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Studies on The Status of Arbuscular Mycorrhizal Fungi on The Fodder Crop *Sorghum bicolor* (L.) Moench

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Abstrak: *Sorghum bicolor* (L.) Moench, biasa dikenali sebagai *maize grass* adalah daripada Famili Poaceae. Ia digunakan sebagai makanan ternakan dan ditanam secara komersil di Daerah Tiruchirappalli di Tamil Nadu, Selatan India. Empat kawasan berbeza telah dipilih untuk kajian ini. Jenis tanah empat kawasan tersebut melingkungi *red sandy*, *red brown sandy* dan *red brown sandy clay*. Walaupun kolonisasi Arbuscular Mycorrhizae (AM) di akar *S. bicolor* adalah positif di keempat-empat kawasan kajian, spesies AM fungi yang berbeza mengkolonikan akar. Peratus kolonisasi akar ialah daripada 52.0% hingga 94.5%. Sebanyak 18 fungi AM telah diasingkan daripada tanah rhizosphere *S. bicolor*, yang mana hanya empat ditemui mengkoloni di akar. Bilangan spora terkumpul ialah 122 hingga 582 per 100 gm tanah. *Glomus aggregatum*, *G. etunicatum* dan *Acaulospora bireticulata* merupakan spesies dominan dan telah menunjukkan 100% frekuensi di kesemua tempat kajian.

Kata Kunci: *Sorghum bicolor*, *Glomus aggregatum*, Fungi AM, Kolonisasi, Spora, Sporocarp

Abstract: *Sorghum bicolor* (L.) Moench, commonly called maize grass, belongs to the family Poaceae. It is used as a fodder grass and is commercially cultivated in the Tiruchirappalli district of Tamil Nadu, South India. Four different localities of the Tiruchirappalli district were selected for the present investigation. The soil types of the four localities ranged from red sandy, red brown sandy to red brown sandy clay. Although the colonisation of Arbuscular Mycorrhizae (AM) in the roots of *S. bicolor* was positive in all the four study localities, the species of AM fungi colonising the roots varied. The percentage of root colonisation ranged from 52.0% to 94.5%. A total of 18 AM fungal species were isolated from the rhizosphere soils of the *S. bicolor*, of which only 4 were found to be colonised in the roots. The total spore counts varied between 122 and 582 per 100 gm of soil. *Glomus aggregatum, G. etunicatum* and *Acaulospora bireticulata* were the dominant forms and these 3 species showed 100% frequency in all the study areas.

Keywords: *Sorghum bicolor, Glomus aggregatum,* AM Fungi, Colonisation, Spore, Sporocarps

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi occur in most vegetation types and constitute an important component of the tropical soil microflora (Smith & Read 1997; Cardoso & Kuyper 2006). They have been shown to increase growth and yield of

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plants. They are also found to have positive effects on plants, such as increased resistance to pathogens (Hampp *et al.* 1999), drought (Davis *et al.* 1992; Nilsen *et al.* 1998; Shi *et al.* 2002) or heavy metal stress (Kothari *et al.* 1991; Van Tichelen *et al.* 1996; Blaudez *et al.* 2000). They have been identified to play roles in both nutrient mobilisation and nutrient cycling. Their distribution and diversity in tropical ecosystems elsewhere appears to be receiving increased attention (Picone 2000; Husband *et al*. 2002; Lovelock *et al*. 2003; Lovelock & Ewel 2005). There were many reports on the association of AM fungi in several cultivated crops (Kurle & Pfleger 1994; Lakshman & Raghavendra 1995). However, the number of studies on the influence of AM fungal status on fodder crop plants is limited in India particularly in Tamil Nadu. *S. bicolor* is of African origin, belonging to the family Poaceae, and is grown in most parts of the world. In India, *S. bicolor* and its varieties are mostly grown in the peninsular region. The grains are rarely for human consumption; instead they are used as a fodder grass and are commercially cultivated in the Tiruchirappalli district. *S. bicolor* needs more phosphorus for its growth and leaf yield. AM fungi are known to play an important role in the growth and development of fodder crop plants, as they help increase the uptake of diffusion limited nutrients (Palipane & Bandara 1985). Inoculation of AM fungi improves the physiological conditions of fodder crops. The present investigation was undertaken to study the species richness, root colonisation and diversity of AM fungi associated with the fodder crop, *S. bicolor* (L.) Moench, from four different localities of the Tiruchirappalli district of Tamil Nadu.

MATERIALS AND METHODS

Four different localities, namely Lalgudi, Thuraiyur, Manapparai and Thottiyam of the Tiruchirappalli district of Tamil Nadu, South India were selected in order to study the AM fungal status of *S. bicolor* (Fig. 1). From each study site, roots and rhizosphere soil samples of 3 to 5 healthy plants were collected at 30 cm of soil depth. Soil samples collected from each site were mixed thoroughly, and a portion of the soil was analysed for various physico-chemical characteristics. Soil pH and electrical conductivity were determined with a digital pH and EC meter (Elico Instruments, India), organic carbon by following the method described by Walkley and Black (1934), total nitrogen (N) and potassium (K) according to the method described by Sankaram (1966), phosphorus (P) was measured by the method of Olsen *et al.* (1954) and micronutrients were determined by Lindsay and Norwell method (1978).

Wet sieving and the decanting method of Gerdemann and Nicolson (1963) were carried out for spore isolation. The total spore count was calculated by counting the number of spores. Based on the microscopic characters, the AM fungal spores were identified. For identification and nomenclatures, synoptic keys of Morton and Benny (1990), Schenck and Perez (1990) and Walker and Trappe (1993) were followed, whereas for species code, Perez and Shenck (1990) was followed. Classification was based on size, shape, colour, surface, structure, general nature of the contents, hyphal attachment and wall details.

Photomicrographs were taken using a Nikon fluorescent microscope (E400, Japan).

For the assessment and quantification of AM fungi, 1-cm-long root segments were washed thoroughly in distilled water and then placed in 10% KOH and heated to 90^{o}C for 15–30 min. They were then washed in distilled water and immersed in alkaline 3% H_2O_2 for 5–10 min. The samples were washed again in distilled water and acidified with 5 N HCl for 2–3 min. The root segments were stained with 0.05% trypan blue in lactophenol for 15–30 min (Phillips & Hayman 1970). Stained root segments were examined under a compound microscope at different magnifications for fungal structures. All AM fungal structures (hyphae, arbuscules and vesicles) that formed in the roots were counted, and the percentages of root colonisation of the segments were estimated by using the formula presented below (Krishna & Dart 1984).

$$\text{Percentage of root colonization} = \frac{\text{Total no. of AM positive segments}}{\text{Total no. of root segments observed}} \times 100$$

Pearson product movement correlation (Sokal & Rohlf 1973) was followed to examine the percentage of AM colonisation and spore density in relation to physico-chemical characteristics of the root zone soil of *S. bicolor*. Estimation was done in triplicates and data were averaged.

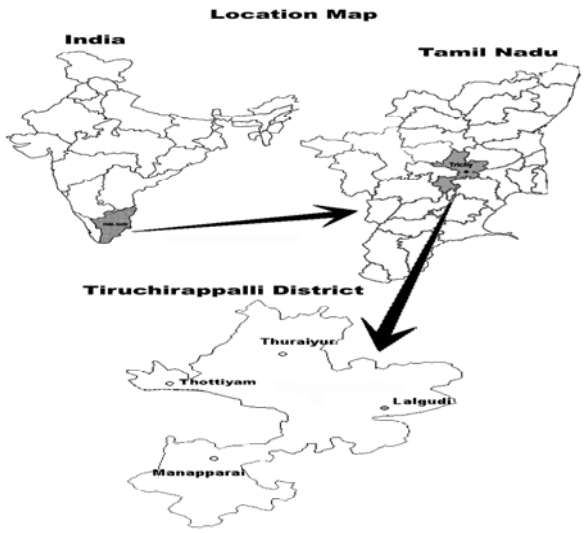


Figure 1: Study sites of Tiruchirappalli District

RESULTS AND DISCUSSION

The physico chemical characteristics of soil samples from all the four study sites are given in Tables 1 and 2. The highest pH value was reported in the soil sample from Thuraiyur (8.4), and the lowest pH value was reported in the sample from Thottiyam (7.4). In Lalgudi and Manapparai, the pH values were 8.3 and 7.7 respectively. The electrical conductivity was very low in the samples from all of the study sites (0.13 to 0.22). The soil sample from Lalgudi showed the high percentage of N, P, K, organic carbon and micronutrients. The lowest amounts of N, P, K and micronutrients were observed in the Thottiyam soil samples. Edaphic characteristics such as soil type (Joshi & Singh 1995; Lekberg *et al.* 2007), soil depth (Zajicek *et al.* 1986; An *et al.* 1990, Oehl *et al.* 2005), soil pH (Ouimet *et al.* 1996; Sidhu & Behl 1997) and soil fertility (Abbott & Robson 1982) have been reported to influence AM sporulation.

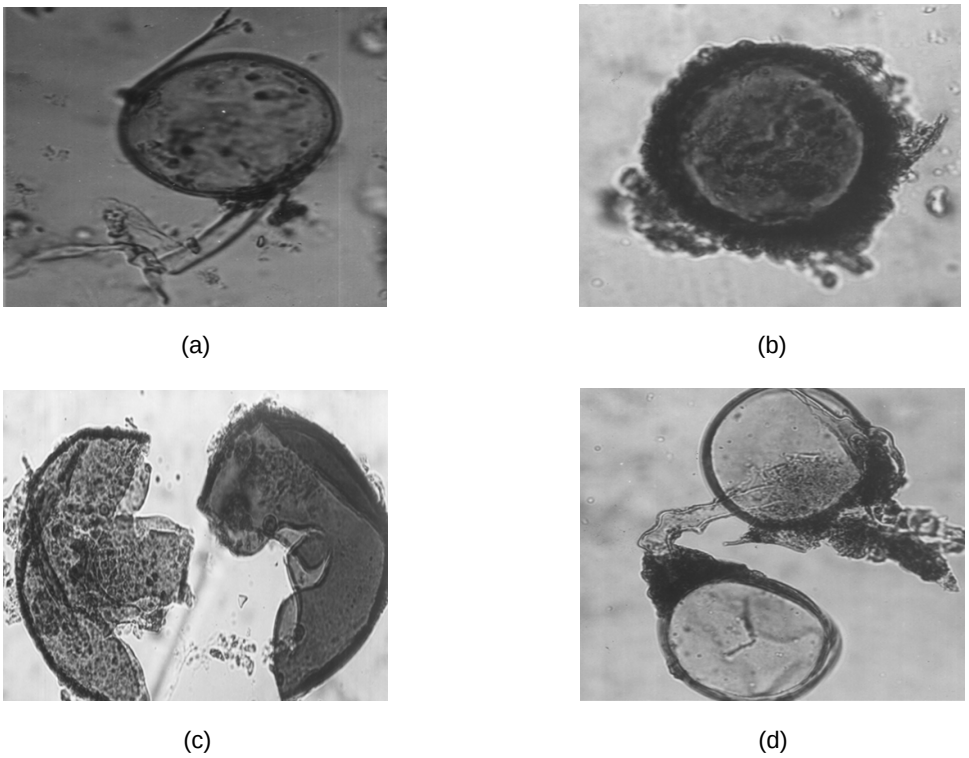
Table 1: Physico-chemical characteristics of soil samples of study sites.

S.No	Study sites	Soils texture	Colour	pH	Ecdsm^{-1}	Organic carbon (%)
1	Lalgudi	Sandy	Brown	8.30	0.13	0.25
2	Thuraiyur	Sandy	Red Brown	8.49	0.22	0.19
3	Manapparai	Sandy	Brown	7.70	0.11	0.21
4	Thottiyam	Sandy Clay	Red Brown	7.40	0.33	0.23

Table 2: Amount of nutrients present in the soil samples of study sites.

	Macronutrients (Kg/ac)			Micronutrients ion (ppm)			
Study sites	N	P	K	Zn	Cu	Fe	Mn
Lalgudi	92.6	5.5	85.0	0.96	0.40	4.15	9.01
Thuraiyur	88.21	4.5	77.5	0.93	0.36	3.23	8.83
Manapparai	80.4	3.5	72.0	0.28	0.30	2.70	8.20
Thottiyam	77.0	2.0	65.0	0.25	0.20	0.26	6.65

A total of 18 AM fungal species belonging to 4 genera (Fig. 2) were detected from the rhizosphere soils of the *S. bicolor* from the 4 study sites (Table 3). Eight species belonged to the genus *Glomus*, seven species to the genus *Acaulospora*, two species of the genus *Sclerocystis* and one species belonging to the genus *Gigaspora*.



(a) (b)

(c) (d)

Figure 2: Different genera of AM fungi obtained from the rhizosphere soils of *S. bicolor. (a) Glomus microcarpum; (b) Acaulospora spinosa; (c) Gigaspora sp.; (d) Sclerocystis sinuosa*

These results are similar to findings from studies in other tropical areas, such as in Central America (Husband *et al.* 2002) and East Africa (Mathimaran *et al.* 2007). Although 18 AM fungal species were isolated from the rhizosphere soil, only 5 species were found to be colonising the roots of *S. bicolor*. They are *G. aggregatum, G. etunicatum*, *G. microcarpum, A. bireteculata* and *A. spinosa.* These AM fungal genera were invariably present in all the four sampling areas with *Glomus* being the predominant species. Wang *et al.* (2008) has reported that the species of *Glomus* are the most widespread and abundant, followed by *Acaulospora* and *Scutellospora* in agricultural soils of the Sichuan Province of mainland China. Similar to the agricultural soils, a study of various natural ecosystems in the Sichuan province revealed more species of *Glomus* than that of *Acaulospora* (Zhang *et al.* 2003). Earlier studies recorded a comparatively smaller number of AM fungal species in Indian soil, with the predominant species being *Glomus* (Ammani *et al*. 1986; Janardhanan *et al*. 1997). Spore density and species richness positively correlated with soil organic carbon and soil pH (Johnson *et al.* 1991; Mohammad *et al*. 2003; Tchabi *et al.* 2008). In addition, Menéndez *et al.* (2001) demonstrated that tillage and cereal monoculture negatively affected the richness of AM fungal species.

Table 3: Various AM fungal species and their frequencies of occurrences in the rhizosphere of *S. bicolor* at four different localities of the Tiruchirappalli district.

S.No		AM fungi identified	Study localities				Frequency (%)
			Sorghum bicolor				
			S1	S2	S3	S4	
I	1.	*Acaulospora spinosa*	+	+	+	+	100
	2.	*Acaulospora laevis*	–	+	+	–	50
	3.	*Acaulospora elegans*	+	+	+	–	75
	4.	*Acaulospora scrobiculata*	+	+	–	–	50
	5.	*Acaulospora bireticulata*	+	+	+	+	100
	6.	*Acaulospora delegate*	+	+	–	–	50
	7.	*Acaulospora trappei*	+	+	–	+	75
II	8.	*Glomus etunicatum*	+	+	+	+	100
	9.	*Glomus occulatum*	+	+	–	+	75
	10.	*Glomus microcarpum*	+	+	+	+	100
	11.	*Glomus ambisporum*	+	+	–	–	50
	12.	*Glomus mossae*	+	+	+	–	75
	13.	*Glomus geosporum*	+	+	+	–	75
	14.	*Glomus* sp	+	–	+	–	50
	15.	*Glomus aggregatum*	+	+	+	+	100
III	16.	*Gigaspora* sp	+	–	+	–	50
IV	17.	*Sclerocystis dussi*	+	+	+	–	75
	18.	*Sclerocystis sinuosa*	+	–	–	–	25

Notes: Lalgudi (S1) Thuraiyur (S2) Manapparai (S3) Thottiyam (S4)
: + = presence of species
– = absence of species

The total AM fungal spore density varied between 120 and 582 per 100 gm of root zone soil (Table 4). Furthermore, *G. aggregatum, G. etunicatum, G. microcarpum, A. spinosa* and *A. bireticulata* showed 100% frequency in all of the four study sites (Table 3). There is an apparent relationship between soil properties and the occurrence of AM fungal species at particular sites. The lowest number of fungal spores recorded in the root zone soil of *S. bicolor* was at Thottiyam while the highest number of fungal spores was at the Lalgudi study site. There was a certain degree of specificity among the different AM fungal spores in their association with root zone soils of *Sorghum* plants at the different localities.

Table 4: Percentage of colonization, spore density and species richness of AM fungi associated with *S. bicolor.*

Study Sites	Root colonization (%)	Vesicles (%)	Arbuscules (%)	Total number of AM fungal spores / 100g soil	Positive for AM fungi in the roots
Lalgudi	94.5	68.6	14.5	582 ± 6.4	*A. bireticulata*
Thuraiyur	82.6	52.5	13.2	490 ± 5.8	*G. etunicatum*
Manapparai	52.5	50.1	8.5	125 ± 3.2	*G. microcarpum*
Thottiyam	52.0	48.0	8.4	120 ± 1.8	*G. aggregatum*

In the present study, the percentage of colonisation of AM fungi in the roots of *S. bicolor* varied between each locality. The extent of AM fungal colonisation in the roots of *S. bicolor* plants ranged between 52.0%–94.5% (Table 4). There was a certain degree of specificity among the different AM fungal species in the four different localities with *S. bicolor*. The percentage of root colonisation was over 80% in *S. bicolor* plants at two of the localities, and below 55% in the other two localities. Saif *et al.* (1977) reported 52%–92% of AM fungal colonisation in vegetable crops while Janardhanan *et al*. (1997) reported 50%–70% in salt marsh plants. The variations in root colonisation may be due to the exudation of toxic metabolites resulting in substances in proximity to the roots which attracts the AM fungi, such as production of easily oxidisable compounds resulting in increased colonisation physiological difference between species (Koske 1987). Although these factors could also influence the process of colonisation, in the present investigation, *S. bicolor* exhibited more than 80% colonisation in two of the study localities. Such a high degree of colonisation could be due to the fact that the study areas were essentially phosphate-deficient, which has been shown to induce high levels of AM fungal species (Selvaraj *et al*. 1994; Muthukumar & Udaiyan 2000).

CONCLUSION

The present investigation demonstrates that colonisation of the AM fungal species in the roots of *S. bicolor* positively occurred in all four of the study localities. Eighteen fungal species were isolated from the rhizosphere soils. The percentage of root colonisation was also very high. Further research must be undertaken to select an efficient strain of AM fungi for the fodder crop *S. bicolor.*

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