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Symbiotic response of sesame (*Sesamum indicum* L.) to different indigenous arbuscular mycorrhizal fungi (AMF) from rice fallows of Kerala, India

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Symbiotic response of sesame (*Sesamum indicum* L.) to five indigenous arbuscular mycorrhizal fungal isolates from the rice fallows of Kerala was studied in pots under glasshouse condition. The isolates varied in their capacity in enhancing the growth characters, yield components and root colonization by AMF during different stages of growth. Among the isolates tested, *G. dimorphicum* was found to be the efficient endophyte in sesame in enhancing most of the parameters tested.

Key words: symbiotic response, sesame, indigenous AMF.

Introduction

Sesame (*Sesamum indicum* L., Fam. Pedaliaceae) is cultivated in tropical, subtropical and southern temperate regions of the world for its seed which is a rich source of edible oil. Studies have shown that the oil lowers cholesterol levels and hypertension in humans (Lemcke-Norojarvi *et al*., 2001; Sankar *et al*., 2004) and reduces the incidence of certain cancers (Hibasami *et al*., 2000; Miyahara *et al*., 2001). The observed effects have been attributed to the chemical composition of the oil characterized by a low level of saturated fatty acids and presence of antioxidants. The grains of sesame are eaten as fried, mixed with sugar or jaggery in the form of sweet meats. Oil cake of sesame is a rich source of protein, carbohydrate and mineral nutrients such as calcium and phosphorus and is eaten avidly by humans.

India ranks first both in the area and production of sesame in the world with an annual area of 2.07 million hectares and total production of 0.76 million tonnes (Anonymous, 2009). In south India the crop is mainly cultivated

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as a summer crop in low land rice fallows poor in nutrients. The poor nutrient availability in sesame soils coupled with a sparse development of root system makes the plant depend greatly on root invading endosymbionts like arbuscular mycorrhizal fungi (AMF) for better growth (Chiramel *et al*., 2006) and enhanced acquisition of nutrients (Gahoonia *et al*., 2005).

High native AMF soil populations during fallow period and better mycorrhizal colonization of roots in upland rice-pulse (*Cajanus cajan* L. and *Arachis hypogea* L.) intercropping systems have previously been demonstrated (Rana *et al*., 2002). Such enhancement of native AMF activities, in terms of root colonization and growth promotion of succeeding crops, by pre-cropping with mycorrhizal crops has been frequently reported (Harinikumar and Bagyaraj, 2005; Grant *et al*., 2009).

AMF inoculum developed from native sources is considered to be more efficient (Oliveira *et al*., 2005), cost effective, adapted to the target ecology, and to have less negative ecological consequences in terms of invasive species introduction as unintended contaminants (Schwartz *et al*., 2006). The objective of the study was to assess the response of indigenous AMF isolates from rice fallows on sesame which is cultivated as a succeeding crop after rice.

Materials and methods

Sesame seeds (var. Tilatara) were sown in plastic pots (3 cm diameter) filled with 4 kg sandy (Entisol) soil (pH 5.6, organic carbon 11.7 g kg^{-1} soil and available P 7.5 µg g^{-1} soil). The soil was sterilized by autoclaving for 2 h prior to sowing the seeds. Five AMF isolates procured from rice fallows of Kerala (Table 1) were multiplied using sterilized sand-soil mix (1:1 v/v) as the substrate and *Sorghum* as the host. After six weeks of growth, shoots of host plants were severed and the substrate containing hyphae, spores and root bits was air dried and used as inoculum. Fifty ml of AM inoculum containing chlamydospores (approximately 200 spores) was placed 2 cm below the soil surface in all pots except control prior to sowing the seeds. Control plants received 50 ml of AM inoculum washing that had been passed through a Whatman 40 filter paper. After emergence of the seedlings, the number of plants was thinned to one per pot and was given need-based irrigation with equal quantities of deionized water. The experiment was setup in a glass house with five mycorrhizal treatments and one non-mycorrhizal control each replicated nine times. The plants were harvested at 25 days interval up to 75 days of growth to measure AM parameters. The sub-samples of root were stored in 50% alcohol till further processing.

Growth and yield components were measured by standard procedures. Leaf area was calculated using a leaf area meter (LI-COR LI 3100). Dry

biomass of plant was recorded after drying the plant at 60^0C to constant dry weight in a hot air oven. Fine roots were stained using 0.02 % trypan blue as described by Phillips and Hayman (1970) and the per cent root colonized was estimated adopting the grid-line intersect method (Giovanetti and Mosse, 1980). The results were subjected to two way analysis of variance (ANOVA) suitable for CRD for the test of significance and the means were separated using Tukey's Honestly Significant Difference (HSD) test using SYSTAT 9.

Table 1. Source of indigenous AMF inoculum for sesame

Location	**Soil type and Taxonomy**	**Culture** No.	**AMF**
Mavelikara	Greyish Onattukara (Entisol)	SDAM 28	*Acaulospora delicata* Walker, Pfeiffer & Bloss
Ramapuram	Greyish Onattukara (Entisol)	SDAM 27	*Acaulospora lacunosa* Morton
Kayamkulam	Greyish Onattukara (Entisol)	SDAM 24	*Glomus dimorphicum* Boyetchko & Tewari
Panthalloor	Brown Hydromorphic (Alfisol)	SDAM 15	*Glomus versiformae* (Karsten) Berch
Thalappara	Brown Hydromorphic (Alfisol)	SDAM 37	*Scutellospora nigra* (Reddeard) Walker & Sanders

Results and discussions

Growth characters like rootlet number, shoot length, leaf number and leaf area were significantly influenced by inoculation with indigenous AMF (Table 2 and 3). However, the isolates varied in their capacity in enhancing these parameters. Mean rootlet number and shoot length were more in *A. lacunosa* inoculated plants while *G. dimorphicum* inoculated plants had the highest leaf number and leaf area. Further, morphological characters showed significant difference with DAS. The M×D interaction was also significant for growth characters except rootlet number/plant. The influence of AMF on increased plant growth is perhaps due to increased P uptake which might have caused cell multiplication and elongation (Sengupta and Chaudhari, 1995). However, there existed variation in their effectiveness, which could be due to the differences in the uptake of P and other nutrient elements in plants inoculated with different fungi (Rakshit and Bhadoria, 2008). This differences may be attributed to (1) differences among AMF for hyphal spread and density away from roots (Bürkert and Robson, 1994), (2) ability of AMF to increase nutrient availability, especially P, in soil through enhanced phosphatase/phytase activity

(Dinkelaker and Marschner, 1992; Khalil *et al*., 1994) and/or excretion of solubilizing materials such as ethylene (Ishii *et al*., 1996), flavonoides (Ishii *et al*., 1997), and growth regulating compounds (Danneberg *et al*., 1992; Thiagarajan and Ahmad, 1994), and (3) ability of AMF to change rhizosphere soil pH (Gianinazzi-Pearson and Azcón–Aguilar 1991; Li *et al*., 1991). Similar differences in the performance of different species of AMF as in the present study have been reported in crops such as *Paspalum notatum* (Mosse, 1972) and sugarcane (Reddy *et al*., 2004).

Table 2. Effect of inoculation with indigenous AMF on rootlet number and shoot length of sesame

Treatment	Rootlet no. plant^{-1}			Shoot length (cm)		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
Uninoculated	9.33^{g}	30.00^{bcdef}	32.66^{abcde}	9.03^{d}	25.70^{c}	34.76^{abc}
A. delicata	19.00^{defg}	42.00^{ab}	33.33^{abcde}	13.20^{d}	27.33^{bc}	36.83^{ab}
A. lacunosa	22.00^{cdefg}	43.00^{ab}	46.00^{ab}	11.16^{d}	35.56^{abc}	39.26^{a}
G. dimorphicum	14.66^{fg}	50.00^{a}	34.33^{abcd}	12.66^{d}	38.83^{a}	37.00^{ab}
G. versiformae	16.33^{efg}	35.00^{abcd}	38.66^{abc}	13.66^{d}	31.13^{abc}	32.33^{abc}
S. nigra	17.33^{defg}	39.33^{abc}	37.66^{abc}	8.20^{d}	32.50^{abc}	38.16^{ab}
ANOVA: M (mycorrhizal treatment), D (days after sowing), M×D (interaction)						
M		***			**	
D		***			***	
M×D		NS			*	

Different letters in a column indicate significant differences ($p \leq 0.05$) using Tukey's HSD Test
*p≤0.05 **p≤0.01 ***p≤0.001 NS not significant

Table 3. Effect of inoculation with indigenous AMF on leaf number and leaf area of sesame

Treatment	Leaf no. plant^{-1}			Leaf area (cm^2 plant^{-1})		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
Uninoculated	4.06^{f}	12.00^{cde}	13.33^{cd}	10.86^{g}	47.28^{defg}	61.72^{def}
A. delicata	6.00^{ef}	11.67^{cde}	16.00^{bc}	18.98^{fg}	52.00^{defg}	76.80^{cde}
A. lacunosa	6.00^{ef}	16.00^{bc}	15.33^{bc}	28.31^{efg}	82.00^{cd}	142.60^{ab}
G. dimorphicum	6.33^{def}	13.33^{cd}	25.00^{a}	24.32^{fg}	67.93^{cdef}	173.00^{a}
G. versiformae	6.67^{def}	11.33^{cde}	22.00^{ab}	22.93^{fg}	58.83^{defg}	133.00^{ab}
S. nigra	6.00^{ef}	14.67^{c}	23.33^{a}	19.30^{fg}	65.30^{cdef}	114.16^{bc}
ANOVA: M (mycorrhizal treatment), D (days after sowing), M×D (interaction)						
M		***			***	
D		***			***	
M×D		***			***	

Different letters in a column indicate significant differences ($p \leq 0.05$) using Tukey's HSD Test
***p≤0.001

Inoculation with indigenous AMF markedly increased biomass in sesame plants over uninoculated control (Table 4). In inoculated treatments, the fresh and dry weight ranged from 0.30 to 4.20 and 0.08 to 1.02 g respectively during the growth stages. Plant biomass varied significantly with plant age which reached a maximum increase at 75 DAS. M×D interaction was significant only in the case of plant biomass (dry). Declerck *et al*. (1995) investigated the growth response of micro-propagated banana plants to AM inoculation. The authors report that inoculation with *Glomus mosseae* and *Glomus geosporum* resulted in significantly higher shoot and root dry weights as compared to the control plants. Fortuna *et al*. (1992) observed large differences in the fresh and dry mass between inoculated and un-inoculated plum plants as a result of differences in the growth behavior of the plants. According to Branzanti *et al*. (1992) and Azcón-Aguilar and Barea (1997) mycorhiza enhances growth of plantlets of selected species and causes earlier resumption in shoot apical growth. Vestberg (1992) found that only 3 of 6 fungal strains tested with 10 strawberry cultivars were highly efficient with regard to significant growth improvements.

Yield components such as pod number, pod weight and seed number were significantly enhanced in treatments inoculated with indigenous AMF (Table 5 and 6). However, in the case of seed weight, the increase has not reached a significant level. Among the various AMF tested, inoculation with *G. dimorphicum* markedly increased the yield components in sesame. Since the reproductive stage of the crop starts at 50 DAS, the yield components could be gauged only at 75 DAS. Increased yield consequential to AM inoculation has been reported in crops such as coffee (Siqueira *et al*., 1998), barley (Khaliq and Sanders, 2000) and *Trifolium alexandrium* (Shokri and Maadi, 2009).

Mycorrhizal colonization (*%F*) was significantly ($p < 0.001$) higher in all the treatments inoculated with indigenous AMF at all stages of growth (Fig. 1). Different isolates colonized sesame roots to different levels ranging from 10 to 94.3%. The highest mean value for *%F* was observed in *A. delicata* inoculated plants (73.66). Irrespective of AM inoculant, the *% F* was highest at mid vegetative growth (50 DAS). M×D interaction was also significant ($p<0.001$) for *% F*. It was observed that the beneficial effect from a particular species of AMF was not always correlated with the extent of root infection.

Table 4. Effect of inoculation with indigenous AMF on biomass production of sesame

Treatment	Plant biomass (g)					
	Fresh			Dry		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
Uninoculated	0.23^{e}	2.20^{bcd}	2.61^{abc}	0.08^{d}	0.39^{c}	0.38^{c}
A. delicata	0.74^{cde}	2.20^{bcd}	3.40^{ab}	0.09^{d}	0.41^{c}	0.63^{b}
A. lacunosa	0.70^{de}	3.40^{ab}	4.20^{a}	0.13^{d}	0.51^{bc}	1.02^{a}
G. dimorphicum	0.60^{de}	3.64^{ab}	3.02^{ab}	0.08^{d}	0.60^{bc}	0.60^{bc}
G. versiformae	0.83^{cde}	2.30^{bcd}	2.80^{ab}	0.09^{d}	0.41^{c}	0.50^{bc}
S. nigra	0.30^{e}	2.20^{bcd}	3.51^{ab}	0.08^{d}	0.50^{bc}	1.01^{a}
ANOVA: M (mycorrhizal treatment), D (days after sowing), M×D (interaction)						
M		**			***	
D		***			***	
M×D		NS			***	

Different letters in a column indicate significant differences ($p \leq 0.05$) using Tukey's HSD Test
**p≤0.01 ***p≤0.001 NS not significant

Table 5. Effect of inoculation with indigenous AMF on pod number and pod weight of sesame

Treatment	Pod no. plant^{-1}			Pod wt. (g plant^{-1})		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
Uninoculated	0.00^{d}	0.00^{d}	1.00^{c}	0.00^{c}	0.00^{c}	0.55^{b}
A. delicata	0.00^{d}	0.00^{d}	2.70^{ab}	0.00^{c}	0.00^{c}	0.61^{b}
A. lacunosa	0.00^{d}	0.00^{d}	2.33^{b}	0.00^{c}	0.00^{c}	0.60^{b}
G. dimorphicum	0.00^{d}	0.00^{d}	3.33^{a}	0.00^{c}	0.00^{c}	1.04^{a}
G. versiformae	0.00^{d}	0.00^{d}	2.70^{ab}	0.00^{c}	0.00^{c}	0.80^{ab}
S. nigra	0.00^{d}	0.00^{d}	2.00^{b}	0.00^{c}	0.00^{c}	0.74^{ab}
ANOVA: M (mycorrhizal treatment), D (days after sowing), M×D (interaction)						
M		***			*	
D		***			***	
M×D		***			**	

Different letters in a column indicate significant differences ($p \leq 0.05$) using Tukey's HSD Test
*p≤0.05 **p≤0.01 ***p≤0.001.

Table 6. Effect of inoculation with indigenous AMF on seed number and seed weight of sesame

Treatment	Seed no. plant^{-1}			Seed wt. (g plant^{-1})		
	25 DAS	50 DAS	75 DAS	25 DAS	50 DAS	75 DAS
Uninoculated	0.00^{d}	0.00^{d}	31.00^{c}	0.00^{c}	0.00^{c}	0.10^{ab}
A. delicata	0.00^{d}	0.00^{d}	28.60^{c}	0.00^{c}	0.00^{c}	0.10^{ab}
A. lacunosa	0.00^{d}	0.00^{d}	38.00^{bc}	0.00^{c}	0.00^{c}	0.14^{ab}
G. dimorphicum	0.00^{d}	0.00^{d}	64.60^{a}	0.00^{c}	0.00^{c}	0.19^{a}
G. versiformae	0.00^{d}	0.00^{d}	51.60^{abc}	0.00^{c}	0.00^{c}	0.16^{ab}
S. nigra	0.00^{d}	0.00^{d}	62.30^{ab}	0.00^{c}	0.00^{c}	0.19^{ab}
ANOVA: M (mycorrhizal treatment), D (days after sowing), M×D (interaction)						
M		**			NS	
D		***			***	
M×D		***			NS	

Different letters in a column indicate significant differences ($p \leq 0.05$) using Tukey's HSD Test
**p≤0.01 ***p≤0.001 NS not significant

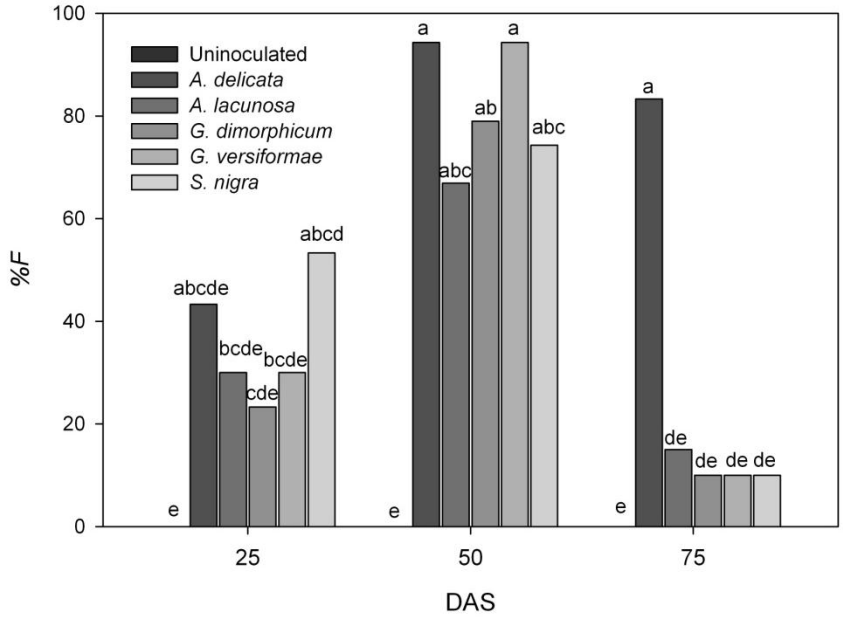

Fig. 1. Effect of inoculation with indigenous AMF on mycorrhizal colonization in sesame. Bars with different letters are significantly different ($p \leq 0.05$) by Tukey's HSD.

For example, sesame inoculated with *G. dimorphicum* had only a mean colonization of 37.44% by AMF but maximum increase in 63% of the measured parameters was recorded with this fungus. As has been observed elsewhere, AMF differ in their ability to enhance growth of the host plant, regardless of the extent of root colonization (Graham *et al*., 1982). One of the

most important factors that influence the efficiency of different AM fungal strains seems to be their external mycelium. The production of external hyphae may vary considerably between AMF (Sanders *et al*., 1977; Abbot and Robson, 1985; Kothari *et al*., 1991). No clear relationship seems to exist between the amount of external hyphae in soil and the growth responses observed in colonized plants (Jakobsen *et al*., 1992; Frey and Schüepp, 1993). Other factors such as the difference in rate of appresorium formation, in hyphal uptake and translocation capacities of nutrients, and in the metabolic activity of the external hyphae, seem to have more influence on the efficiency of AMF (Jakobsen *et al*., 1992, Frey and Schüepp, 1993; Giovannetti and Citernesi, 1980, 1993).

In general, the indigenous AMF improved the growth and yield characters of sesame though their efficiency varied. Among the AMF, *G. dimorphicum* emerged out as the efficient isolate in improving majority of the tested parameters. The study thus sheds light into the importance of proper selection of efficient AMF for the right crop and environment.

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