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SELECTION OF AN EFFICIENT INDIGENOUS ARBUSCULAR MYCORHIZAL FUNGUS FOR *COFFEA ARABICA* L. OF NILGIRI DISTRICT, TAMILNADU, INDIA.

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ABSTRACT

The present study was undertaken to screen and select an efficient Arbuscular Mycorrhizal Fungi and also to study its effect on growth, biomass and nutrition in *Coffea arabica* L. of Nilgiri District. A poly bag trial was conducted at Government Arts College, Ooty. Seven dominant native AM fungi *Acaulospora scorbiculata, Gigaspora margarita, Glomus aggregatum, Glomus fasciculatum, Glomus geosporum, Glomus mosseae,* and *Scutellospora heterogama* isolated from different coffee plantations of Nilgiris were tested for their symbiotic efficiency against coffee plants. The growth and biomass in 6 months, 10 months, 14 months and 18 months and nutrient level of 18 months seedlings of seven different AM fungi inoculated plants were recorded. In general, inoculated plants showed increased plant height, number of leaves and biomass compared to control plants without am fungi inoculum. The plant growth, biomass and nutrient were maximum in plants inoculated with *Glomus mosseae* followed by those inoculated with *Glomus aggregatum.* Considering the various parameters such as plant growth, biomass and nutritional status of the plant, it was observed that *Glomus mosseae* is the best AM symbiont for *C. arabica* L. used in this experiment.

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INTRODUCTION

The biofertilizers have proved that its application increase the biomass and productivity of a wide range of cereals and medicinal crops[1]. Biofertilizers have some limitations in most of the plantation crops as they are long duration crops. In most of the plantation crops, preliminary trialing has been carried out to explore the potential to use biofertilizers for enhancing growth and yield. The use of biofertilizers is effective in developmental stage either in nursery or in field than the synthetic fertilizers. Most of the plant community and plant productivity are thought to be influenced by mycorrhizae[2]. On the other hand, mycorrhizae were considered as most common species of symbiotic association, with arbuscular mycorrhizae (AM)[3].

AMF are soil borne fungi that form associations with the roots of plants by forming diverse symbiotic structures. AMF is a potent biofertilizer, nutrient remedifier, eco-friendly and used in agriculture, forestry and horticulture. AMF group has recently been erected to the status of a monophyletic phylum, the Glomeromycota [4]. Arbuscular Mycorhizal Fungus (AMF) also termed as mineral mobilizers are of great importance has been found to be very effective for growth, nutrient especially in phosphorus uptake of plantation crops [3]. The use of AMF as natural fertilizers is found to be beneficial for the improvement of sustainable agriculture in nutrient deficient soil[5,6].

The genus Coffea belongs to the Rubiaceae family, which includes about 100 species [7]. In several developing countries such as Africa, Asia and America, coffee was the significant source of income [8]. However, only two species namely *Coffea arabica* L. and *Coffea robusta* L. are commercially cultivated in Nilgiri District. For many generations, Coffee was a backyard crop and has been part of Indian life. Later coffee was cultivated as a cash crop under forest covers by British invaders [9,10]. The positive effects of AMF on development of coffee seedling in nursery and its benefits after transplantation in fields have received great attention [11,12].

Many studies described the present status of mycorrhizal fungi in coffee in the various ecosystems [10,13-15]. It was reported that the deeper soil layers of coffee plants showed greater numbers of spores and also it may be due to greater numbers of roots at those depths [16-20]. The use of efficient mycorrhizal inocula in coffee nurseries may be a promising technology for the production of healthy and vigorous coffee plantlets, thus increasing survival after field transplantation. Nevertheless, knowledge about the role and benefits of mycorrhizae in this important economic crop is still very sparse. Hence the present study was undertaken to screen and select an efficient AMF for *Coffea arabica* L. and also to study its effect on growth, biomass and nutrition in Coffee plants of Nilgiri District.

MATERIALS AND METHODS

A poly bag trial was conducted at Government Arts College, Ooty. Poly bags of 30 cm height and 10 cm diameter were filled with sterile sand: soil (1:1) at 1 kg/sleeve. The soil had 20 kg of P_2O_5/ha (NH_4F + HCl extractable) with a pH of 5.2 and 5% NPK fertilizers were given at the recommended level (60:60:60 kg NPK/ha) as urea, super phosphate and muriate of potash respectively. Pot cultures of AM fungal inoculums were maintained in onion (*Allium cepa* L.). The inoculum was placed 3 cm below the soil as a thin layer based on the number of infective propagules (*Acaulospora scorbiculata*, *Glomus aggregatum*, *Glomus fasciculatum*, *G. feugianum*, *Glomus mosseae*, *Gigaspora margarita*, and *Scutellospora heterogama* at 1g/sleeve respectively).

Coffee seeds (*Coffea arabica* L.) were selected and germinated in sterilized soil, where the seedlings of high vigorous growth in the nursery; high yield potential; average quality and resistant to drought were raised in sterilized nursery soil bed were transplanted in the poly bags at the rate of one in each poly bag. The plants were maintained for upto 18 months after sown. Growth parameters such as plant height, number of leaves, plant fresh and dry weight for 6 months, 10 months, 14 months and 18 months were recorded after harvest at regular intervals. Percentage root colonization and AM fungal spore number in root zone soils were also determined [21,22]. The nitrogen content of shoot and root was determined by microKjeldhal method as outlined by Jackson [23]. Shoot and root P concentration was estimated by Vanadomolybdate phosphoric yellow colourmethod [23]. Shoot and root K content was estimated by the Flame Photometric method [23]. Total Zn, Ca, Mn, Mg and Fe contents of root and leaves were determined after HCl digestion. Using suitable dilutions of the digested extract, absorbance at 2139, 3247, 2795, 2382 and 2483 A^o were read on a Shimadzu AA630-11 model atomic absorption spectrophotometer to measure the amount of Zn, Ca, Mn, Mg and Fe respectively [24]. Nutrient utilization efficiency was calculated by using the formula of Siddiqui and Glass (1981)[25]. Data were subjected to analysis of variance for a completely random design (CRD) with five replicates. Treatment means were further separated by DMRT for significant difference $P<0.05$ [26].

RESULTS

A poly bag trial was conducted in Government Arts College, Ooty to study the influence of inoculation of different AM fungi on growth and biomass of production of *Coffea arabica* in sand : soil of 1:1 ratio with pH 5.2. Seven dominant native AM fungi isolated from different coffee plantations of Nilgiris were tested for their symbiotic efficiency against coffee plants. The growth, biomass and nutrient of coffee seedlings was influenced by different AM fungi was recorded.

In general, inoculated plants showed increased plant height, number of leaves and biomass compared to control plants without am fungi inoculum. The growth, biomass and nutrient in 6 months, 10 months, 14 months and 18 months of seven different AM fungi inoculated plants were recorded. Among the seven inoculated treatments, the plants of 18 months inoculated with *Glomus mosseae* showed maximum shoot and root length (25.9 cm and 36 cm respectively), next to this the plants inoculated with number of leaves (22 leaves) and fresh and dry biomass of shoot and root (19.55, 6.99, 8.18 and 3.54 g/plant respectively) which differed significantly from all other treatments(Table 1,2,3 and 4)

Table 1. Influence of native AM fungi on plant growth response of *C. arabica* (Mean of five replicates).

S.No	Inoculation treatment	Plant height (cm) in different growth stage							
		6th Month		10th Month		14th Month		18th Month	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
1.	Control	12.1[a]	12[a]	14.0[a]	15[a]	18.6[a]	18[a]	21.5[a]	20[a]
2.	*Acualospora scorbiculata*	12.2[a]	14.4[b]	15.2[b]	17.4[b]	20.1[c]	23.4[cd]	23.2[b]	25.3[b]
3.	*Gigaspora margarita*	12.3[a]	13.9[b]	14.3[a]	17.2[b]	19.5[b]	20[c]	21.7[a]	22.2[b]
4..	*Glomus aggregatum*	14.3[c]	19.5[e]	17.2[c]	22.5[d]	21.4[d]	32[ef]	24.5[c]	34.1[d]
5.	*Glomus fasciculatum*	14.1[c]	17.4[d]	17.1[c]	20.5[d]	21.3[d]	30.5[e]	24.7[c]	32.6[c]
6.	*Glomus geosporum*	12.8[b]	15.2[c]	15.9[b]	18.2[c]	21.2[d]	26.2[d]	23.6[b]	28.3[bc]
7.	*Glomus mosseae*	14.8[d]	27.1[f]	17.4[c]	30.1[e]	22.2[e]	34[f]	25.9[d]	36[d]
8.	*Scutellospora heterogama*	12.1[a]	12[a]	14.1[a]	15[a]	19.5[b]	19[b]	21.5[a]	21[a]
	SEM ±	0.40	1.77	0.51	1.76	0.43	2.21	0.57	2.21
	CD (P=0.05)	0.5	3.2	0.8	3.2	0.5	4.8	0.8	4.8

Means in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

Table 2. Influence of native AM fungi on growth of leaves/plant of *C. arabica* (Mean of five replicates).

S.No	Inoculation treatment	Number of leaves/plant in different growth stage			
		6th month	10th month	14th month	18th month
1.	Control	6[a]	10[a]	12[a]	14[a]
2.	*Acualospora scorbiculata*	8[b]	12[b]	14[b]	18[c]
3.	*Gigaspora margarita*	6[a]	10[a]	12[a]	16[b]
4.	*Glomus aggregatum*	10[c]	14[c]	16[c]	20[d]
5.	*Glomus fasciculatum*	10[c]	14[c]	16[c]	20[d]
6.	*Glomus geosporum*	8[b]	12[b]	14[b]	18[c]
7.	*Glomus mosseae*	12[d]	16[d]	18[d]	22[e]
8.	*Scutellospora heterogama*	6[a]	10[a]	12[a]	16[b]
	SEM ±	0.79	0.79	0.79	0.93
	CD (P=0.05)	1.2	1.2	1.2	1.4

Means in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

Table 3. Influence of native AM fungi on plant biomass of *C. arabica* (Mean of five replicates).

S. No	Plant biomass (g/plant) Inoculation treatment	6th Month				10th Month			
		Shoot		Root		Shoot		Root	
		FW	DW	FW	DW	FW	DW	FW	DW
1.	Control	2.42	1.05	0.33	0.18	2.73	1.71	1.20	0.46
2.	*Acualospora scorbiculata*	3.11	1.40	0.68	0.31	3.36	1.86	1.54	0.61
3.	*Gigaspora margarita*	3.07	1.22	0.53	0.22	3.11	1.79	1.45	0.52
4.	*Glomus aggregatum*	3.74	1.98	1.08	0.44	4.36	2.06	1.77	0.86
5.	*Glomus fasciculatum*	3.15	1.97	0.91	0.42	4.34	2.02	1.62	0.76
6.	*Glomus geosporum*	3.11	1.81	0.76	0.41	4.05	1.94	1.59	0.74
7.	*Glomus mosseae*	4.13	2.05	1.95	0.84	5.16	2.10	1.80	0.93
8.	*Scutellospora heterogama*	3.02	1.11	0.46	0.24	2.84	1.73	1.22	0.49
	SEM ±	0.18	0.15	0.18	0.07	0.30	0.05	0.08	0.06
	CD (P=0.05)	1.5	1.5	1.5	0.12	1.6	0.12	0.12	0.12

FW – Fresh Weight; DW – Dry Weight.

Table : 4. Influence of native AM fungi on plant biomass of *C. arabica* (Mean of five replicates).

S. No	Plant biomass (g/plant) Inoculation treatment	14th Month				18th Month			
		Shoot		Root		Shoot		Root	
		FW	DW	FW	DW	FW	DW	FW	DW
1.	Control	4.43	2.01	1.61	1.12	10.65	3.55	5.01	2.35
2.	*Acualospora scorbiculata*	6.91	2.67	2.13	1.63	14.47	3.93	5.42	3.11
3.	*Gigaspora margarita*	4.96	2.56	2.02	1.55	14.21	3.87	5.13	2.84
4.	*Glomus aggregatum*	8.27	3.82	2.41	1.86	17.24	4.70	7.99	3.29
5.	*Glomus fasciculatum*	7.33	3.10	2.38	1.84	16.97	4.08	6.93	3.20
6.	*Glomus geosporum*	6.16	2.96	2.27	1.73	16.08	3.96	6.23	3.11
7.	*Glomus mosseae*	8.61	3.99	3.43	1.98	19.55	6.99	8.18	3.54
8.	*Scutellospora heterogama*	4.45	2.14	1.83	1.17	13.35	3.77	5.01	2.57
	SEM ±	0.59	0.25	0.19	0.11	0.97	0.39	0.47	0.14
	CD (P=0.05)	1.8	1.6	1.5	1.3	2.4	1.6	1.8	1.5

FW – Fresh Weight; DW – Dry Weight.

The plant growth and biomass was maximum in plants inoculated with *Glomus mosseae* followed by those inoculated with *Glomus aggregatum* and *Glomus fasciculatum*. Both shoot and root biomass was maximum in plants inoculated with *Glomus mosseae* followed by *G. aggregatum* (Table 1,2, 3 and 4).

The lowest plant growth, biomass and nutrient was noticed in uninoculated control plants. Mycorrhizal inoculation resulted in significant increase in shoot and root N (26.25 mg/plant), P (85.59 mg/plant) and K (0.32 mg/plant) in 18th month coffee plant (Table 5). Highest N. P and K content was recorded in plants inoculated with *Glomus mosseae* which differed significantly from other treatments, followed by the plants inoculated by *G. aggregatum*.

Table 5.: Influence of native AM fungi on macro and micro nutrient content in 18th month leaves of *C. arabica* (Mean of five replicates).

S.No.	Inoculation treatment	Macro nutrient content (mg/plant)			Micro nutrient content (ppm/plant)			
		N	P	K	Zn	Ca	Mg	Fe
1.	Control	20.65^{a}	40.10^{a}	0.212^{a}	0.50^{a}	0.26^{a}	0.21^{a}	0.34^{a}
2.	*A. scorbiculata*	22.28^{c}	68.12^{c}	0.240^{c}	0.52^{b}	0.29^{b}	0.31^{c}	0.53^{c}
3.	*Gigaspora margarita*	22.25^{c}	51.10^{b}	0.232^{b}	0.52^{b}	0.28^{b}	0.30^{c}	0.52^{c}
4.	*Glomus aggregatum*	24.25^{e}	75.40^{e}	0.262^{d}	0.61^{d}	0.32^{d}	0.40^{d}	0.62^{d}
5.	*G. fasciculatum*	23.80^{d}	71.90^{d}	0.242^{c}	0.62^{d}	0.31^{c}	0.49^{d}	0.79^{e}
6.	*G. geosporum*	23.56^{d}	71.16^{d}	0.245^{cd}	0.59^{c}	0.30^{c}	0.33^{c}	0.55^{c}
7.	*G. mosseae*	26.25^{f}	85.59^{f}	0.320^{e}	0.64^{d}	0.34^{d}	0.52^{e}	0.85^{f}
8.	*S. heterogama*	21.19^{b}	50.07^{b}	0.202^{a}	0.51^{a}	0.27^{a}	0.26^{b}	0.44^{b}
	SEM ±	0.64	5.44	0.01	0.02	0.01	0.04	0.06
	CD (P=0.05)	4.6	18.2	1.2	1.2	1.2	1.3	1.3

Means in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test

Inoculation with AM fungi greatly influenced the uptake of micronutrients such as Zn (0.64 mg/plant), Ca (0.34mg/plant), Mg (0.52 mg/plant) and Fe (0.85mg/plant) (Table 3). Least growth, biomass and nutrient were observed in plants inoculated with *Scutellospora heterogama.* Lowest number of spores was recorded in the root zone of plants inoculated with *Scutellospora heterogama* (Table 1,2 and 3).

Mycorrhizal root colonization was also maximum in plants inoculated with *Glomus mosseae* followed by plants inoculated with *G. aggregatum*. The results of the present study clearly brought out that *Glomus mosseae* is the efficient AM fungi selected from screening, performed better in improving plant growth and nutrition.

DISCUSSION

Though AM fungi are not host specific, recent studies indicate host preference by AM endophyte [27,28]. Chiramel, *et al*. (2006)[29] and Rajeshkumar, *et al*. (2008)[28] stressed the need for selecting efficient native AM fungi for plant species. The present study conducted with an intention of selecting and screening for efficient AM fungi with indigenous origin for coffee plant has also resulted in varied plant responses viz, growth, biomass and nutrient to different AM fungi. It is well known that improved nutritional status of a plant also improve plant growth [12].

Coffee seeds inoculated with Mycorrhiza showed diverse variations than the coffee seeds without mycorrhizal inoculation for most of the growth parameters. Enhanced plant growth and biomass production due to inoculation with native AM fungi had been reported earlier in other plants [29-32]. The response of *coffea arabica*, preinoculated with different AM fungal species was found to be varied. AMF inoculation resulted in a considerable increase in height, biomass and nutrient content of Coffee plants. Selvaraj, *et al*., (2009)[32] reported that the response of plants to inoculation with different AM fungi was found to vary. Seedlings grown in the presence of *G. mosseae* showed an increase in shoot and root length, number of leaves as well as in fresh and dry weights, followed by those grown in the presence of *G. aggregatum* than the other treatments. Anusuya and Senthil Kumar (2003)[33] reported the inoculation of *Glomus mosseae* received the highest AM colonization and spore number.

The uptakes of N, P, K recorded with Glomus species, established that heritable factors play important role in translocation of mineral elements [34,35]. The level of increase in plant nutrient content varied among the fungi. The nutritional status viz., nitrogen, phosphorus, potassium, zinc, calcium, magnesium and iron content of coffee plants reported was also significantly higher in plants raised in soil inoculated with AM fungi. In earlier studies it was reported that he different AM fungal strains differ in the level of increase in nutrient uptake, biomass and plant growth [36].

CONCLUSION

This study clearly indicates that a proficient biological response of coffee plants towards different AM fungi, with *G. mosseae* conferring superior benefits compared to all other AMF inoculated plants. The results clearly indicate that the use of efficient mycorrhizal inocula in coffee plants at nurseries may be a recommended for the production of healthy coffee plantlets, and also increase survival after field transplantation.AM fungal inoculation can also significantly reduce synthetic fertilizer in seedling production. The best suited mycorrhiza can be mass cultured *in-vitro* and utilized commercially.

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