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Original Research Paper

Synergistic Effect of Arbuscular Mycorrhizal Fungi and *Rhizobium* Inoculation on *Dalbergia sissoo* Roxb. in Unsterile Soil

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Arbuscular mycorrhizal fungi (AMF)
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ABSTRACT

Dalbergia sissoo Roxb. has been attributed as a desirable leguminous species used in regulating the distributed lands and improving deteriorated waste lands in India. *Dalbergia sissoo* seedlings were grown in earthen pots containing unsterilized soil inoculated with *Glomus fasiculatum, Rhizobium* or a combination of symbionts. After 120 days of growth, plant height, dry weight, root dry weight, percent mycorrhizal colonization, nitrogen and phosphorus contents were quantified. Plant with either a combination of mycorrhizal fungi and R*hizobium* grew taller and produced higher dry matter of root and shoot than infected with *Rhizobium* species alone or control plants. Presence of *Rhizobium* increased nitrogen content of the above ground plant. Foliage and inoculation with mycorrhizal fungi increased the phosphorus content. Mycorrhizal fungi and *Rhizobium* were found to be synergistic with respect to nitrogen fixation and per cent of root colonization.

INTRODUCTION

Although man and higher plants live immersed in atmosphere which has 78% nitrogen, they ironically are unable to use this element in its gaseous form. Instead, they must rely on other sources to provide them with fixed nitrogen in either oxidized or reduced form, which is suitable for further metabolism. From an agronomic point of view, the most important of these sources are bacteria of the genus *Rhizobium*, which live symbiotically within root nodules of leguminous plants (Phillips et al. 1978) and fix the atmospheric nitrogen into nitrate. Leguminous trees and shrubs have been suggested for nodulation and the resources of fuel and wood (Hogberg & Kvarnstrom 1982, Salo 1985, Lakshman 1996).

Most of the land plants require arbuscular mycorrhizal fungi (AMF) to achieve maximum growth in nutrient deficient soils by an increased uptake of soil phosphorus (Barea & Azcon-Aguilar 1983). These fungi may have no effect on plant growth in the absence of applied phosphorus in highly phosphorus deficient soils or when phosphorus supply is not limiting growth of non-mycorrhizal plants (Aarons & Ahmed 1987). Legumes have phosphorus requirements for optimum growth nodule formation and nitrogen fixation (Mosse 1977a). In sterilized soil, Crush (1974) reported increased nodulation growth in *Centrsema pubescens* and *Stylosantnes guyanensis*. Beneficial responses to AM fungi inoculation in sterilized in natural soils have also been obtained on French bean (Daft & El-Giahmi 1976) and peanut (Krishna & Bagyraj 1984).

Literature survey revealed that very limited studies have been carried out on interaction between *Rhizobium* and AM fungi on tree species, but little information is available as to its symbiotic relationship, therefore, this paper reports the growth response of *Dalbergia sissoo* Roxb. to AM fungal inoculation with *Rhizobium* in pot experiments in unsterilized soil.

MATERIALS AND METHODS

Experiments were conducted in earthen pots (25 × 30 cm) with unsterile soil. The used sandy loam was with pH 6.9, phosphorus deficient and practically devoid of *Rhizobium*, but had an indigenous AMF population of 239/50g soil. *Dalbergia sissoo* seeds were surface sterilized with 2% potassium hypochlorite and germinated in pots containing sterile sand. One week old seedlings were transplanted to the experimental pots. The following treatments were taken.

1. Unsterile soil infested with *Glomus fasciculatum*
2. Unsterile soil infested with *Rhizobium* species
3. Unsterile soil infested with *Glomus* and *Rhizobium*
4. Unsterile soil without any inoculation

Rhizobium inoculation was done by treating the seeds with a peat based culture before sowing. Mycorrhizal inoculation was done by placing the seeds over a thin layer of mycorrhizal inoculum (10g mixed inoculum) at the time of sowing. The mycorrhizal inoculation consists of roots and soil from a pot of culture of *Sorghum bicolor*, which was infected with *Glomus fasciculatum* and grown for four months. The inoculum contained hyphae, vesicles and chlymadospores of *Glomus fasciculatum*. There were four replicates for each treatment. At the two intervals of 60 and 120 days after sowing, observations on plant weight, dry weight of shoot and root, number of leaves, number of nodules, percent root colonization, spore number per 50g soil and phosphorus content (Jackson et al. 1972) of shoot was determined. Total nitrogen was determined by the microKjeldahl method (Bremner 1960). Percent of mycorrhizal colonization of root was determined by the root slide technique (Nicolson 1974) after clearing the roots with 10% KOH and stained with 0.05% trypan blue in lactophenol. Number of *Glomus fasciculatum* spores were determined by the wet sieving and decanting technique.

RESULTS AND DISCUSSION

The results of the study are shown in Fig. 1 and Tables 1 and 2. The above ground dry weight produced by *Dalbergia sissoo* plants was greatest when *Glomus* and *Rhizobium* were present together as compared with the plants infected with *Glomus fasiculatum* or *Rhizobium* alone and uninoculated control plants (Table 1). The presence of both mycorrhizal fungi and N_2-fixing bacteria stimulated the above ground mean growth, but this growth was not significantly greater than *Glomus*-inoculated plants.

The below ground portion of the plants responded differently to the various treatments. The control plants had limited root development, compared with the other treatments (Table 1). This may be the result of very low available P level in soil. Phosphorus stimulates root development to a greater extent than shoot. The magnitude of root production by plants inoculated with *Rhizobium* and *Glomus* together was significantly more than all other treatments.

In general, plant height was increased and proportional to the number of leaves. Dual inoculation with both the symbionts resulted in significantly more height, but response was not significantly different from plants infected with *Glomus.* Separate inoculations with *Rhizobium* and *Glomus* produced similar responses in plant height and both the treatments produced plants taller than control (Fig. 1).

Inoculation with *Glomus* and *Rhizobium* alone or a combination of *Glomus* and *Rhizobium* produced significantly ($P = 0.05$) more leaves than uninoculated control plants. Dual inoculation with *Glomus* and *Rhizobium* produced more leaves than the control and *Rhizobium* treatments, but there

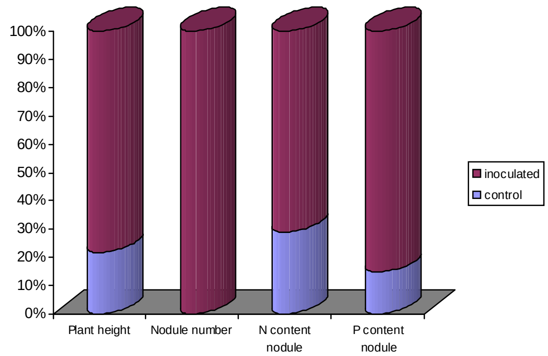

Fig. 1: Effect of AM fungus and *Rhizobium* interaction on *Dalbergia sissoo* with regard to plant height, nodule number, and nitrogen and phosphorus of nodules for 120 days.

were no differences between plants inoculated with *Glomus* when compared with plants inoculated just with *Rhizobium.* This may be explained by the increased level of plant available N supplied by the symbiotic bacteria present or the formation of nodules. The increased level of available N may have caused a proliferation of roots as a result of N concentrating in the root cells and hastening cell division and elongation. Total root production was significantly less when *Glomus* was present as compared with *Rhizobium*. Perhaps this response is a result of mycorrhizal fungi increasing the surface area and, thus, the efficiency of roots to absorb nutrients and, therefore reducing the amount of root material required by the plant. There is much evidence in the literature that the presence of mycorrhizae decreases the root : shoot ratio by increasing above ground production and possibly by reducing the heed for below ground production. Crush (1974) reported reduced root : shoot ratio when *Glomus* was present as compared with *Rhizobium*-inoculated plants. The root : shoot ratios calculated in the present study indicate that they decrease with the presence of mycorrhizal fungi.

The presence of *Glomus* or *Rhizobium* shows a positive influence on N content of infected plants (Mosse 1977a). Results in this study show that *Rhizobium* significantly increased N content and *Glomus* significantly increased P content.

Table 1: Effect of AM fungus and *Rhizobium* interaction on *Dalbergia sissoo* with regard to plant height, dry weight of root and shoot, nodule number and nitrogen and phosphorus of nodules for 120 days.

Treatment	Plant height (cm)	Dry weight root (g)	Dry weight shoot (g)	Nodule number/plant	% nitrogen in shoot (dry)	% phosphorus in shoot (dry)
Control	17.8 a	5.1 b	0.78 a	_	1.23 a	0.10 a
Rhizobium	36.3 b	7.8 b	1.6 b	2.3 a	1.42 a	0.14 a
Glomus fasciculatum	41.2 c	9.4 c	2.2 b	2.4c	1.33 b	0.215
Glomus+Rhizobium	49.7 c	11.6 b	4.3 c	5.4 b	1.69 c	0.27 c

* Values not followed by identical letters in each vertical column are significantly different at P > 0.05 by DMRT

Table 2: Effect of AM fungus and *Rhizobium* interaction on *Dalbergia sissoo* with regard to number of leaves, root/shoot ratio and spore number for 120 days.

Treatment	Number of leaves/plant	Root/shoot ratio (%)	Percent root colonisation	Average spore number in 50g soil
Control	17.8 a	10.1 b	0.78 a	-
Rhizobium	36.3 b	7.8 b	11.6 b	12.0 a
Glomus fasciculatum	41.2 c	9.4 c	52.2 b	98.7 c
Glomus +Rhizobium	49.7 c	9.6 b	57.2c	103 b

Values not followed by identical letters in each vertical column are significantly different at P > 0.05 by DMRT.

Nitrogen levels in shoots were significantly higher in *Rhizobium* inoculated plants when compared with plants that were not infected (control) or infected with *Glomus*. The presence of both the symbionts increased N level in above ground plant only slightly over the single inoculation with *Rhizobium*. The response of inoculated plants in accumulating P in shoots was also recorded. Plants infected with *Glomus* had higher levels of P than *Rhizobium* inoculated plants. Dual inoculation resulted in the highest P level. It is possible that the response of *Dalbergia sissoo* in accumulating P may be directly related to the mycorrhizal infection level. The mean level of plants inoculated *Glomus* was 52% and dual inoculated plants had 57.2%, a significantly greater infection level (Table 2). In unsterilized soils, the positive growth responses could in part be due to the low inoculum potential or effectiveness of the introduced mycorrhizal fungi (Laksman & Ratageri 2005). Dual inoculation of VA mycorrhizae and *Rhizobium* beneficial to *Pithecollobium dulce* has also shown that *Glomus fasiculatum* was very efficient strain in stimulating uptake, plant growth and nodulation of several legumes in many unsterile tropical acid soils. The principal effect of mycorrhiza may have other secondary effects, possibly of hormonal nature (Mosse 1977b). In conclusion, the present study brings out that an effective AM fungus *Glomus fasiculatum* with *Rhizobium* inoculation could contribute to the efficiency of such a system, especially in phosphorus deficient soils, even though native endophytes may be present.

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