INFLUENCE OF DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI AND SHADE ON GROWTH OF SANDAL (*SANTALUM ALBUM*) SEEDLINGS

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BINU NK, ASHOKAN PK & BALASUNDARAN M. 2015. Influence of different arbuscular mycorrhizal fungi and shade on growth of sandal (*Santalum album*) seedlings. Sandal (*Santalum album*) is a precious tree valued for its fragrant heartwood known as East Indian sandalwood. Establishment of sandal seedlings in natural condition and when planted is difficult as the seedlings are exposed to varying shade levels. Hence, we studied the response of sandal seedlings to inoculation with cultures of three selected species of arbuscular mycorrhizal fungi (AMF), subjected to four shade levels and two host species. The results showed an increase in seedling height, number of leaves, leaf area and shoot weight of sandal seedlings due to AMF inoculation, especially in those inoculated with *Glomus mosseae*. During initial phases of seedling growth, high shade (75%) was essential but in later stages 50% shade was better. Interactions between 50% shade and mycorrhizae were the best especially for seedlings inoculated with *G. mosseae* (average root colonisation 68%). Relative water content of inoculated seedlings grown under 50% shade was the best (89.5%). Sandal–AMF association had improved the growth of sandal seedlings.

Keywords: Shade and mycorrhizal interaction, sandal host, agroforestry system, water status

INTRODUCTION

Santalum album is mainly distributed in south India and the Indonesian islands. Other species of *Santalum*, namely, *S. yasi*, *S. lanceolatum*, *S. austrocaledonium* occur in parts of Australia, Caledonia and Polynesia. Global demand for sandalwood is about 5000–6000 tonnes year^{-1} and that of its oil is 100 tonnes year^{-1} (Joshi & Arun Kumar 2007). However, the production of sandalwood has declined from 3176 tonnes year^{-1} in 1960–1965 to 1500 tonnes year^{-1} in 1997–1998 and eventually to 500 tonnes year^{-1} in 2007 and 100 tonnes year^{-1} in 2011–2012. Sandal heartwood prices have increased from USD6 tonne^{-1} in 1900 to USD1050 tonne^{-1} in 2000 and to USD60,000 tonne^{-1} in 2007 (Joshi & Arun Kumar 2007). The production of sandal in India is decreasing annually at the rate of 20% since 1995 (Ananthapadmanabha 2000). This necessitates growing of sandal outside conventional forest areas.

Sandal planted as part of homestead or agroforestry system has to survive under varying levels of shade. Therefore, an understanding of the responses of sandal towards various shade levels is necessary to gauge the suitability of sandal as understorey species in agroforestry systems. Sandalwood tree is an obligate root hemiparasite. This means that they obtain some of their water and simple nutrients from other plants even though they are able to manufacture their own complex compounds (Wanntorp & De Craene 2009).

Plant interaction is a major determinant of sandal growth in agroforestry systems (Kumar 2011). Initially shade tolerant, mature sandal trees do not tolerate overhead shade. Seedlings of sandal grown in shade show better performance than in full sunlight (Barrett & Fox 1994). Studies of the association between arbuscular mycorrhizal fungi (AMF) and growth of seedlings show that spores extracted from rhizosphere of sandal are predominantly of *Glomus* and *Gigaspora* species. (Subbarao et al. 1990, Thappar et al. 1992). Sandal seedlings inoculated with *Glomus* sp.

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performed better than uninoculated seedlings in terms of water relations and nutrient content (Nagaveni et al. 1998). Relative water content measures plant water status and indicate the metabolic process in tissues and lethal water level of leaf (Sinclair & Ludlow 1985). The levels of AMF infection have been strongly linked to the amount of available light and therefore the production of photosynthate by the plant (Harley & Smith 1983). However, little is known about the ecological role of different fungi at different shade levels. It is well established that colonisation by AMF assures good survival and growth of many tree seedlings on various sites. Since sandal is difficult to establish and its growth is very slow, we intend to investigate if AMF association can help in its establishment and growth. Hence, the present experiments were carried out to study the influence of shade, host and AMF inoculation on the growth of sandal seedlings.

MATERIALS AND METHODS

The investigation was conducted at the College of Forestry, Kerala Agricultural University in the Thrissur district of Kerala, India (10° 32' N, 76° 10' E). Three isolates of AMF commonly seen in the rhizosphere region of sandal and two hosts of sandal were selected for the experiments. Growth of the sandal seedlings were compared by subjecting the seedlings to four different shade levels (75, 50, 25 and 0%). A 2 × 3 × 4 factorial experiment was laid in a completely randomised design with seven replications. The different treatments are given in Table 1. Sample size was 56 for every shade level. The experiment was conducted for 8 months. The required shade levels were created by erecting net houses covered from all sides using appropriate shade nets. Light intensities were verified and regulated by measuring the photosynthetically active radiation using quantum sensor.

Sandal seeds were collected from Marayoor Forest Range, a seed stand of the Kerala Forest Department. Sandal seeds, after pretreatment with gibberellic acid, were sown in sterilised sand kept in plastic trays. These were placed in a greenhouse and watered regularly with sterile water. The potting media used in this experiment were sieved soil and sand in 1:1 ratio which were solarised before use. It has been demonstrated that high temperature induced during solarisation is able to significantly reduce indigenous AMF (Chen et al. 1991, Bendavid-Val et al. 1997).

Table 1 Different treatment combinations given to sandal seedlings each at 75, 25, 50 and 0% shade levels

Treatment combination
Sandal + casuarina + *Glomus mosseae*
Sandal + red gram + *G. mosseae*
Sandal + casuarina + *G. fasciculatum*
Sandal + red gram + *G. fasciculatum*
Sandal + casuarina + *G. intraradices*
Sandal + red gram + *G. intraradices*
Sandal + casuarina
Sandal + red gram

Spores of *Glomus fasciculatum* and *Glomus intraradices* were obtained from Tata Energy Research Institute, New Delhi while spores of *Glomus mosseae* were obtained from University of Agricultural Sciences, Bangalore. All the three species of AMF were multiplied in sterilised sand and soil mix (1:1) media in plastic basins planted with sorghum seedlings. After 3 months, spores were isolated from soil samples by wet sieving and decanting method (Gerdemann & Nicolson 1963) and observed under microscope for AMF spore count. It was observed that 1 g of soil contained approximately 25 spores. Infection on sorghum roots by the three species of AMF was tested by clearing and staining method (Phillips & Hayman 1970). Solarised soil was immediately transferred to the greenhouse and polythene bags (21 cm × 15 cm) of gauge 250 µm were filled with potting media leaving 4-cm space at the top. For each treatment, 10 g of AMF inoculum (approximately 250 spores) were placed in the polybag and covered with 1:1 mixture of sterilised sand and soil up to 2 cm above the inoculum. Pre-germinated sandal seeds were dibbled in these polybags, one in each bag, to a depth of 2 cm. After 1 week the pre-germinated seeds of casuarina (*Casuarina equisetifolia*) and redgram (*Cajanus cajan*) were dibbled in these polybags according to the treatments. The seedlings were transferred to shade houses as per treatment and arranged randomly under each shade level. Seedlings were irrigated regularly with sterile water.

Height, collar diameter and number of leaves were recorded for each seedling at monthly intervals. Total biomass, per cent of AMF colonisation, root length and leaf area were recorded after 6, 7 and 8 months of planting. Relative water content was recorded after 8 months. Sandal seedlings from each treatment were sampled 6, 7 and 8 months after planting for biomass studies. Length of roots was observed after separating the stem portion from the collar region. Shoot and root samples were dried for 2 days in the shade after which they were dried in an oven at 70–80 °C for 48 hours. Dry weights of shoot and root samples were measured using a precision balance. Leaves of seedlings were separated from the shoot and the leaf area was determined using the graphical method. Fine roots from the seedlings were observed under compound microscope and presence of vesicles, arbuscules or hyphae was taken as positive for infection of AMF and the total per cent of AMF association was calculated. Leaves were selected randomly from sandal plants from two replications at each shade level to measure their relative water content (Barrs & Weatherley 1962).

The data were analysed statistically using analysis of variance for each shade level separately. Then combined analysis of all observations at four shade levels was done using randomised complete block design over location (Panse & Sukhatme 1978).

RESULTS

There were variations in height, number of leaves, leaf area, shoot weight and relative water content of sandal seedlings due to AMF inoculation and shade treatment (Tables 2–6). There was significant ($p = 0.05$) increase in height of seedlings inoculated with AMF grown under 50, 25 and especially 75% shade from 7 months after planting (Table 2). Inoculated seedlings grown under shade also showed significant increase in height. However, no significant increase in height was observed for inoculated seedlings grown under 0% shade. Initially, number of leaves increased for seedlings under all treatments but decreased from four months onwards. The decrease in number of leaves was less in seedlings inoculated with *G. mosseae* grown under 50 and 25% shade (Table 3). Significant increase in leaf area of sandal seedlings was recorded for inoculated seedlings under all shade levels. Maximum value was observed for *G. mosseae*-inoculated seedlings grown under 50% shade (Table 4). The highest shoot weight was recorded in seedlings inoculated with *G. mosseae* grown under 50 and 25% shade (Table 5). Relative water content was higher for seedlings inoculated with AMF, particularly those inoculated with *G. mosseae* grown under 50 and 25% shade (Tables 6 and 7). Seedlings inoculated with *G. mosseae* showed superior growth under all levels of shade as well as in full sunlight. This study showed that seedlings inoculated with *G. mosseae* under 50% shade showed maximum growth in height, number of leaves, leaf area, shoot weight and relative water content.

Seedlings grown under 75% shade showed significant increase in height for the first two months. After 8 months of planting, maximum height was observed for seedlings raised under 50% shade, followed by those under 25, 75 and 0% shade (Table 2). Seedlings grown in shade showed higher number of leaves for the first 4 months after planting compared with seedlings grown in full sunlight. After 8 months, seedlings grown under 25% showed maximum number of leaves followed by those grown under 50, 0 and 75% shade (Table 3). Shoot weight (Table 5) and relative water content (Table 7) of seedlings grown under 50% shade was significantly higher when compared with the rest of the shade levels. The AMF and shade did not show any significant differences in collar girth, root weight and root length of sandal seedlings. Host species also did not show any significant differences in any of the observed growth parameters. Inoculation with AMF showed increase in percentage of colonised root of sandal seedling which increased with time. Root colonisation was maximum for seedlings inoculated with *G. mosseae* and grown under 50% shade (Table 8).

DISCUSSION

Sandal is a very slow-growing species and the surface area of its root system is small compared with other species (Srinivasan et al. 1992).

Table 2 Influence of arbuscular mycorrhizal fungi (AMF) inoculation on height (cm) of sandal seedlings grown under various shade levels

AMF	Shade level (%)	Month after planting							
		1	2	3	4	5	6	7	8
Glomus fasciculatum	75	8.2	10.2	12.9	13.3	13.7 b	14.2 b	14.6 ab	14.8 ab
G. intraradices		7.7	10	12.4	12.4	13.0 b	14.6 b	15.2 ab	16.2 a
G. mosseae		9.1	12.3	14.3	15.7	15.8 a	16.4 a	16.3 a	16.8 a
Uninoculated		9.2	11.8	12.1	12.6	12.8 b	12.9 b	13.1 b	13.5 b
Mean		8.5 a	11.1 a	12.9 b	13.2 b	14.0 b	14.4 b	14.9 b	15.0 b
G. fasciculatum	50	7.5	11.1	13.7	14.4	15.1	15.2	16.5 ab	17.0 a
G. intraradices		8.7	11.3	13.5	14.9	15.8	16.7	17.0 a	17.5 a
G. mosseae		8.1	10.6	13.2	13.9	14.1	14.8	15.7 ab	17.3 a
Uninoculated		7.6	10.7	12.5	13.3	13.6	14.1	14.9 b	13.5 b
Mean		7.9 b	10.9 a	13.5 a	14.0 a	14.7 a	15.8 a	16.4 a	16.9 a
G. fasciculatum	25	8.3	10.6	12.0	13.0	13.9	14.3	15.4 ab	15.2 ab
G. intraradices		6.2	9.1	11.6	12.6	13.2	14.1	15.0 ab	15.7 ab
G. mosseae		6.9	9.9	12.9	14.4	14.4	15.2	17.3 a	17.2 a
Uninoculated		6.9	9.1	10.5	12.2	12.2	13.3	13.5 b	13.8 b
Mean		7.1 c	9.9 b	11.5 c	13.0 b	13.9 b	14.5 b	15.3 ab	15.8 bc
G. fasciculatum	0	8.2	9.2	11.2	11.8	11.8	12.7	12.7	13.3
G. intraradices		5.3	7.6	10	10.9	10.9	11.4	13.0	13.5
G. mosseae		6.5	7.7	10.3	11.7	11.1	13.2	13.9	14.5
Uninoculated		6.7	7.7	11.4	11.3	11.3	12.4	12.8	13.2
Mean		6.7 c	7.9 c	10.7 d	11.4 c	12.1 c	12.5 c	13.3 c	13.6 c

Values with similar alphabets within a column do not vary significantly, values without alphabets are insignificant

Our study showed improved growth of sandal seedlings in response to AMF inoculation. Height, leaf area and shoot weight of sandal seedlings increased due to inoculation. The increase in mycelium increases root surface area so the efficiency of the root is increased. This was observed for the roots of AMF-inoculated seedlings, thereby increasing the efficiency of roots in absorption of minerals and water, hormone production, nitrogen production and resistance to disease (Gianinazzi & Gianinazzi 1983). AMF augment root system surface area and increase its absorption efficiency. AMF inoculation improved growth of forest tree seedlings in species such as *Tectona grandis* (Durga & Gupta 1995), *Dalbergia sisoo* (Singh et al. 1998), *Pterocarpus marsupium* (Sharma et al. 1996) and *Azadirachta indica* (Sumana & Bagyaraj 2003).

Sandal seedlings inoculated with *G. fasciculatum*, *G. aggregatum* and *G. caledonium* showed better performance than the uninoculated seedlings (Nagaveni et al. 1998). In our study, maximum response was observed for seedlings grown in soil inoculated with *G. mosseae* regardless of shade levels. AMF colonisation was also higher for *G. mosseae* compared with the rest of the species. So the growth response observed in sandal seedlings inoculated with *G. mosseae* may be related to higher colonisation of AMF. Decrease in the number of leaves observed after 4 months of planting might be due to heavy rains in the months of June and July during the period of the experiment which encouraged pathogenic fungal activity on leaves. Consequent decrease in the leaf area and shoot weight was observed. The decrease was less for *G. mosseae*-inoculated seedlings which might be due to better water relations of the seedlings, as evident from their high relative water content. The present investigation revealed that there was considerable interaction between AMF and shade levels. Growth of sandal seedlings and

Table 3 Influence of arbuscular mycorrhizal fungi (AMF) inoculation on the number of leaves of sandal seedlings grown under various shade levels

AMF	Shade level (%)	Month after planting							
		1	2	3	4	5	6	7	8
Glomus fasciculatum	75	8.4	11.0	9.9	13.6	9.9	7.0	5.6	3.3 b
G. intraradices		5.9	8.5	9.1	12.5	10.8	7.9	6.1	4.5 b
G. mosseae		7.6	10.1	10.6	12.7	11.2	8.4	7.3	7.5 a
Uninoculated		6.1	8.6	9.6	11.4	9.6	6.7	5.9	3.7 b
Mean		7.5 ab	9.5 a	9.8 a	12.8 a	10.3 b	8.8 b	6.8 c	4.8 c
G. fasciculatum	50	7.0	9.3	9.1	12.6	10.3 b	10.5 b	10.1 b	8.2 b
G. intraradices		7.5	9.9	11.3	13.3	12.3 b	11.8 b	10.6 b	9.0 b
G. mosseae		8.4	10.7	10.5	14.2	15.3 a	14.8 a	14.4 b	13.3 a
Uninoculated		9.0	10.3	8.9	13.6	12.2 b	11.1 b	7.3 c	7.2 c
Mean		8.0 c	10.0 a	10.0 a	13.4 a	12.3 a	10.5 ab	9.9 ab	9.4 ab
G. fasciculatum	25	7.0	9.2	8.5	11.9	12.0	12.8 a	12.5 a	11.8 a
G. intraradices		7.5	8.6	10.5	12.4	12.2	11.8 ab	11.2 a	9.8 a
G. mosseae		8.4	8.7	9.6	13.3	12.9	12.6 ab	12.2 a	12.3 a
Uninoculated		8.0	9.8	8.2	11.6	10.8	9.9 b	7.5 b	6.0 b
Mean		8.0 c	9.1 b	9.2 b	12.3 a	11.9 b	11.3 b	10.9 b	10.0 b
G. fasciculatum	0	7.0	8.1	7.7	10.9	9.7	8.3 a b	7.1 b	8.0 b
G. intraradices		7.5	7.9	7.2	11.4	10.8	9.7 a b	7.3 b	7.1 b
G. mosseae		6.4	7.4	8.3	11.8	11.2	10.9*	10.1 a	11.1 a
Uninoculated		6.5	7.4	8.1	9.1	9.6	8.6 b	6.9 c	6.5 b
Mean		6.9 b	7.7 b	7.8 b	10.8 c	9.3 c	8.9 b	8.1 ab	8.2 b

Values with similar alphabets within a column do not vary significantly, values without alphabets are insignificant

Table 4 Influence of arbuscular mycorrhizal fungi (AMF) inoculation on leaf area (cm^2) of sandal seedlings

AMF	Shade level (%)											
	75			50			25			0		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
Glomus fasciculatum	27.5 b	15.3 c	9.3 b	28.8 a	28.8 a	19.4 b	22.4 b	26.0 b	23.5 b	9.8 b	12.4 b	12.8 b
G. intraradices	28.2 b	15.9 b	11.6 d	28.8 b	21.0 b	20.2 b	27.7 a	25.2 b	21.8 b	9.3 b	14.5 b	11.2 b
G. mosseae	38.5 a	23.3 a	21.1 a	39.6 a	40.9 a	38.7 a	29.4 a	27.7 b	26.6 a	15.1 a	17.0 a	17.9 a
Uninoculated	18.0 c	10.5 c	6.4 b	14.0 b	8.0 c	8.2 c	17.0 c	11.3 c	9.1 c	10.3 b	9.1 c	7.5 c

Values with similar alphabets within a column do not vary significantly

AMF colonisation were best under 50% shade level. Diffused sunlight is necessary for growth of sandal seedlings especially during the first year of growth (Troup 1921). Colonisation and growth of *G. mosseae* itself may have also benefited from the shade. More photosynthate made available to sandal root/AMF and/or the beneficial effect of shade on AMF colonisation and growth may have resulted in improved growth of sandal seedlings. Positive relation between shoot growth parameters and AMF colonisation may also be related to these reasons. AMF colonisation on roots is strongly linked to the amount of sunlight, which in turn will decide the production of photosynthate by the plant resulting in increased carbon allocation to the root system which

Table 5 Influence of arbuscular mycorrhizal fungi (AMF) inoculation on the shoot weight (g) of sandal seedlings

AMF	Shade level (%)											
	75			50			25			0		
	Month after planting											
	6	7	8	6	7	8	6	7	8	6	7	8
Glomus fasciculatum	0.28 ab	0.25	0.20	0.31 b	0.29 b	0.27 b	0.30 a	0.28 a	0.30 a	0.13	0.16	0.17
G. intraradices	0.27 b	0.24	0.22	0.25 d	0.27 b	0.29 b	0.25 a	0.26 ab	0.28 a	0.12	0.15	0.14
G. mosseae	0.33 a	0.26	0.21	0.45 a	0.46 a	0.42	0.30 a	0.32 a	0.31 a	0.17	0.18	0.17
Uninoculated	0.20 c	0.21	0.21	0.28 c	0.27 b	0.28 b	0.14 b	0.15 b	0.17 b	0.10	0.11	0.11
Mean	0.27 b	0.19 c	0.21 bc	0.32 a	0.32 a	0.31 a	0.23 b	0.25 b	0.27 b	0.12 c	0.13 c	0.15 c

Values with similar alphabets within a column do not vary significantly, values without alphabets are insignificant

Table 6 Influence of arbuscular mycorrhizal fungi (AMF) inoculation on the relative water content of leaves of sandal seedlings

AMF	Relative water content (%)
Glomus fasciculatum	87.6 a
G. intraradices	87.9 a
G. mosseae	87.7 a
Uninoculated	86.8 b

Values with similar alphabets do not vary significantly

Table 7 Influence of shade on the relative water content of leaves of sandal seedlings

Shade level (%)	Relative water content (%)
75	85.2 c
50	89.5 a
25	89.1 a
0	86.3 b

Values with similar alphabets do not vary significantly

stimulates AMF colonisation (Harley & Smith 1983). These reasoning are further corroborated by the observations on seedlings grown under full sunlight which did not show significant response to AMF colonisation. This indicated the adverse effects of bright sunlight on growth of AMF as well as sandal seedlings, resulting in poor carbon supply to AMF, thus, suppressing its colonisation.

The present study revealed that for all the growth parameters, during the initial seedling phase, high shade (75%) was needed but during the later stages of seedling growth, medium shade (50%) was best. However, it has been reported that plant height, leaf number, crown width and stem diameter do not change significantly at various shade levels, while leaf area is higher for sandal seedlings grown under shade (Barrett & Fox 1994). Inoculating seedlings with AMF did not affect collar girth, root weight and root length of sandal seedlings. Similar results were reported for black pepper (Ashithraj 2001) and cowpea (Beena 1999). Root weight of sandal seedlings inoculated with *G. mosseae* was higher under all shade levels, even though it did not vary significantly. Similar results were observed in safed musli (*Chlorophytum borivilianum*) treated with some common AMF species (Dave et al. 2011). Influence of host species on the growth of sandal seedlings was not significant. *Casuarina equisetifolia* (Varghese 1996) and *C. cajan* (Srinivasan et al. 1992) were good hosts and improved growth of sandal seedlings. However, red gram is considered as short-term host while casuarina, long-term host. The long-term influences of casuarina on sandal are yet to be documented.

Relative water content was higher in seedlings inoculated with AMF compared with uninoculated seedlings. Seedlings grown under 50 and 25% shade showed high values of relative water content. This indicated that the seedlings had better plant water status. Thus, in the long run, metabolic process and growth of sandal seedlings were superior in these seedlings (Sinclair & Ludlow 1985). Seedlings grown in full sunlight and 75% shade had the lowest relative water content. In full sunlight, seedlings are water-stressed because of high transpiration

Table 8 Percentage of arbuscular mycorrhizal fungi (AMF) colonisation on sandal seedlings grown at four shade levels

AMF	Shade level (%)											
	75			50			25			0		
	Month of planting											
	6	7	8	6	7	8	6	7	8	6	7	8
Glomus fasciculatum	28	37	38	39	55	53	17	42	43	28	32	48
G. intraradices	44	53	57	40	48	53	20	46	48	25	35	51
G. mosseae	48	57	52	42	56	68	48	55	62	32	34	52
Uninoculated	2	4	4	5	8	6	4	6	8	6	11	14

loss. With high shade level, absorption of water may be less resulting in low relative water content. Our observations confirmed that both these contrasting extreme environments were not suitable for sandal seedlings. High light intensity results in its interception, more than light saturation point for photosynthesis, which increases leaf temperature. To dissipate this heat, plants may transpire more (Landsberg 1986). Absorption capacity of the root system of plant depended on intensity of sunlight, which in turn determined carbon allocation to roots and root system development. In high shade levels, low carbon turnover and low carbon allotted to root system may result in poor growth and absorption of water, resulting in low relative water content and poor growth of sandal seedlings.

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