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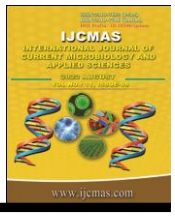

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Isolation and Identification of Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Black siris (*Albizzia odoratissima* (L.F.) Benth)

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A B S T R A C T

Arbuscular mycorrhizal fungi (AMF) represent one of the major components of soil microbiota with a potential to not only aid in the establishment of the host plant, but also to enhance the overall plant growth. Despite increased understanding of the mycorrhizae, the mycorrhizal association, distribution and relative abundance for the majority of forest tree species are lacking. In the present study, we have isolated and identified the diverse species of arbuscular mycorrhizal fungi associated with the rhizosphere of *Albizzia odoratissima* (Black siris) located at an intensively managed site in the Forest College and Research Institute, Mulugu, Telangana, India. Rhizosphere soil samples collected from four different sites at the study location were processed for isolation of AMF, which were then identified using microscopic examination of morphological features. The AM fungi observed in the soil samples included 4 species of *Glomus*, and one species each of *Acaulospora*, *Gigaspora*, and *Scutellospora*. The most common and dominant mycorrhizal fungi associated with roots was found to be *G. mosseae* with an average spore density of 35/100g of soil. Microscopic observations of root samples confirmed the presence of arbuscules as well as vesicles. Overall findings suggest that the rhizosphere of *A. odoratissima* is highly rich in myco- biodiversity in the FCRI campus, but intensive management of appears to limit the distribution of mycorrhizae to the deeper layers of soil, resulting in less diversity.

Introduction

Every photosynthetic plant requires water and minerals for synthesizing its' organic components (Johnson and Jansa, 2017). When the water and mineral resources are in limited supply in the soil, a syiotic interaction between plants and fungi can greatly enhance the performance of plant roots to acquire water and mineral nutrients from deeper layers of the soil through the fungal mycelium, this association is called as Mycorrhizae (Johnson and Jansa, 2017). Mycorrhizae are the ubiquitous important soil micro-organisms improve plant health and help in their establishment A Navarro Garcia *et*

al., (2011). These organisms known to infect approximately 2,50,000 species of plants including the plants from diversified ecosystems (Van der Heijdan *et al.,* 2015).

The mycorrhizal fungi produce organic acids along with other molecules that metabolize the organic compounds and aid in the solubilization of mineral nutrients (Smith and Read, 2008).

Additional benefits of mycorrhizal symbiosis include the maintenance of host plant growth when subjected to both abiotic and biotic stress (Faria *et al.,* 2013).

Albizzia odoratissima, popularly known as Black Siris is lesser-known and not well-studied species among the forest species that have potential importance for timber. It belongs to the family Fabaceae with large natural occurrence in Indian Sub-continent, Myanmar, Thailand, Laos, Vietnam, East Asia, and Southern China.

It occurs in tropics and sub-tropic climatic regions such as open areas, dry deciduous forests, secondary forests, fire-damaged forests, and hill-mixed evergreen forests. The tree is extensively planted as a shade tree in Tea and Coffee plantations. The tree generally prefers well-drained soils and is a light demander even though it requires shade at the initial stage (Fern *et al.,* 2014).

Growth studies of *A. odoratissima* upon inoculation with microorganisms are scanty. However, recent reports demonstrated the association with nitrogen-fixing Rhizobium bacterium, which produces abundant quantities of nodules (Fern *et al.,* 2014).

Yet, *Albizzia odoratissima* was classified as a mycorrhizal dependent species, leading us to believe this tree also forms an association with mycorrhizae (Habte *et al.,* 1994). Thus, the present study aimed to determine the type of mycorrhizal species associated with *A. odoratissima* and their relative abundance.

Materials and Methods

Study area

The study was conducted at Forest College and Research Institute (FCRI), Mulugu from December 2021 to February 2022. On the Agro-ecological zone map of Telangana, the study site forms part of Central Telangana Zone with hot semi-arid climatic conditions. The block plantations of *A. odoratissima* were established in 1998 and cohabited with plantations of *Gmelina arborea*, *Dalbergia latifolia*, and *Pterocarpus santalinus* species and were subjected to intensive management practices, including mechanical ploughing.

AMF observations on *Albizzia odoratissima*

Before isolation and identification of AM spores associated with *A. odoratissima*, we experimented with the roots of *A. odoratissima*, to know whether the tree forms symbiotic relationship with *Arbuscular mycorrhiza* or not by following available methodology (Phillip and Hayman, 1970). The roots picked from the rhizosphere were cut into 1-2 cm lengths and cleaned thoroughly with water and kept in 10% (w/v) KOH solution and autoclaved for 1 hour, to remove any lignin material, if present in the root materials. Then the roots were again cleaned with running water and kept in 2% HCl for 1 hour. Again, the roots were washed thoroughly under running water and immersed in a trypan blue solution for some time. The roots were placed on glass slides and observed under the microscope. The root sections were observed for the presence of hyphae, arbuscules, and vesicles.

Soil collection process

Soil samples from the *A. odoratissima* rhizosphere were taken at 2 points diagonally at the depth of 15-30 cms by clearing the upper soil layer from litter, bushes, and grass. The sample collected from each tree was mixed together to get a homogenous composite sample of about 500g (Mahulette *et al.,* 2021). The sample was then packed in a properly

labelled zip lock bag and stored in the refrigerator at 4oC until the next step. Along with the soil samples, small root particles such as tertiary roots and root hairs were also collected for identification of colonization with AMF.

Isolation of spores from rhizosphere

Briefly, the procedures pertaining to the isolation and identification of AM spores were carried out at the Pathology Laboratory, Department of Forest Resource Management and Conservation, FCRI.

AM fungal spores from the rhizosphere samples were isolated after wet sieving, followed by decantation (Gerdemann and Nicolson, 1963). The procedure was undertaken to separate spores from the soil sample for easy identification of the AMF genus.

200 grams of soil sample was suspended in lukewarm water and the suspension was stirred at an interval of 3-5 minutes for 15 minutes to make sure the spores were completely dissolved in the solution.

The solution was allowed to rest for 5 minutes to let the soil particles settle at the bottom of the beaker. Then the solution was passed through a set of stratified sieves ranging from 1mm to 45 µm.

The residues were collected from the last three sets i.e., 105 µm,75µm, 45 µm by pumping water under high pressure through an orifice on diagonally opposite sides of the sieve, and the contents of each sieve were transferred to separate Petri-dishes.

The shallow suspension was observed with a stereomicroscope to calculate spore density (Mahulette *et al.*, 2021) and for the identification of spores. The spores and micro sporocarps were picked up with a Pasteur pipette and transferred to the slides for further observations.

$$\text{Spore density} = \frac{\text{Number of spores}}{\text{Weight of analyzed soil}}$$

Identification of spores

AM fungi were identified based on the morphology of the spores; thus, mycorrhizae that are not in the form of spores were not recognized. Identification of spores was done according to Schenck and Perez (1988) and descriptions provided by INVAM 2006.

AM fungi root colonization

Colonies of AM fungi associated with the root segments were studied following a well-defined procedure (Habte *et al.*, 1994). The % of root colonization was determined using the below equation:

$$\text{AM colonization (\%)} = \frac{\text{Total no.of infected root segments}}{\text{Total no.of root segments examined}} \times 100$$

Results and Discussion

Observation of AM association on *Albizzia odoratissima*

Preliminary microscopic observations revealed that *A. odoratissima* was associated with endomycorrhiza, in the form of arbuscules and vesicles Fig 1[a-c]. Arbuscules are the most important structures in mycorrhizal symbiosis (Smith and Read, 2008), formed by internal hyphae that aid in the exchange of nutrients between plant roots and fungi (Sukmawati *et al.*, 2021), while the vesicles act as a storage house of carbohydrates. These results indicate that *A. odoratissima* is indeed a mycorrhizal-dependent host with symbiotic association, although earlier studies focused on other species of Albizzia (Habte *et al.*, 1994).

Isolation and identification of AM fungi

AM spores isolated from the rhizosphere of *Albizzia odoratissima* revealed some differences in the type and density of the spores at the four sites within the block plantation. Albizzia roots in all the sites

sampled were colonized by AM fungi, without exceptions as shown in Table 1. The AM fungi included 4 species of Glomus, and one species each of *Acaulospora*, *Gigaspora*, and *Scutellospora*. The most common and dominant mycorrhizal fungi associated with *A. odoratissima* roots was *G. mosseae*, followed by *G. aggregatum*. *Scutellospora* was the less abundant species in all the samples collected. Spore count ranged from 148-188 per 100 g of soil. The spore density is considered high if the number of specific spores ranges from 16-140/ 100g of soil (Warouw and Kainde, 2010).

The spore density of each of the AMF at all the four sites can be considered rich, except for *Acaulospora* at site I and *Scutellospora* at sites I, II and IV. Overall, all four sites exhibited a rich diversity of mycorrhiza, with varied abundance.

Percentage of colonization

Each tree's rhizosphere was examined using at least 10 root samples. The percentage of root infections by AMF presented in Table 2 was calculated by microscopic observations. All root samples unequivocally exhibited the presence of arbuscles, vesicles or hyphae (Wulandari *et al.*, 2013) and therefore the AM colonization was found to be 100%. Observed incidence of colonization in all root samples clearly implies the mycorrhizal dependency, despite being a member of Leguminosae.

Table.1 Diversity and abundance of AMF spores associated with *Albizzia odoratissima* from different sites in the FCRI campus

AMF species	SiteI	Site II	Site III	Site IV
G. mosseae	36	30	34	**40**
G. fasciculatum	26	30	32	**24**
G. aggregatum	32	34	35	**33**
G. intraradices	21	23	28	**19**
Acaulospora	13	24	18	**22**
Gigaspora	20	18	22	**27**
Scutellospora	0	15	19	**0**
Total no. of spores	**148**	**174**	**188**	**165**

Numbers against each AMF species indicate the observed number of sporesper100 g of soil at four different sites (I to IV).

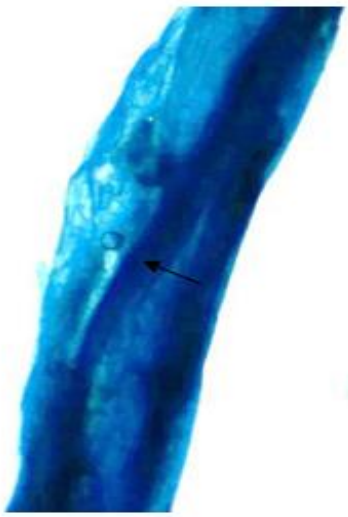
Table.2 Percentage root colonization of roots of *Albizzia odoratissima* by AMF in the FCRI campus.

Site	Vesicles	Arbuscules	Hyphae	% of colonization
SiteI	+	+	+	**100**
SiteII	+	+	+	**100**
SiteIII	+	+	+	**100**
SiteIV	**+**	**+**	**+**	**100**

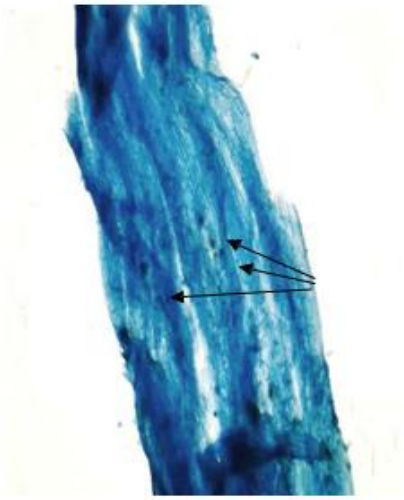
+ indicates Presence, - indicates Absence (not detected) in the root segments

Fig.1 Root sections of *Albizzia odoratissima* stained with trypan blue for visualization of spores and arbuscules. (a) 20X magnification, Arrow indicates the presence of spore; (b)arrows indicate the presence of arbuscules, (c)arrows indicate the internal hyphae inside the cortical cells

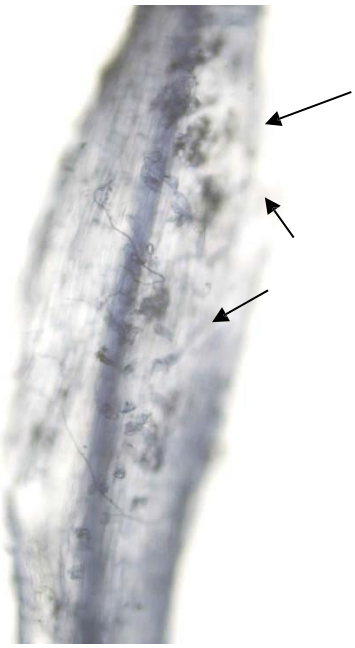
a



b



c



In this study, analysis of the diversity and abundance of AM spores associated with the rhizosphere of *A. odoratissima* affirms that *Glomus mosseae* and *G. aggregatum* were the most dominant, whereas *Scutellospora spp*. were least observed or barely present at two of the experimental sites from where the soil was collected. Intensive management such as ploughing or other disturbances might result in a lower incidence of *Scutellospora* spores at 15-30 cm depth, which tend to be located deeper at 50-70 cm (Oehl *et al.*, 2005).

Notwithstanding this, the distribution of *Scutellospora* was reported to be limited compared to the Glomus *spp*. (Ibou *et al.*, 2021; Sukamawati *et al.*, 2021). More no. of Glomus spores in the rhizosphere is due to easy adaptation (Delvian, 2010) and can produce more no. of spores and easy germination in less time i.e., only 4-6 days (Wang and Jiang, 2015). Furthermore, the abundance pattern of various AMF species is known to vary depending on the host species, as well as the soil characteristics such as pH and texture (Lara-Capistran *et al.*, 2021).

Although Albizzia is known to associate with Rhizobium for nitrogen fixation, it appears to depend on the mycorrhiza for absorption of water, nutrients and minerals. However, it remains to be tested if inoculation with AMF to Albizzia under nursery conditions promotes growth.

The study was conducted to determine the diversity of *Arbuscular mycorrhizae* associated with *A. odoratissima*. The results from the study demonstrated Glomus was dominant with an average spore density of 35/100g of soil. The rhizosphere of the *A. odoratissima* is rich in myco-biodiversity on the FCRI campus. All the analysed root samples have shown AMF formation with internal structures like arbuscles, vesicles, and internal hyphae. The findings suggest that intensive management is likely to decrease the distribution of mycorrhizae and limit its distribution to the deeper layers of soil leading to the less diversified mycobiota at the rhizosphere.

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