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DIVERSITY OF MYCORRHIZAL SPORE POPULATION DYNAMICS AND QUANTIFICATION IN RHIZOSPHERIC SOIL OF *TECTONA GRANDIS* L.

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ABSTRACT:

Arbuscular mycorrhizal (AM) fungi are known to be well distributed and these fungi can be isolated from a wide variety of natural habitats and are particularly abundant in cultivated lands. Little work has been carried out regarding their distribution in Teak located in Dharwad district of Karnataka, India. A total of Thirty six arbuscular mycorrhizal (AM) fungal spores were identified and the genera observed in the recovered spores are-*Glomus, Acaulospora, Sclerocystis, Gigaspora, Scutellispora and Entrophospora*. The highest AM fungal spore population was observed in Mavinakoppa and lowest in Holthikoti. Similarly, the highest AM fungal per cent root colonization in Mavinakoppa and lowest in Holthikoti. *Glomus* species is most predominant among the recorded AM fungal spores from rhizospheric soil of *Tectona grandis*.

KEYWORDS: *Arbuscular mycorrhiza, Rhizospheric, Root colonization, Spore population, Tectona grandis.*

INTRODUCTION:

The term mycorrhiza, which literally means 'fungus-root', was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank. All mycorrhizal associations are symbiotic, since then a vast majority of land plants have been reported to form symbiotic associations with fungi. The AM fungi are the most intensively studied types of mycorrhizae because they are present in most

agricultural and natural ecosystems and play an important role in plant growth, health and productivity (Gianinazzi *et al.,* 1990). Mycorrhizal plants are often more competitive and exhibit enhanced tolerance against biotic and abiotic stresses compared to non-mycorrhizal plants and 80% of land plant species and 92% land plant families, were shown to have mycorrhizal associations Wang and Qiu (2006). The benefits afforded to the plants from mycorrhizal symbioses can be characterized agronomically by increased growth and yield and ecologically by improved fitness (i.e., reproductive ability).

There are only a few genera belonging to *Cruciferae*; *Chenopodiaceae* and *Cyperaceae* where they are not found due to the presence of glucosinolates and their hydrolysis products isothiocyanates in and around the roots (Glenn *et al.,* 1988), which are toxic to the growth of fungi. About 150 species of the genera *Acaulospora, Enthrophospora*, *Gigaspora, Glomus, Sclerocystis* and *Scutellospora* have been recognized as forming symbiotic associations with plants. These fungi form morphologically distinct resting spores in the soil and can be multiplied in the presence of a hostplant. Some of these spores can be surface sterilized and used to produce new spores in axenic seedlings or root organ culture (Mosse and Hepper, 1975). The close relationship of AM fungi with their host plants is mirrored by their obligatory biotrophic status. In absence of a host, their growth is limited to a relatively short time (20-30 days) after which many modifications in fungal morphology point to a cessation of hyphal growth. Presence of the root allows development of vegetative mycelium, which, under favorable conditions, can colonize 60-90% of the length of the root system (Becard and Piche, 1989; Bianciotto and Bonfante, 1995).

Teak (*Tectona grandis*) is an important timber tree species grown in tropical and sub-tropical countries. It is well known worldwide for its good quality wood used for making furniture's, panel work, railway carriages etc. *Tectona grandis*, is used in the treatment of laxative, for piles, leucoderma, dysentery, headache, burning pain over the region of the liver, expels worms from the body, is an expectant, cures inflamed eyelids, inflammatory swellings, indigestion, pain with burning in the stomach and removes itchiness of the skin.

MATERIAL AND METHODS:

Study site and Sample collection

Occurrence of Arbuscular Mycorrhizal (AM) fungal association is investigated in planted areas of *Tectona grandis* plants in places such as; Dharwad, Gungargatti, Holthikoti, Manasur and Mavinakoppa in Dharwad district of Karnataka, India. It is geographically Dharwad district is lying in between 14^0 $15^{'}$ and 15^0 $5^{'}$ North longitude and 74^0 $49^{'}$ and 76^0 $21^{'}$ East latitude. Root and rhizospheric soil sample were collected from *Tectona grandis*. These samples were returned to the laboratory and the fine roots in each sample were removed, rinsed with tap water and fixed in

formalin acetic alcohol (FAA), for determination of root colonization. The soil samples were then air dried in the shade at laboratory temperature for spore counting.

Isolation and Quantification of spores

The spores were separated from the soil by wet sieving and decanting technique (Gerdman and Nicolson, 1963). Fifty grams of freshly collected soil sample is put into one to two liters of plastic beakers. Usually rhizosphere soils are rich in AM fungal spores. Beaker size can be changed depending on the soil sample size. Soil is suspended with about 500ml to 1 liter of tap water. Soil macro-aggregates should be crushed with glass rod. After 30 minutes of settling down of soil particles, the upper layer of soil suspension is poured into the sieve (600 µm, 500 µm, 300 µm, 250 µm, 105 µm, 75 µm and 45 µm) to retain the spore of 45-250 mm size. The procedure should be repeated until the upper layer of soil suspension is transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out by modified method of Gaur and Adholeya (1994). Later single spore or sporocarps were easily picked up from the sample with the help of syringe or fine point brush and mounted on a glass slide with a drop of polyvinyl lactophenol (PVL) and a cover slip was placed. Subsequently, recovered spores were identified with the help of manual and different taxonomic keys proposed by different workers (Schenck and Perez, 1990; Frank and Mortan, 1994). The following characters are considered for identification sporocarp, spore morphology, size, shape and peridium of spore, sporocarp colour, wall ornamentation, subtending hyphae and mode of attachment.

Evaluation of AM Fungal colonization

Arbuscular mycorrhizal fungal structure in roots is usually not observed without appropriate staining. Freshly collected root samples should be washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots. Roots are treated with 10 % KOH solution for 30 min to 1-2 hours in a hot bath, depending on thickness of root structure. Treated roots are washed with water and treated with 2 % HCl solution. Acidified root samples are stained with 0.05 % trypan blue (or acid fuchsin) in lactic acid for 10-15 min in a hot bath or for a few hours without heating. The roots are destained with lactic acid or lacto-glycerol and are now ready for microscopic observation. The stained roots may be observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope. Fungal structures are stained and can be easily recognized (Phillips and Hayman, 1970).

RESULTS AND DISCUSSION:

The rhizosphere soil samples were collected from five localities of Dharwad district of Karnataka. Most of the collected rhizosphere samples exhibited varied range of spore population in localities of the soil profile (Table 1).The soil samples were subjected to the recovery of AM fungal spores. The

soil samples of different location showed different types of spores (Table 2). The genera observed in the recovered spores are- *Glomus, Acaulospora, Sclerocystis, Gigaspora, Scutellispora and Entrophospora*. In the present study it was observed that in many cases more than one appressorium is located at an entry point. In most cases, adjacent appressoria probably resulted from the branching of single external hyphae before or after contact with the root, which is in accordance with Brundrett *et al.,* (1985). Occasionally numerous branches were produced by intercellular hyphae and a convoluted or "comb" like structure was observed. Frequent branching of the intercellular hyphae, probably ensures a uniform distribution of the endophyte in the host root. Identification of AM fungi in conjunction with ecological information is of primary importance to interpret their distributions in natural and disturbed sites.

The present survey conducted to study the spore distribution and population dynamics of AM fungal spores in Dharwad district of Karnataka. The present investigation highest spore density was observed in the soil with rich organic carbon, which is in accordance with Dickman *et al.,* 1984. Hence it is suggested that AM fungal species is not host specific and the variation may be due to soil edaphic factors. During growing season of the plants percentage of infection is low which might be due to the absence of small roots at this stage or by lack carbohydrates available for the fungus (Douds *et al.,* 1986). The young and root feeder roots showed arbuscules, making additional nutrient exchange possible, whereas extensive vesicles formation is seen only at the end of the seasons, thus arbuscules functioning as nutrient exchange as reported by Allen (1991).

In the present study the formation of vesicles was observed at later stage of growth, containing storage lipid droplets, which are similar in structure and possibly function as soil borne spores of the fungus (Biermann *et al.,* 1983). Most descriptions of AM fungi based on the predominance of intercellular or intracellular hyphae spread by the endophyte. It is possible that in association with host, the endophyte spreads by intercellular coils. All the plants growing under natural condition possessed AM fungal spores as a regular component of the soil microflora. And spore total density records were lower than those of earlier workers (Ebbers *et al.,* 1987 and Bever *et al.,* 1996). This made to assume that these differences are mainly due to the different ecosystems studied. The total AM fungal spore number at different localities varied and the highest AM fungal spore population was observed in Mavinakoppa (188.326 per 50 gm of soil) and lowest in Holthikoti (80.668per 50 gm of soil). Similarly, the highest AM fungal per cent root colonization in Mavinakoppa (68%) and lowest in Holthikoti (31%). There was a wide variation in spore number especially in *Glomus* species followed by *Acaulospora* species.

Glomus species was the dominant in total AM fungal spore populations screened in most of the localities. On the other hand *Acaulospora, Gigaspora, Entrophospora, Sclerocystis* and *Scutellispora*

genera were predominant. *Glomus* species were most common AM fungi found in Citrus soil of Spain was reported by Camprubi and Calvet, (1996) while Desouza *et al.*, (2002) reported the similar results from *Citrus* orchards of Brazil. Lakshman *et al.*, (2004) observed the *Glomus* species was predominant. Schalamuk *et al.*, (2006) also reported similar results while working with a wheat cultivation system. Deeepak *et al.*, (2007) reported *Glomus* species was found that among glomealian fungi. *Glomus* species were most common AM fungi found in Citrus mycorrhizospere soil of Vidarbha region (Sanjay, 2008). Sharada *et al.*, (2008) and Prakash *et al.*, (2009) reported that *Glomus* species was dominant and recovered from all the study sites. AM fungal species belonging to genus *Acaulospora, Gigaspora, Glomus* and *Sclerocystis* were recorded, with the genus G*lomus* being predominant over others (Tabin *et al.*, 2009). Such differentiation of AM communities could have important implications for soil feedback and the composition of the regenerating tree community.

CONCLUSION:

The AM fungi at different levels of their organization (inter-interspercific population), conservation and efficient utilization of their biodiversity are of crucial importance for sustainable plant production system. Further research in plant communities with different levels of structure and in different seasons will be important to obtain a better understanding whether and how plant community structure affects the distribution of AM fungal spores and any reduction in the richness of populations of AM fungi or in their functional diversity could be important consequences for the equilibrium of natural plant community structure.

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Table 1. Showing the physico-chemical characteristics of soils and population of AM fungal spores and per cent of root colonization in *Tectona grandis* L. at various places of Dharwad district

Parameters	Gungargatti	Holthikoti	Dharwad	Manasur	Mavinakoppa
Soil	Black soil	Sandy loam	Red soil	Red soil	Red soil
pH (1:2.5)	7.230d	6.433de	7.067d	7.267d	7.133d
Moisture (%)	315.333a	325.000a	313.333a	329.333a	330.667a
Conductivity (Fc)us/cm	3.543de	3.873f	3.613e	4.210e	4.447e
Total organic carbon (%)	0.813e	0.800g	0.807f	0.623f	0.567f
Nitrogen (%)	0.057e	0.060g	0.043f	0.080f	0.077f
Potassium (%)	6.533d	7.793d	7.400d	6.697d	7.620d
Phosphorus (%)	4.313de	4.163ef	4.433e	4.323e	4.493e
Magnesium (%)	0.140e	0.132g	0.133f	0.130f	0.133f
Calcium (%)	0.453e	0.461g	0.511f	0.610f	0.417f
Zinc (ppm)	3.650de	3.500f	3.410e	3.710e	3.653e
Copper (ppm)	0.017e	0.027g	0.013f	0.017f	0.027f
Manganese (ppm)	0.780e	0.770g	0.797f	0.867f	0.740f
Iron (ppm)	8.620d	7.847d	8.317d	9.001d	8.410d
AM fungal spore/50gm of soil	110.333b	80.668b	96.667b	122.333b	188.326b
Percent colonization (%)	42.333c	31.000c	41.667c	51.667c	68.000c

Means values followed by the same letter within a column do not differ significantly at $P<0.05$ according to DMRT.

Table 2. Showing the AM fungal spores recovered from Dharwad district in five places with respect to the rhizosperic soil of *Tectona grandis* L.

Locality	Spore type	Shape	Spore diameter (µm)	Colour of wall layers	Number of wall layers
S1, S2, S3, S4, S5	*Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker and Koske	Globose-Subglobose	75-150x35-100	Light brown	Single layered
S2, S5	*Glomus flavisporum* (M. Lange & Lund) Trappe & Gerdemann	Globose	149-202x95-152	Yellowish brown	Double layered
S1, S3	*Glomus fragilistratum* Skou & Jakobsen	Globose	108-191	Yellow	Double layered
S4, S5	*Glomus geosporum* (Nicolson & Gerdemann) Walker	Globose-Subglobose	110-290x100-290	Yellowish brown	Single layered
S2, S4	*Glomus pallidum* Hall	Globose-Subglobose	32-78x28-68	Pale yellow	Single layered
S1, S2, S4	*Glomus tenebrosum* (Thaxter) Berch	Globose or Subglobose	(200-)240(-270) x(205-)230(-270)	Dark brown	Single layered
S3, S4, S5	*Glomus albida* Walker & Rhodes	Globose	143-330(-350)	Yellow-golden yellow	Single layered
S1,S2, S5,	*Glomus formosanum* Wu & Chen	Globose-Subglobose	360-500x450-500	Reddish brown	Single layered
S2, S3, S4	*Glomus macrocarpum* Tulasne & Tulasne	Globose-Subglobose	(90-)120(-140)x(70-)110(-130)	Yellowish brown	Single layered
S1, S2	*Glomus caledonium* (Nicolson & Gerdemann) Trappe & Gerdemann	Globose-Ellipsoid	124-394	Yellowish brown	Double layered
S1, S2, S4	*Glomus maculosum* Miller & Walker	Globose-Subglobose	(95-)135-178(-220)	Pale straw	1-3 layered
S1	*Glomus multicauli* Gerdemann & Bakshi	Elliptical	149-249x124-162	Dark brown	Single layered
S5	*Glomus australe* (Berkeley) Berch	Globose-Subglobose	(120-)160(-180)	Yellowish brown	Double layered
S2, S3, S4	*Glomus gerdemannii* Rose, Daniels & Trappe	Globose-Subglobose	140-198x149-230	Yellowish brown	Five layered
S1, S5	*Glomus globiferum* Koske & Walker	Globose-Subglobose	150-260x150-270	Red brown	1-4 layered
S1, S2, S3, S5	*Glomus microagreegatum* Koske, Gemma & Olexia	Globose	30(-50)x(15-)30(-40)	Brownish-yellow	1-2 layered
S2, S3, S4	*Glomus occultum* Walker	Globose-Subglobose	15-100x20-120	Hyaline white	1-2 layered
S1, S4, S5	*Glomus taiwanensis* Wu & Chen	Chlamydospores	40-85	Yellowish brown	Double layered
S1, S3, S4, S5	*Glomus mossae* (Nicolson & Gerdemann) Gerdemann & Trappe	Globose-ellipsoid	105-310x110-305	Brownish yellow	Double layered
S2, S3, S4, S5	*Glomus fistulosum* Skou & Jakobsen	Globose-Subglobose	(78-)120-160(-200)	Yellowish brown	Double layered
S3, S4, S5	*Glomus halon* Rose & Trappe	Globose-Subglobose	200-280	Yellowish brown	Double layered
S1, S2, S4	*Glomus botryoides* Rothwell & Victor	Globose-Subglobose	145-250	Radish brown	Double layered
S3, S4, S5	*Glomus fuegianum* (Spegazzini) Trappe & Gerdemann	Globose-Subglobose	65-80	Radish brown	Single layered
S2, S3, S5	*Glomus clarum* Nicolson & Schenck	Globose-Subglobose	68-290	Yellowish brown	Single layered

Locality	Spore type	Shape	Spore diameter (µm)	Colour of wall layers	Number of wall layers
S1, S4	*Acaulospora denticulata* Sieverding & Toro	Globose-Subglobose	(112-)130-170(-175)	Red brown	1-4 layered
S1, S2, S3	*Acaulospora scrobiculata* Trappe	Globose	100-240x100-220	Greenish yellow	Four layered
S4	*Acaulospora thomii* Hu	Globose-Subglobose	425-475	Yellowish brown	Single layered
S3, S5	*Acaulospora foveata* Trappe & Janos	Globose	250-300x185-250	Yellowish brown	Three layered
S2, S5	*Acaulospora delicata* Walker, Pfeiffer & Bloss	Globose-Subglobose	80-125(-150) x80-110(-140)	Yellowish cream	Double layered
S1, S2, S3, S4, S5	*Acaulospora laevis* Gerdemann & Trappe	Globose-Subglobose	119-300x119-520	Red brown	Three layered
S3	*Acaulospora taiwania* Hu	Globose-Subglobose	425-475	Dull yellowish brown	Double layered
S1, S4	*Gigaspora albida* Schenck & Smith	Globose	232-252x234-250	Greenish yellow	1-6 layered
S2, S4	*Gigaspora ramisporophora* Spain, Sieverding & Schenck	Globose-Subglobose	200-450x143-501	Yellowish brown	Three layered
S1, S2, S3, S4, S5	*Gigaspora margarita* Becker & Hall	Globose	260-480	Hyaline-white	Four layered
S3	*Gigaspora decipiens* Hall & Abbott	Globose	320-490	Yellowish	Three layered
S1, S2, S3, S4, S5	*Sclerocystis dussii* (Patouillard) von Hohnel	Chlamydospores	50-80x32-54	Brown	Single layered
S4	*Sclerocystis pachycaulis* Wu & Chen	Chlamydospores	170-230x175-270	Yellowish brown	Double layered
S3, S4	*Sclerocystis pakistanica* Iqbal & Bushra	Chlamydospores	65-205x32.5-55	Dark brown	Single layered
S2	*Sclerocystis taiwanensis* Wu & Chen	Chlamydospores	40-85x22-42.5	Yellowish brown	Double layered
S5	*Scutellispora erythropa* (Koske & Walker) Walker & Sanders	Globose-Subglobose	170-551x205-660	Pale yellow	4 or 5 layered
S1	*Scutellispora scutata* Walker & Diederichs	Globose-Subglobose	350-667x350-713	Hyaline	Six layered
S4	*Scutellispora calospora* (Nicolson & Gerdemann) Walker & Sander	Globose-ellipsoid	114-285(-311)x110-412	Pale greenish yellow	Four layered
S1, S2, S5	*Entrophospora schenckii* Sieverding & Toro	Globose-Subglobose	(37-)50-60(-77)	Hyaline	Three layered

S1= Gungargatti; **S2**= Holthikoti; **S3**= Dharwad; **S4**= Manasur; **S5**= Mavinakoppa