selection and identification of plants: The plants were identified based on their physical characteristics and features and were selected based on their availability and richness in the selected forest area.

Sample Collection and Storage: Root samples were collected in triplicate from all four study sites. Collected roots were kept in locked polyethylene bags. Roots were brought to the laboratory and labelled accordingly. After labelling roots were processed for the clearing staining process and the remaining roots were stored at 4^0C for further use.

Root processing and assessment for colonization

The Rapid Clearing and Staining Method[17] was used to assess DSE colonization in the randomly chosen root segments of the 1cm-long. The root slide technique[5] was used to evaluate the root colonization using the following formula:

$$\text{Percentage of DSE Colonization} = \frac{\text{No. of Root Segments with infection}}{\text{Total no. of Root studied}} \times 100$$

Statistical analysis

All the data were recorded and analyzed with the help of MS Excel for the calculation of Average and Standard Error. Micrographs were taken with Magnus MX2Li LED (Binocular Microscope) and Image view (Camera software) was used for measurement and image processing. All the root structures were identified based on previous studies.

Results and Discussion

This is the first report on the occurrence of DSE in the roots of invasive plants from the Tropical Dry Deciduous Forest of Central India. Most screened roots contained cells that were colonized by loose hyaline to dark septate melanized hyphae (Fig.1 A-I). Microsclerotia were observed in different shapes and shades, some of them were darker compared to others, and some of them had extending hyphae at the base (Fig. 2 A-L). In all the collected root samples nine out of 10 invasive plant species were colonized by intraradical melanized septate hyphae and microsclerotia, the hyphae were dark brown with thick lateral walls and frequently invaded the cortical or epidermal cells with septa. Single hyphae were accompanied sporadically by sclerotia. The frequency of DSE occurrence and the percentage of root colonization was high in most of the invasive plants studied except *A. sessilis* (Table-1). The roots of *A. sessilis* (Family-Amaranthaceae) were devoid of mycelium and hyphae and had no colonization of DSE in all study sites (Fig. 1 A). The invasive plant *A. sessilis* belongs to the family Amaranthaceae which was earlier reported as a non-mycorrhizal plant from aquatic habitat[20]. Although it was mentioned that out of nine citing only six studies reported the presence of only AMF and no DSE in *A. sessilis* roots in Agroforestry systems of Brazilian Atlantic Forest[3].

Out of ten plants, DSE colonization was most prominent in *S. tora* and had the highest percent colonization (86.39±1.52%) among the root samples collected from Belgahna Forest followed by Ratanpur Forest, Bilaspur Forest, and Kota Forest. (Table-1). The hyphal colonization percent of DSE was minimum 57.33±1.45% in *U. lobata* of the Belgahna Forest site. Microsclerotia formation was not uniform in all invasive plants at all study sites. The percent of occurrence of microsclerotium was a minimum of 07.33±1.45 in *A. radicans* and a maximum of 54.62±1.20 in the root sample of *S. tora* from the Bilaspur Forest site (Table-1). The DSE was detected in all study sites under tropical conditions, but the pattern of hyphal coiling, presence of melanized hyphae, and formation of microsclerotium was not uniform (Figs. 1-2). Similar findings on varied DSE colonization were also reported earlier[10,12]. Colonization of DSE may vary with sampling sites[7], season[12], host plants, edaphic factors[4], and climatic conditions[13].

Dark septate endophyte structures were observed inside epidermal cells of selected invasive plants, they were different in size shape and, colour. *A. radicans, A. conyzoides,* and *B. lacera* had intracellular Hyaline microsclerotium in the cortical cells (Fig.2: A & C). Whereas *C. odorata* had both intracellular hyaline microsclerotium and intercellular mature, pigmented, toruloid microsclerotium (Fig. 2: D-E). *E. scaber* had many small tufted and mature microsclerotium with hyphal attachments (Fig.2: F-G). *M. coromandelianum* had comparatively bigger and darker microsclerotium with distinctive cells (Fig. 2: H). Long tubular conglomerated microsclerotium along with the development of oval-shaped microsclerotium were seen in *S. tora* (Fig. 2: I-J). *S. rhombifolia* and *Urena lobata* had very dark, compact, and irregular shapes microsclerotium with hyphal development around the microsclerotium (Fig.2: K &L).

Occurrences of hyaline hyphae to melanized hyphae of DSE in inter- or intra-cellularly in the root of host plants have been reported worldwide[14,21]. Hyaline microsclerotia stained and appear blue in colour and microsclerotia with dark-coloured mycelium remain unstained in the same root zone of the different host plant. The frequency of microsclerotia was poor in the root samples collected from Kota Forest in comparison to the three study sites. It was mentioned that microsclerotium light-colored and dark-colored mycelium were seen in the same root[8]. The variability in the occurrence of the structure of microsclerotium may be due to the habit of the host plant[2,6,19]. The formation of melanin in the hyphal cell wall may be progressed by the gradual exposure of the host plant to harsh climatic conditions and the intensity of melanin formation would extend the viability and adaptability of the plant in such climatic conditions[2,22,25].

The DSEs of plants grown in harsher edaphic and climatic conditions had thicker hyphae and shorter septa. These traits may have protected the host from drought stress by facilitating the more efficient absorption and translocation of scarce nutrients that would not have otherwise reached the plant's roots[16,18]. Similar structures were observed in the roots of invasive plants from the tropical forest of Chhattisgarh which also suffers high temperatures and drought-like conditions and had a great number of DSE colonization, important DSE propagules, hyphae, and microsclerotia are believed to help plants store substrates and absorb nutrients, improving the host's resistance to dry circumstances[24].

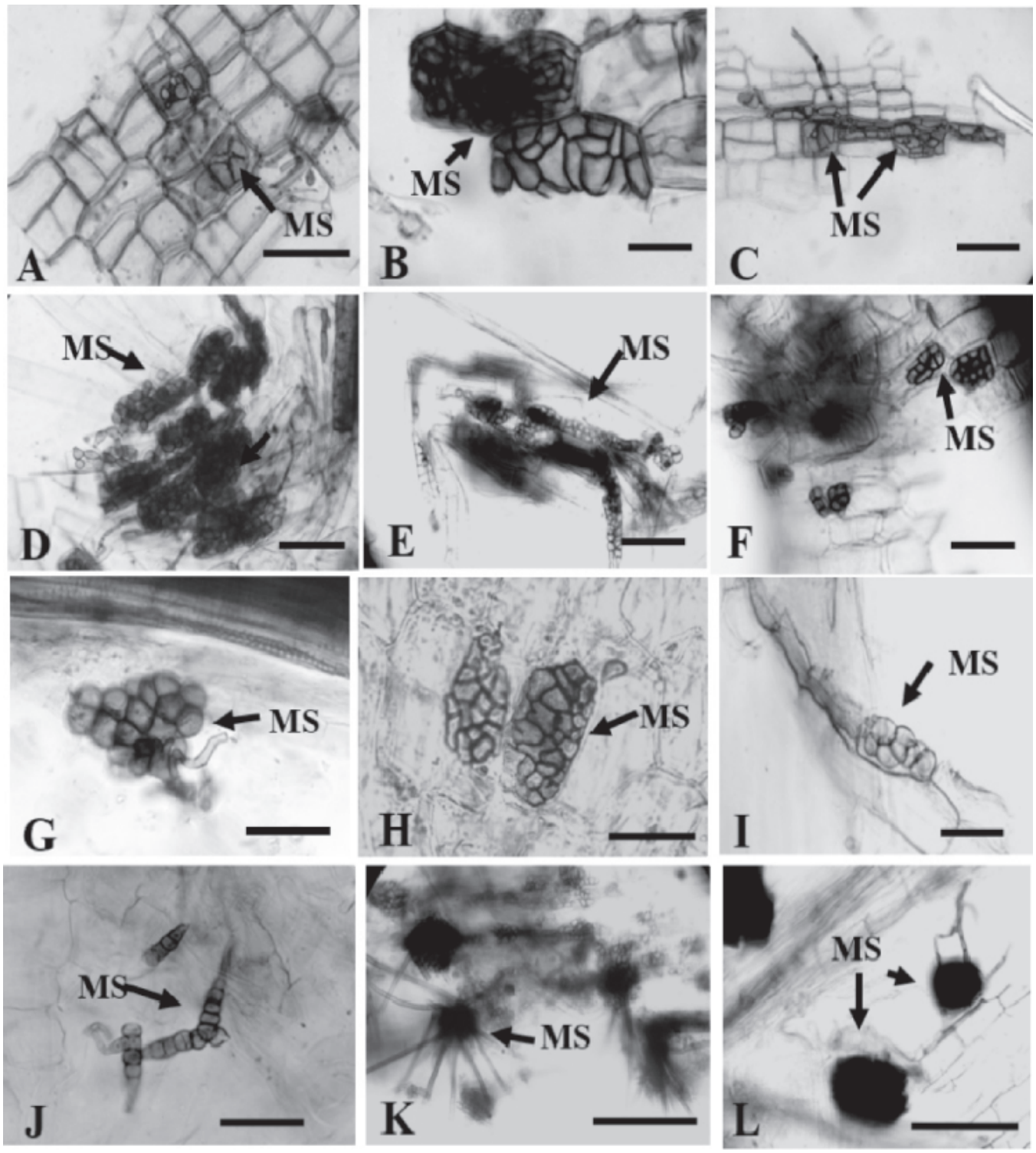

Fig. 2: (A) *A. radicans*-Hyaline microsclerotium, (B) *A. conyzoides*- Melanized microsclerotium, (C) *B. lacera*- Hyaline microsclerotium, (D-E) *C. odorata*- Septate hyphae with moniliform cells & Microsclerotium, (F-G) *E. scaber*- Microsclerotium, (H) *M. coromandelianum*- Microsclerotium, (I-J), *S. tora*- Developing microsclerotium, (K) *S. rhombifolia*- Microsclerotium and Septate melanized hyphae (L) *U. lobata*- Microsclerotium; Scale 50µm; MS-Microsclerotium.

The studied invasive plants are well grown in all study sites in the Tropical Dry Deciduous Environment and compete with the native plants for nutrition uptake and space. The high intensity of the DSE in the invasive plants may be one of the potential reasons for their survival and growth in the Tropical Dry Deciduous Forest of Central India. Further research is required to understand the mechanism and process of DSE

colonization in the roots of host plants under various climatic conditions which could help for the exploration and exploitation of DSE species in the field of forest and agriculture.

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