J. Mycopathol. Res. ***58(1&2)*** *: 51-55, 2020;* (ISSN 0971-3719)

Root colonization with Arbuscular mycorrhizal fungi and Dark septate Endophytes in Tea plants

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Received : 24.03.2020 Accepted : 22.04.2020 Published : 27.07.2020

Root colonization with arbuscular mycorrhizal fungi (AMF) and dark septate endophyte (DSE) were studied in fifteen tea varieties, of which six UPASI varieties (UP-2, UP-3, UP-8, UP-9, UP-26 and BSS-2) and nine Tocklai varieties (TV-18, TV-9, T-17, TV-22, TV-23, TV-25, TV-26, TV-29 and TV-30) being grown in Tea Germplasm Bank (15 year old bush in experimental field) of Department of Botany, University of North Bengal. The physical nature of arbuscules, vesicles, intraradical hyphae and dark septate endophyte associations were studied extensively to determine the colonization impact of these tea varieties. Highest percentage of root colonization (86-88%) were noticed in some UPASI varieties of which biclonal seed stock (BSS-2) yielded highest root colonization. Besides, among nine Tocklai tea varieties tested, TV-29 yielded highest (87%) root colonization. Paris type hyphae are abundant in all the varieties that come from *Glomus* sp. along with some coiled arbuscular structure that proves the infections of some *Gigaspore* species. The mycelium of dark septate endophyte (DSE) was observed in all the tea varieties but most extensively was in BSS-2, UP-3, UP-8, TV-18, T-17, TV-22 and TV-26.

Key words : Arbuscular mycorrhizal fungi, dark septate endophyte, *Glomus*, *Gigaspora*, root colonization.

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze.), is the major plantation crop of North- East India and forms the back bone of the economy of this region. It is a perennial and survives for more than 100 years. After water, tea is the most widely consumed beverage in the world. Tea contains catechins, a type of antioxidant. In a freshly picked tea leaf, catechins can comprise up to 30% of the dry weight. Catechins are highest in concentration in white and green teas, while black tea has substantially fewer due to its oxidative preparation. It has a cooling, slightly bitter, astringent flavour which is enjoyed by many people. The major tea growing areas of India are Darjeeling, Terai and Dooars of West Bengal, Assam and Nilgiri (Kerala and Tamil Nadu). The high quality and distinct flavour and aroma of Darjeeling tea is a result of unique climate, soil, altitude and processing methods prevalent in Darjeeling. In tea plantations, with the reduction in the permissible levels of chemicals which can be used, there is urgent need for identification and selection of beneficial microbes which have the potential to control diseases and also increase productivity. Arbuscular mycorrhizas are by far the most prevalent of all mycorrhizal categories with more than 80% of all plant species showing an association involving a few fungal genera in the Glomeromycota. Mycorrhizas increase nutrient uptake from the soil. Also it can be used in the biocontrol of pathogenic fungi and nematodes (Chakraborty, 2019). Dark septate endophytes (DSE) are a group of hetero-geneous endophytic fungi which are characterized by melanized hyphae within plant roots. Critically the role of DSE is still not understood. The occurrence of arbuscular mycorrhizal fungi and association of dark septate endophytes in tea root system of Darjeeling, Tocklai and UPASI varieties are discussed in the present study.

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MATERIALS AND METHODS

Host Plants

Fifteen tea varieties, of which six UPASI varieties (UP-2, UP-3, UP-8, UP-9, UP-26 and BSS-2) and

nine Tocklai varieties (TV-18, TV-9, T-17, TV-22, TV-23, TV-25, TV-26, TV-29 and TV-30) being grown in the experimental field (15 year old bush) and some tea gardens from Siliguri Foothills (26.7223°N,88.4248°E), Darjeeling Hills (27.0436°N, 88.2644°E) and Jalpaiguri Terai (26.5200°N,88.7300°E) were also studied to explore the diversity and mycorrhization and the presence of DSE.

Isolation of Arbuscular Mycorrhizal Spores

Spores of arbuscular mycorrhizal fungi were isolated from rhizosphere soil of tea by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Approximately 250 ml of soil was suspended in 1 litre water. Heavier particles were allowed to settle for a few minutes and the liquid was decanted through sieves of decreasing size (BS 60, BS 80, BS 100, BS 150 and BS 200). Pores are fine enough to remove the larger particles of organic matter, but coarse enough to allow the desired spores to pass through. The suspension that passed through these sieve was saved and stirred to re-suspend all particles. The heavier particles were allowed to settle for a few seconds and the liquid decanted again through the sieve and spores collected by fine brushes and were kept in different petriplates according to their size and colours.

Purification of AMF spores

Further purification of AMF spores was done by sucrose gradient centrifugation method. In sucrose gradient centrifugation (Daniels and Skipper, 1982), spores and minimal amount of organic particles were further purified by suspending sieving in 40% sucrose solution and centrifuging at 2000 rpm (approximate 370 x g) for 1 minute. The supernatant (with spores) was passed through a sieve of BS 200 mesh and rinsed with distilled water to remove sucrose residue. With the help of a simple microscope (20X) parasitized spores, plant debris etc were separated and clean spores were stained with Poly Vinyl Lacto Glycerol and studied microscopically. For further use the spores were stored in Ringer's Solution (8.6gm NaCl, 0.3gm KCl, 0.33gm $CaCl_2$ in 1 ltr. of boiled distilled water) at -15 to -20 ºC or in sterile distilled water.

Identification of AMF spores

Spore samples were separated according to their morphology, size, colour, shape, wall thickness, wall layers, and other accessory structures like hyphal attachment etc. for the purpose of identification. The spores were identified up to species level with the help of standard keys (Walker 1992). Spores were critically examined with special reference to variation in vesicles (size, shape, wall thickness, wall layers, position and abundance), hyphal branching patterns, the diameter, structure (especially near entry points) and the staining intensity of hyphae.

Spore count

Rhizosphere soil (100g) was taken and suspended in 250 ml water. Wet sieving and decanting method was used for isolation of spores. Total number of spores was then counted and spore percentage of different genera was obtained.

Histopathology

Association of AM fungi within the root tissues was observed according to Phillips and Hayman (1970). Roots were cut into 1cm or smaller pieces and washed in tap water. It was boiled in 2% KOH in hot water bath for 1 hour. The KOH was decanted and the roots washed with water for 2-3 times. 1% HCL was added and kept for 30 minutes. After decanting the HCL the sample was washed thrice in tap water and cotton blue, lactic acid and glycerol was added in the ratio 1:1:1 to stain the internal structures of AMF inside the root segments i.e. arbuscules, vesicles, auxiliary cells, and boiled in water bath for 1 hour. The excess stain was decanted and sample placed in 50% glycerol for distaining. The roots were then crushed under pressure in slide and covered with cover slip for microscopic observation.

RESULTS AND DISCUSSION

Colonization of Mycorrhiza in plant roots

Root samples as well as soil samples were collected from each variety and data were recorded. Root endophytes with dark septate hyphae (called dark septate endophytes or DSE) are common in many tea roots and may provide benefits to their hosts (Jumpponen and Trappe, 1998). Results revealed that percentage root colonisations (86-88%) were noticed in two UPASI varieties (UPASI-9 and BSS-2), of these biclonal seed stock variety BSS-2 yielded highest root

colonization. Besides, among nine Tocklai tea varieties tested, TV-29 yielded highest (87%) root colonization. The predominant spores that were found in tea rhizosphere are given in Table 1 and Figures 1 and 2. Both arum and paris type hyphae are abundant in all the varieties. Root samples taken from each of the fifteen varieties were examined under microscope and mycorrhization was documented (Figure 3). The physical nature of arbuscules; vesicles, intraradical hyphae etc were studied extensively to determine the colonization impact of these tea varieties. During the onset of germination of AM spores a fine structure called the prepenetrating apparatus (Genre *et al*. 2005) is produced that ultimately triggers the entry into the host cell. In tea roots artificially inoculated with *Glomus mosseae* spores follow the same pattern during the entry and approximately 20-25 days are required for sporulation. The arbuscules characters that were found during the investigation in tea roots are full of diversities. Spore population is highest in TV-29, TV-30, BSS-2, UP-2, UP-3 and UP-8 (Table 2). The most abundant genera of AMF in tea roots are *Glomus*, followed by *Acaulospora*, *Gigaspora* and *Scutellospora* .

Colonization of dark septate endophytes (DSE) in plant roots

Dark septate endophytes (DSE) are a group of hetero-geneous root-associated endophytic fungi which are characterized by melanized intercellular and intracellular runner hyphae and so-called

Table 1: Population of AMF spores found in tea (*Camellia sinensis*) plant

Name of AM Fungi	Siliguri Foothills 26.7223°N,88.4248°E	Darjeeling Hills 27.0436°N,88.2644°E	Jalpaiguri Terai 26.5200°N,88.7300°E
Acaulospora bireticulata	++	++	++
A. scrobiculata	++	++	++
A. spinosa	+	-	+
Glomus aggregatum	+	+	+
G. constrictum	++	++	++
G. fasciculatum	+	-	+
G. intraradices	+	-	+
G. mosseae	++	++	++
G. albidum	+	+	+
G. ambisporum	+	+	+
Gigaspora rosea	+	++	+
Gi. gigantea	++	++	++
Gi. margarita	+	++	+
Scutellospora rubra	++	++	++
S. pellucida	++	++	+
S. calospora	-	+	-
S. persica	+	+	+
Sclerocystis	+	-	-

[(++) = high, (+) = low and (–) = absent.]

Table 2: AMF and DSE associations of Tea varieties

Tea variety	pH of soil	No. of spores / 100gm soil	% colonization	No. of vesicles/root	Vesicle / cm root	DSE
BSS-2	6.8	142	88	123	03	+++
UP-2	6.5	125	64	88	04	+++
UP-3	7.2	123	55	94	01	+++
UP-8	6.8	132	48	79	04	+++
UP-9	7.6	85	86	113	06	+++
UP-26	6.5	75	47	61	02	+
TV-18	6.9	65	50	72	05	+++
TV-9	7.2	45	50	77	01	+
T-17	6.6	50	58	90	02	+++
TV-22	6.8	49	68	122	02	+++
TV-23	6.5	78	49	95	04	+
TV-25	7.2	72	35	88	01	+
TV-26	6.8	87	47	104	03	+++
TV-29	7.6	95	87	116	06	+
TV-30	6.5	134	69	112	01	+

((+++) = Abundant (+)= Moderate)

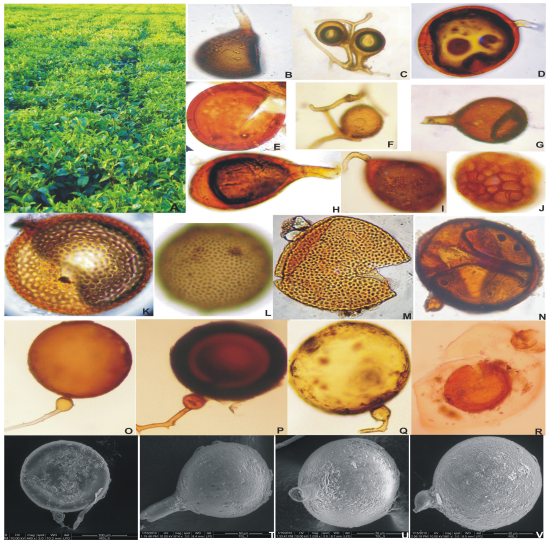

Fig.1 : Spores found in Tea rhizosphere; Fig.A. Tea garden; B. *Glomus mosseae* (mature); C. *Glomus fasciculatum*; D. *Glomus* sp; E. *Glomus* sp.; F. *G. mosseae* (young); G. *Glomus constrictum*; H. *Glomus badium.*; I. *Glomus* sp; J. *Glomus* sporocarp; K. *Acaulospora bireticulata*; L. *Acaulospora spinosa*; M. *Acaulospora* sp; N. *Gigaspora gigantean* O. *Gigaspora margarita* ; P. *Scutellospora calospora* ; Q. *Scutellospora* sp. ; R. *Scutellospora rubra* and SEM photo of *Glomus* (S,T, and U) and *Gigaspora* (V) spores

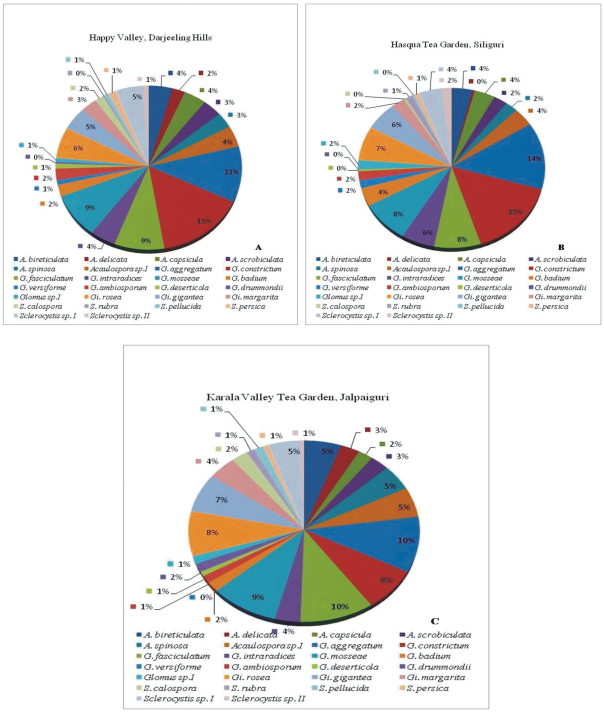

Fig.2 : Population of AMF isolated from tea rhizosphere from (A) Happy Valley Tea Garden, Darjeeling Hills, (B) Hansqua Tea Garden, Siliguri and (C) Karala Valley Tea Garden, Jalpaiguri.

microsclerotia (aggregation of dark, thick-walled, closely packed inflated cells) within epidermis and cortex of plant roots (Wilson *et al.*, 2004, Mandyam and Jumpponen, 2005, Muthukumar *et*

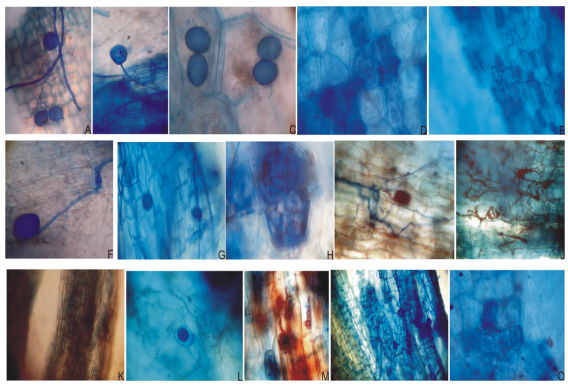

Fig.3 : Histopathological study of all the 15 tea varieties showing different shapes of vesicles, hyphae, arbuscules etc. (A). BSS-2; (B).UP-2; (C). UP-3; (D). UP-8; €. UP-9; (F). UP-26; (G).TV-18; (H). TV-9; (I). T-17; (J). TV-22; (K). TV-23; (L). TV-25; (M). TV-26; (N). TV-29; and (O). TV-30.

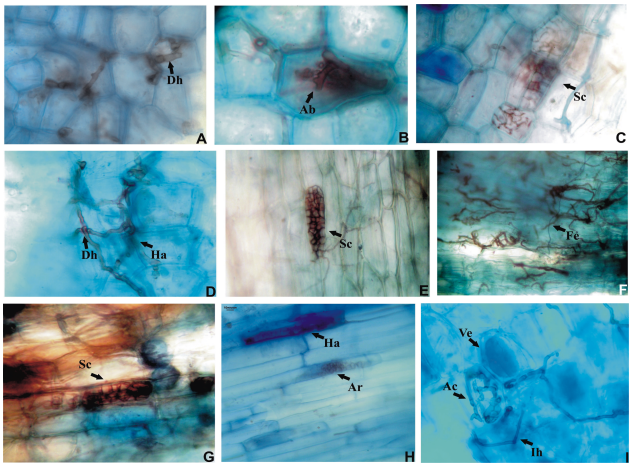

Fig.4 : A-H Different structures of endophyte obtained from tea varieties. (A). BSS-2; (B).UP-3; (C).UP-8; (D).TV -18; (E).T-17; (F). TV-22; (G). TV-26; (H). UP-2 and (I). UP-9. [Dh- Dark hyphae, Ab- Arbuscules, Sc – Sclerotium formed by DSE, Ha – Hyphae growing intercellular, Fe – Fine endophytic mycelium, Ar – Terminally formed arbuscules, Ve – Vesicle formed inside a cortical cell, Ac – Arbuscules formed in coils, Ih – Intercellular hyphae.]

al., 2006, Silvani *et al.*, 2008). Moreover, their existence has also been found prevalent in hydrophytic plants (Wang and Zhao, 2005). Although, confusions of their taxonomic affinity and obscure effects on hosts remain a problem due to little understanding of their teleomorph (most DSE rarely sporulate or remain absolutely sterile under culture conditions) and their variable impacts on hosts ranging from positive to negative in different experimental conditions (Horton *et al.,* 1998). Dark septate endophytes (DSE) comprise a miscellaneous group of root-inhabiting fungi. It is defined DSE as conidial or sterile ascomycetous fungi that colonize living plant roots without causing apparent negative effects such as tissue disorganization. This definition is likely to include

a plethora of fungi whose functions and taxonomic affinities remain unknown. The dark septate hyphae (DSH) also formed arbuscules that are coiled in structure. Hyphae, originating from a spore or another colonized root, usually grow along the epidermis, often following the groove between epidermal cells. Plant cell responses clearly associated with this stage have not been described yet. Abundant DSE has been found in UPASI and Tocklai tea varieties (Table 2). Intercellular and intracellular runner hyphae and microsclerotia were found abundant in tea varieties - BSS-2, UP-2, UP-3, UP-8, UP-9, TV-18, T-17, TV-22 and TV-26 (Figure 4)

From the results of the present study it can be concluded that there is a high incidence of arbuscular mycorrhiza (AM) and dark septate endophytic (DSE) fungal associations in all the tea varieties of which nine were highly colonised by DSE which represent a taxonomically and ecologically diverse group of fungi. Their interactions with plants range from parasitic to symbiotic, depending on fungal species, host plants and environmental conditions. AM symbiosis also increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (Aliasgarzad *et al.*, 2006, St-Arnaud and Vujanovic, 2007, Maya *et al*. 2013, Khati and Chakraborty, 2019).

Appropriate management of mycorrhizae in agriculture should ultimately result in a substantial reduction in chemical use and production costs. Extensive research works are required to create a database of mycorrhizal species and DSE colonizing these plants and to determine their efficiency in promoting growth and health benefits.

REFERENCES

Aliasgarzad, N., Neyshabouri, M.R. and Salimi, G.(2006). Effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on drought stress of soybean. *Biologia*. **61**: 324–328.

Chakraborty, B.N. (2019).Arbuscular mycorrhizal fungi and mycoeehiza helper bacteria: Molecular detection and their role in symbiosis and crop production. *J. Botan. Soc. Bengal* **73** : 1-20.

Genre, A, Chabaud, M. and Timmers, T. (2005). Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**:3489–3499.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogone* extracted from soil by wet sieving and decanting. *Trans.Br. Mycol. Soc*., **46**: 235–244.

Horton, T.R., Cázares, E. and Bruns, T.D. (1998). Ectomycorrhizal vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza* **8**:11-18.

Jumpponen, A. and Trappe, J. M. (1998). Dark septate endophytes : a review of facultative biotrophic root-colonizing fungi. *New Phytologist* **140**: 295–310.

Khati, S. and Chakraborty, B.N. (2019). Immunodetection of *Drechslera oryzae* causing brown spot of rice, its root colonization with arbuscular mycorrhizal fungi and their use for induction of resistance. *J. Botan.Soc.Bengal* **73**: 43-52.

Mandyam, K. and Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Stud. Mycol*., **53**: 173-189

Maya, M.A. and Matsubara, Y. (2013). Influence of arbuscular mycorrhiza on the growth and antioxidative activity in cyclamen under heat stress. *Mycorrhiza.* **23**:381–390.

Muthukumar, T., Senthilkumar, M., Rajangam, M., Udaiyan, K. (2006). Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. *Mycorrhiza* **17**:11-24.

Phillips, J. M. and Hayman, D. S.(1970) Improved procedure for clear staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc*., **55**: 158–161.

Silvani, V.A., Fracchia, S., Fernández, L., Pérgola, M. and Godeas, A. (2008). A simple method to obtain endophytic microorganisms from field-collected roots. *Soil Biol. Biochem*. **40**: 1259-1263.

St-Arnaud, M. and Vujanovic, V. (2007). Effects of the arbuscular mycorrhizal symbiosis onplant diseases and pests. In: *Mycorrhizae in Crop Production,* (Eds). Hamel, C. and Plenchette, C., New York: Haworth, pp. 67–122.

Walker, C. (1992). Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)-a possible way forward. *Agronomie*, **12**: 877-897.

Wang, K. and Zhao, Z.W. (2005). Occurrence of Arbuscular Mycorrhizas and Dark Septate Endophytes in Hydrophytes from Lakes and Streams in Southwest China. *Int. Rev. Hydrobiol*. **91**:29-37

Wilson, B.J., Addy, H.D., Tsuneda, A., Hambleton, S. and Currah, R.S. (2004). *Phialocephala sphaeroides* sp. nov., a new species among the dark septate endophytes from a boreal wetland in Canada. *Can. J. Bot.*, **82**: 607-617.