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ASSOCIATION OF ARBUSCULAR MYCORRHIZAL FUNGI WITH MAIDA CHAAL (*Litsea glutinosa* Lour) SEEDLINGS

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ABSTRACT
An investigation was carried out to study the natural development of arbuscular mycorrhiza (AM) and its effect on the performance of Maida chaal (*Litsea glutinosa*) seedlings. Seeds were collected from different forest areas of Central India and were raised at nursery of Tropical Forest Research Institute, Jabalpur. Data was recorded on shoot and root length, collar diameter, number of AM fungi spore and root colonization. Analysis of variance revealed the significant differences between the seedlings of different localities for the shoot length (cm), root length (cm) and root shoot ration. Maximum seedling height and collar diameter was obtained in Balaghat seed source followed by Chhindwara and Jabalpur seed sources. Root colonization in this threatened tree ranges from 64.4 to 80.0%. Twenty eight fungi were identified from the rhizospheric soil amongst them *Glomus* was the most dominant. Correlation study confirms that mycorrhization of seedlings by AM fungi significantly supported growth of seedlings raised from different seed sources. In conclusion, application of AM fungi during raising seedlings is helpful for growth of Maida chaal seedlings in nurseries.

KEYWORD: AM species, Root colonization, Symbiosis, Seedling growth.

INTRODUCTION

Mycorrhizal fungi differ from other plant–fungus associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant. Mycorrhizal fungi interact with plants at different levels and plants can be grouped into obligate mycorrhizal, facultative mycorrhizal and non-mycorrhizal (Brundrett, 2004). Arbuscular mycorrhiza fungi (AM) colonize plant feeder root forming an extended mycelial net that provides multiple benefits to the host plant including greater water and nutrients uptake (specially P, Cu and Zn); protection and better survival under stress environmental conditions such as salinity, drought, acid soils, presence of toxic elements or root pathogens (Smith and Read, 1997) influencing plant growth, crop quality and adaptability to stress conditions (Sharma and Yadav, 2013).

Maida chaal (*Litsea glutinosa* Lour.) is an important medicinal plant. The bark of this species is one of the most popular of native drugs and is considered capable of relieving pain, arousing sexual power, good for stomach in treatment of diarrhea, dysentery, fractured limbs and some other diseases (Kritikar and Basu, 1981). Recently, *L. glutinosa* has also been investigated as a source of essential oils, arabinoxylans and other components with antiseptic properties (Prusti *et al.*, 2008; Qin Wen Hui *et al.*, 2012). Maida Lakri is also used for Agarbatti making industry, which is valued around Rupees 1000 crores with an annual growth rate of 10–15% and provides employment to around 1.5 million people. This industry consumes whole production of the bark of *L. glutinosa* along with other two species of the same family, *i.e.* *Persea macrantha* and *Litsea monopetala* (Rath, 2003; Mohammad *et al*., 2016).

Due to increase in demand, the trees were extensively damaged for the extraction of bark. This indiscriminate exploitation of the stem bark made the species as threatened and endangered causing grave concern about the loss of wild germplasm. The plant is listed as red listed plant and considered as critically endangered in the state of Andhra Pradesh, India (Reddy and Reddy, 2008) and as endangered species in Philippines. For the conservation of this threatened species, it is necessary to include this species in plantation programmes of Forest Departments and for which mass availability of health seedlings is requisite. Mohammad *et al.* (2016) reported the protocol for seed germination and Verma and Verma (2015) reported wilt disease in nursery. Since, the arbuscular mycorrhiza play an important role on growth and establishment of plants, the present investigation was carried out to investigate the association of AM fungi with Maida chaal and its association with growth parameters.

MATERIALS & METHODS

Study Site

The study site, Tropical Forest Research Institute, Jabalpur is situated between 23°5'37" to 23°6'10"N latitude and 79°59'49" to 79°59'42"E longitude. The average elevation of the site is 411 meter (1348 ft) from sea level and is situated in the Mahakoshal region of Madhya Pradesh in central India. The temperature of Jabalpur varies from 9ºC to 43ºC. The average annual rainfall over the area is 1358mm.

Raising of seeding

The seeds of Maida chaal were collected from four different forest areas namely Balaghat (N21˚32'22.5" E80˚37'50.6"), Chhindwara (N22˚24'28.2" E78˚42'21.4"), Jabalpur (N23°5'37" E79°59'49") and Keonchi (N22˚37' 49.0" E81˚46'56.6"). The seedlings were raised at nursery of Genetics and Plant Propagation Division, Tropical Forest Research Institute, Jabalpur. Standard package of practices were followed to raise healthy seedlings (Fig. 1).

Collection of soil sample

Soil samples were collected from the rhizospere of selected seedlings of different localities. Seedlings were selected randomly for this purpose. Collected soil samples were homogenized in laboratory for further investigation. Total height and collar diameter of selected seedlings was recorded.

Isolation and identification of arbuscular mycorrhiza fungi

Collected soil sample were processed for isolation of AM spore. To extract AM spores, wet sieving and decanting technique as described by Sylvia (1994) was followed. Identification of AM fungi on the basis of morphology of their resting spores carried out by referring taxonomic manual of Schenck and Perez (1987) and http://www.zor. zut.edu.pl/Glomeromycota/Classification.html.

Determination of root colonization

Development of Mycorrhizal fungi in term of root colonization was detected by staining the root. The method prescribed by Phillips and Hayman (1970) was followed.

$$\% \text{ root colonization} = \frac{\text{Number of root bits infected with AM}}{\text{Total number of root bits examined}} \text{ X } 100$$

Statistical analysis

Data obtained on plant height, diameter of seedlings at collar height and root colonization by AM fungi was subjected to analysis of variance using OPSTAT (Sheoran *et al.*, 1998). Correlation between plant heights, diameter and root colonization was also determined. Relative spore density (RSD) of identified AM fungal spore was calculated as follows:

$$\text{RSD} = \frac{\text{Spore density of a particular species}}{\text{Spore density of all species}} x\ 100$$

RESULTS

Maximum shoot length, root length, root and shoot length ratio and collar diameter was observed in Balaghat seed sources followed by Chhindwara and other sources. Highest root colonization by AM fungi was recorded in Balaghat seed sources followed by Keochi, Chhindwara and Jabalpur (Table 1 and Fig 2). All the characters under investigation found to be highly correlated with each other. Root colonization by AM fungi exhibited highly significant and positive correlation with the height (r=0.916) and collar diameter of the seedling (r=0.821) (Table 2).

TABLE 1: Performance of *Litsea glutinosa* seedlings raise from different seed sources

S. No.	Sites of seed collection	Shoot Length (cm)	Root length (cm)	Root: Shoot	Collar Diameter (cm)	Spore /100g	Root colonization (%)	A	V	H
1.	Balaghat	50.12 ±5.86	32.45 ±3.31	0.822 ±0.15	1.008 ±0.09	168 ±33.17	80 ±3.86	√	√	√
2.	Chhindwara	43.98 ±5.89	26.27 ±4.6	0.805 ±0.24	1.03 ±0.05	131 ±19.96	66.67 ±2.22	×	√	√
3.	Jabalpur	39.84 ±2.2	22.66 ±1.75	0.719 ±0.18	1.01 ±0.06	143.33 ±26.02	64.44 ±2.22	×	√	√
4.	Keonchi	37 ±1.45	18.2 ±0.83	0.727 ±0.23	0.99 ±0.33	146 ±19.08	71.11 ±5.88	×	√	×
CD at *P = 0.05*		4.789	1.28	0.066	N/A	N/A	N/A	-	-	-

Significant at *P* = 0.05; A= arbuscule, V=vesical, H=hyphae

TABLE 2: Pearson Correlation coefficient of growth response in *Litsea glutinosa* seedling

	Shoot length	Root length	Root: Shoot	Collar diameter	Spore number	Root colonization
Shoot length	1					
Root length	0.967**	1				
Root : Shoot	0.767**	0.717**	1			
Collar diameter	0.719**	0.676**	0.814**	1		
Spore number	0.923**	0.861**	0.833**	0.812**	1	
Root colonization	0.916**	0.911**	0.821**	0.821**	0.939**	1

Significant at *P* = 0.01

AM fungi identified from *L. glutinosa* seedlings include: *Acaulospora bireticulata* F.M. Roth Well & Trappe, *A. denticulata* Sieverd. & S. Toro., *A. foveata* Trappe & Janos, *A. trappei* R.N. Ames & Linderman, *A. lacunosa* J.B. Morton, *A. scrobiculata* Trappe, *Gigaspora* sp., *Glomus aggregatum* N.C. Schenck & G.S. Sm. Emend. Koske, *G. callosum* Sieved., *G. clavisporum* (Trappe) R.T. Almeida & N.C. Schenck, *G. fasciculatum* (Thaxt.) Gerd. & Trappe emend. C. Walker & Koske, *G. geosporum* (T.H. Nicolson & Gerd.) C. Walker, *G. heterosporum* G. S. Sm. & N.C. Schenck, *Glomus microcarpum* Tul. & C. Tul, *G. minutum* Blaszk., Tadych & Madej, *G. multiforum* Tadych & Blaszk, *G. reticulatum* Bhattacharjee & Mukerji, *G. rubiformi* Gerd. & Trappe, *G. versiforme* (P.

Karsten) Sm. Berch, *G. walkeri* Blaszk. & Renker, *G. xanthium* Blaszk., Banke, Renker & Buscot, *G. constrictum* Trappe, *G. deserticola* Trappe, Bloss & J.A. Menge , *G. intraradices* N.C. Schenck & G.S. Sm., *G. macrocarpum* Tul. & C. Tul., *G. mosseae* T.H. Nicolson & Gerd.) Gerd. & Trappe, *Paraglomus lactum* (Blaszk.) Renker, Blaszk. & Buscot and *Scutellospora nigra* (F.M. Redhead) C. Walker & F.E. Sanders.

TABLE 3: Relative spore density of AM spore

S. No.	Name of spore	Relative spore density	S. No.	Name of spore	Relative spore density
1.	*Acaulospora bireticulata*	3.9168	15.	*Glomus heterosporum*	1.9584
2.	*Acaulospora denticulate*	0.2448	16.	*Glomus intraradices*	4.5288
3.	*Acaulospora foveata*	2.3256	17.	*Glomus macrocarpum*	13.5863
4.	*Acaulospora lacunose*	4.5288	18.	*Glomus microcarpum*	3.5496
5.	*Acaulospora scrobiculata*	0.9792	19.	*Glomus minutum*	0.3672
6.	*Acaulospora trappei*	0.9792	20.	*Glomus mosseae*	14.4431
7.	*Gigaspora sp.*	0.3672	21.	*Glomus multiforum*	3.1824
8.	*Glomus aggregatum*	5.9976	22.	*Glomus reticulatum*	2.2032
9.	*Glomus callosum*	2.3256	23.	*Glomus rubiformi*	0.2448
10.	*Glomus clavisporum*	0.9792	24.	*Glomus versiforme*	4.6512
11.	*Glomus constrictum*	6.3647	25.	*Glomus walkeri*	7.2215
12.	*Glomus deserticola*	1.4688	26.	*Glomus xanthium*	4.7736
13.	*Glomus fasciculatum*	7.4663	27.	*Paraglomus lactum*	0.1224
14.	*Glomus geosporum*	0.7344	28.	*Scutellospora nigra*	0.4896

FIGURE1. Maida chaal seedlings in nursery

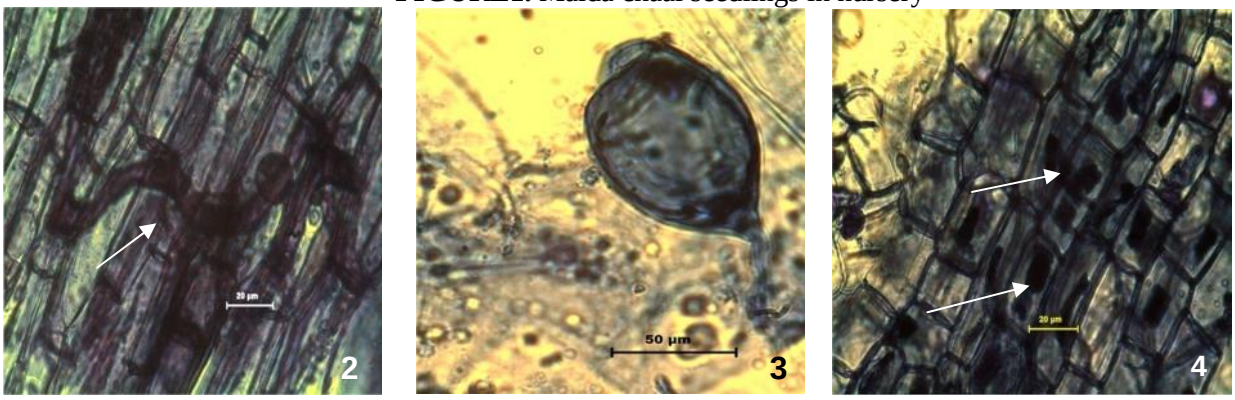

FIGURE 2-4. Root colonization by AM fungi in Maida chaal seedlings (A) hyphae, (B) development of vesicles, (C) arbuscule

Relative spore density of identified AM fungi was measured. Minimum spore density was observed in *Paraglomus lactum* followed by *Acaulospora denticulata, G. rubiformi, Gigaspora* sp. and maximum density was observed in *G. mosseae* followed by *G. macrocarpum, G. fasciculatum, G. walkeri, G. constrictum, G. aggregatum* (Table 3).

DISCUSSION

In the present study maximum plant height, diameter of seedlings at collar height was obtained in Balaghat–seed source followed by Chhindwara and Jabalpur (Table 1). Maximum root colonization by AM fungi was obtained in Balaghat followed by Keochi, Chhindwara and Jabalpur. AM fungi root colonization was significantly correlated with shoot length (r=0.916), root length (r=0.911), root:shoot ratio (r=0.821), diameter (r=0.821) and spore number (r=0.939) (Table 2). Significant differences in these parameters were observed in different seed sources. Similar result was observed in *Pterocarpus marsupium* seedlings raised from different seed sources of Chhattisgarh, India and found positive correlation between

presence of AM fungi and growth of seedling (Verma *et al*., 2016a).

AM symbiosis is an important phenomenon responsible for plant growth by providing better absorption of nutrients, especially phosphorus and provide disease resistance (Perrin, 1990). In the present investigation, enhancement in growth and diameter of seedlings may due to activities of AM fungi as earlier reported by many workers (Cooper and Tinker, 1978; Ames *et al*., 1984) and other microorganisms (Garbaye, 1994). AM fungi also enhanced phosphorus uptake and synergistically affects the plants health and growth (Gaur and Rana, 1990). Kamal Prasad (2006) has also reported the positive synergistic effect of AM fungi and phosphate solubilising bacterium on growth of *Azadirachta indica* in nursery soil. Verma and Jamaluddin (1994) reported that seedlings with better root colonization also help to withstand the water stress conditions. Verma *et al*. (2016) observed that AM fungi was growing and multiplied in mine land soil.

Root colonization in *L. glutinosa* ranges 64.4 to 80% and 28 AM fungi were identified belonging to 5 genera, namely *Acaulospora* 6 species, *Gigaspora* 1 species, *Paraglomus* 1 species, *Scutellospora* 1 species and *Glomus* 18 species. *Glomus* species were dominant in rhizosphere soil of *L. glutinosa*. *Glomus* sp. was noted to be the most abundant and it was followed by *Acaulospora* sp. Distribution of *Glomus* sp. with respect to wild, soil and environmental conditions and host species has been reported by earlier workers (Jha *et al*., 1994; Ruiz-Lozano, 2003; Pande and Tarafdar, 2004; Vivas *et al*., 2005; Sharma *et al*., 2009**;** Cano and Bago, 2005; Kullu and Bahera, 2012). The dominance of this genus has been ascribed due to its smaller sized spores, which are reported to take shorter duration to sporulate and reproduce (Nandakwang *et al.*, 2008). Verma *et al*. (2014) also reported arbuscular mycorrhization in another important tree species, *Ougeinia oojeinensis* belonging to the same family with root colonization ranges from 45 to 58% in central India. They have identified 12 AM fungi from rhizosphere of this species. In the present study, Balaghat and Keonchi seed sources had better root colonization by AM fungi and reported to be better growth for seedlings. Such seedlings may perform better when transplanted in the field. AM fungi may reduce in phosphorous fertilizer application.

CONCLUSION

In this investigation very good root colonization was observed with roots of *Litsea glutinosa* seedlings and also root colonization exhibited positive correlation with growth parameters. AM fungi may be included in the package of practices for raising healthy seedlings of this threatened species.

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