Forests, Trees and Livelihoods, 2006, Vol. 16, pp. 269–275
1472-8028 $10

RESEARCH NOTE
GROWTH RESPONSE OF *ACACIA PLANIFRONS* W. *et* A. TO ARBUSCULAR MYCORRHIZAL FUNGI AND NITROGEN FIXING BACTERIA UNDER NURSERY CONDITIONS

A. KARTHIKEYAN[1] AND T. MUTHUKUMAR[2]

[1]*Institute of Forest Genetics & Tree Breeding, Coimbatore – 641 002, Tamil Nadu, India*
[2]*Department of Botany, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India*

ABSTRACT

An experiment was conducted to determine the influence of arbuscular mycorrhizal (AM) fungi and *Rhizobium* sp. individually as well as in combinations, on the growth response of *Acacia planifrons* W. *et* A. seedlings in sterilized soil under greenhouse conditions. The native AM fungi *Glomus fasciculatum* (Thatcher) Gerd. & Trappe emend. Walker & Schenck & Smith emend. Koske and *G. geosporum* (Nicol & Gerd.) Walker were isolated from the rhizosphere of *A. planifrons* and multiplied in the roots of *Zea mays* (L.). The *Rhizobium* sp. was isolated from the root nodules of *A. planifrons* and cultured in yeast extract mannitol broth. These cultures were used to inoculate the seedlings of *A. planifrons* individually and in combinations. The results showed that co-inoculation of AM fungi and *Rhizobium* sp. increased the growth, biomass and nutrient uptake of *A. planifrons* more than single inoculations of AM fungi or *Rhizobium* sp. AM fungi and *Rhizobium* sp. inoculated seedlings had a higher accumulation of nitrogen (N), phosphorus (P) and potassium (K) than un-inoculated controls. The seedlings inoculated with triple root symbionts, *G. fasciculatum* + *G. geosporum* and *Rhizobium* sp. resulted in the maximum plant growth and nodular biomass production. Lower root to shoot ratios and increased seedling quality index were also obtained in these combinations.

Key words: *Glomus fasciculatum*, *Glomus geosporum, Rhizobium* sp, seedling quality, nutrient uptake, root symbionts.

Introduction

Arbuscular mycorrhizal (AM) symbiosis is an important factor in the establishment of good seedlings through an increase of nutrient uptake (Harley and Smith 1983) and the fungi are considered as beneficial microorganism for forest trees. The symbiosis also

- assists in the movement of water to plant roots (Parke *et al.* 1983),

Author for correspondence: Dr. A. Karthikeyan, Silviculture Division, Institute of Forest Genetics & Tree Breeding, P.O. Box: 1061;R.S. Puram, Fax: 91-422-2430549, Coimbatore – 641 002, Tamil Nadu, India. E mail: karthikeyana@ifgtb.res.in karthikarumugam@hotmail.com

- offers resistance against pathogens (Dehne 1982),
- increases seedling growth rate, vigour and their survival after transplanting (Brandeau 1970),
- promotes plant uniformity and reduction of transplanting damage (Biermann and Lindermann 1983),
- alleviates drought stress during transplantation (Michelsen and Rosendahl 1990), and
- increases the mineralization of organic phosphorus (P) (Jayachandran *et al.* 1992) and uptake of P.

The nitrogen (N) fixing bacteria *Rhizobium* sp. also play a role in improving seedling quality of woody legume species (Michelsen 1992). So using AM fungi and *Rhizobium* sp. should be expected to increase the survival of woody legumes after planting in the field as the nodulated and mycorrhizal plants are adapted to cope with nutrient deficient situations (Harley 1973, Harley and Smith 1983, Johansen *et al.* 1993).

Therefore seedlings of the leguminous tree *Acacia planifrons* W. *et* A. were inoculated with the root symbionts – AM fungi and *Rhizobium* sp. – in the nursery to improve their quality. *A. planifrons* was used because of its importance in social forestry. This tree has a regular umbrella shape and short bole with spreading branches. The wood is hardy, heavy and used for agricultural implements and as fuel. The pods are eaten by cattle. It thrives in low moisture soil conditions and has potential for the reclamation of wastelands due to its ability to survive in stressful environments (Udaiyan *et al.* 1996). It is often used in social forestry and afforestation programmes in Tamil Nadu (India) because of its importance in fulfilling the day-to-day needs of local people for fuel wood. It is a good shade provider due to its umbrella shape and is usually planted on roadsides.

Materials and methods

Native AM fungal species, *Glomus fasciculatum* Gerd. & Trappe and *G. geosporum* (Nicol. & Gerd.) Walker, isolated from the rhizosphere of *A. planifrons*, growing in Coimbatore, India, were multiplied and maintained in sterile soil media (alfi soil:sand) with *Zea mays* (L.) under green house conditions for three months as pot cultures. *Rhizobium* sp. isolated from the nodules of the same plant was cultured and maintained in yeast extract mannitol broth. Healthy, fully matured seeds of *A. planifrons* of uniform weight were collected, washed and soaked in hot water at 60°C for 30 minutes. The treated seeds were sown in polythene pots (14 × 27 cm) containing 3 kg of sterilized (100°C for 3hr at 103.5×10^3 Pa) soil: sand mixture (1:1). Later, five days old seedlings – some 7 cm tall – were transplanted to polythene pots containing sterilized soil:sand media, at the rate of one seedling per pot.

The AM inoculum consisted of 100 g of soil from pot cultures of *Z. mays* containing chlamydospores of *G. fasciculatum* and *G. geosporum*, was placed 5 cm below the soil surface in each polythene bag. Inoculation of *Rhizobium* sp. was achieved by applying 20 ml of rhizobial suspension to each bag of the rhizobial treatment.

Seven treatments were applied

(i) control
(ii) *G. fasciculatum*,
(iii) *G. geosporum*
(iv) *Rhizobium* sp.
(v) *Rhizobium* sp. + *G. fasciculatum*
(vi) *Rhizobium* sp. + *G. geosporum*
(vii) *Rhizobium* sp. + *G. fasciculatum* + *G. geosporum*

Each was replicated five times. The potted seedlings were arranged in a randomized block (RDB) design under green house conditions at 31.6°C (± 2.8), 72% (± 5.2) Relative Humidity (RH) and watered regularly to maintain turgidity.

Sixty days after emergence, the seedlings were harvested with their entire root system intact. The roots were washed free of soil and the following parameters recorded:

- nodule numbers,
- shoot and root lengths and their respective dry weights after drying in a hot air oven at 80°C for 72 hr.
- total kjeldahl nitrogen (N) on kjeltec Auto analyzer (1030):
- phosphorus (P) by vanadomolybdate phosphoric yellow colour method,
- potassium (K) content by flame photometer (Jackson 1973),
- seedling quality Index using the formula of Dickson *et al.* (1960) viz.
  Seedling quality index (SQI) = Seedling dry weight (mg)/[Seedling height (cm)/Root collar diameter (mm) + Shoot dry weight (mg)/Root dry weight (mg)].

A portion of the harvested root samples was processed for microscopic observation following the procedure of Phillips and Hayman (1970) and the percent of AM infection was determined by the root slide method of (Read *et al.* 1976). The data of treatment means were subjected to analysis of variance and the means were separated using Duncans's New Multiple Range Test (IRRISTAT 1993).

Results

After sixty days, the triple root symbionts (*G. fasciculatum* + *G. geosporum* + *Rhizobium* sp.) inoculated seedlings had significantly higher values of all the parameters measured than those of other treatments and the control, except for

TABLE 1

Growth and Biomass of *Acacia planifrons* in different inoculation treatments with AM fungi and /or *Rhizobium* sp.(mean of 5 replicates)

Sl. No.	Treatments	Shoot length (cm)	Root length (cm)	No. of leaves/ plant	Collar dia-meter (mm)	No. of nodules/ plant	Plant Biomass (Dry weight mg/plant)		Nodules Dry weight (mg) plant	R/S ratio	Tissue nutrient content (mg/g)						SQI*	AM fungal coloni-zation (%)
											N		P		K			
							root	shoot			root	shoot	root	shoot	root	shoot		
1.	Control	14.1^{a}	27.6^{a}	20^{a}	0.9^{a}	–	55.6^{a}	76.2^{a}	–	0.72^{d}	8^{a}	14^{a}	1.65^{a}	2.1^{a}	6.^{a}	15.9^{a}	6.72^{a}	–
2.	*Glomus fasciculatum*	15.4^{b}	29.12^{a}	24^{b}	1.20^{b}	–	71.2^{c}	29.82^{b}	–	0.24^{a}	10^{b}	20.5^{b}	2.1^{b}	2.5^{b}	15.0^{b}	25.5^{b}	21.4^{b}	90^{a}
3.	*G. geosporum*	17.2^{c}	29.82^{a}	21.2^{a}	1.23^{b}	–	74.0^{b}	233.8^{ab}	–	0.31^{b}	12.5^{b}	27^{b}	1.9^{b}	3.1^{b}	16.5^{b}	22.5^{b}	18^{b}	92^{a}
4.	*Rhizobium sp.*	24.8^{d}	39.09^{c}	37.4^{c}	1.40^{bc}	8^{a}	73.2^{b}	301.4^{b}	3^{a}	0.24^{ab}	15^{b}	34^{c}	3.0^{b}	3.9^{c}	18.5^{c}	28.0^{c}	12.5^{a}	–
5.	*G. fasciculatum* + *Rhizobium* sp.	26.8^{e}	30.4^{b}	30^{b}	1.47^{c}	8^{a}	**173.0^{d}**	273.8^{b}	13.2^{b}	**0.63^{c}**	18^{c}	35.5^{d}	3.3^{cd}	4.0^{d}	23.7^{d}	31.4^{d}	22.5^{c}	88^{a}
6.	*G. geosporum* + *Rhizobium* sp.	29de	35^{bc}	45.5^{d}	1.45^{c}	10^{a}	161.4^{c}	361.4^{c}	11^{b}	0.44^{bc}	18^{c}	37^{d}	2.75^{c}	4.5^{d}	38.1^{e}	35.0^{d}	23.5^{c}	86^{a}
7.	*G. fasciculatum* + *G. geosporum* + *Rhizobium* sp.	**40.2^{f}**	**44^{d}**	**55^{e}**	**1.90^{d}**	**15^{b}**	164.0^{d}	**426.8^{d}**	**15^{c}**	0.38^{bc}	**22^{d}**	**41^{e}**	**4.0^{e}**	**4.75^{e}**	**35.5^{e}**	**42.5^{e}**	**30.5^{d}**	**100^{b}**

Values not followed by identical letters in each column are significantly different as determined by Duncan's New Multiple Range Test ($p < 0.05$). The highest value of a parameter is given in bold

*Seedling quality index.

the root/shoot ratio that was less favourable than those of the single treatments (ii) with *G. fasciculatum* and (iv) with *Rhizobium* sp. The results are summarized in Table 1.

The N, P and K contents in the seedlings inoculated with AM fungi were higher than the control seedlings. Treatment (vii) triple root symbionts inoculated seedlings showed more nutrient accumulation in the shoots and roots

The seedling quality index was higher in the seedlings inoculated with AM fungi and *Rhizobium* sp., either individually or in combinations over control. Again treatment (vii) triple root symbionts, inoculated seedlings had significantly increased seedling quality index.

High percentages of root infection were observed in single AMF inoculations of *G. fasciculatum* and *G. geosporum*. (treatments ii & iv) and with dual root symbionts (*Rhizobium* sp. and AM fungi) inoculations (treatments v & vi), but triple root symbionts inoculations resulted in significantly still higher percentage of root colonization than other treatments.

Discussion

Results of this study showed that inoculation of *A. planifrons* seedlings with native *Rhizobium* sp. or AM fungi (*G. fasciculatum* and *G. geosporum*) either individually or in combinations increased the seedling growth and biomass over un-inoculated control. The study showed that AM fungi could improve plant growth associated with increased uptake of P (Gerdemann 1975). Inoculations with *Rhizobium* sp. and AM fungi had a significantly positive effect on growth and nutrient uptake in *A. planifrons*. Similar results were reported earlier for dual root symbionts employed treatment (Patterson *et al.* 1990).

Under nursery conditions triple root symbionts inoculations of *G. fasciculatum*, *G. geosporum* and *Rhizobium* sp. resulted in the highest growth and biomass production. This may be a reason for plants that prefers more than one root symbiont. Nodule numbers also were higher in triple root symbionts inoculations indicating that mycorrhizal plants can produce significantly more nodules than non-mycorrhizal ones in accord with Azcon and Barea (1978) and Kucey and Paul (1983). Higher concentrations of leghaemoglobin and acetylene in nodular tissue has been reported by Kucey and Paul (1983). Earlier studies have also indicated that AM fungi can enhance nodulation with *Rhizobium* sp. (Daft and El. – Giahmi 1975). Seedlings inoculated with AM fungi resulted in increased shoot to root ratio by increasing above ground production and possibly by reducing the need for production below ground since the function of nutrient acquisition is taken over by AM fungi (Smith 1980). Daft and El-Giahmi (1975) also reported increased shoot to root ratio when AM was present as compared with *Rhizobium* sp. inoculated plants.

The increased accumulation of mineral nutrients such as N, P and K in the plant as a result of tripartite symbiotic association (legume – *Rhizobium* sp. and AM fungi) may be attributed to the role of extramatrical hyphae of AM fungi making inaccessible nutrients available to the roots (Gerdemann 1975). Generally, *Rhizobium* sp. will increase N content and AM fungi increase P content in seedlings. However, seedlings infected with AM fungi and *Rhizobium* sp. had higher P and N contents than individual inoculations of AM fungi or *Rhizobium* sp. (Redante and Reeves 1981; Udaiyan *et al.* 1997). An increased K status of seedlings in dual and triple root symbionts may suggest that K acts as an enzyme activator and greatly stimulates nodules through improved AM fungal hyphae activity, in addition to the absorption and translocation of NH_4 from the soil to the plant root. It also facilitates the transfer of N (Barea *et al.* 1987). Increased root collar in triple root symbiont inoculated seedlings reflects their improved quality associated with massive AM fungal root colonization (Ikram *et al.* 1992).

The triple root symbionts significantly increased the growth, biomass and nutrient uptake in this experiment and resulted in improved seedling quality index of *A. planifrons.*

Conclusions

The AM fungi and *Rhizobium* sp. association increased the efficiency of the shoot and root system in providing the seedlings with essential levels of P and N for growth. Therefore, for quality seedling production of leguminous trees like *A. planifrons,* due consideration should be given to symbiotic microorganisms such as AM fungi and *Rhizobium* sp rather than chemical inputs. The simple root symbionts inoculation technology could be adopted in nurseries to improve the rate of growth and quality of seedlings produced without chemical fertilizers, thus avoiding their associated costs and dangers to the environment, water supplies, local inhabitants and their domestic stock.

REFERENCES

Azcon-Aguilar C and Barea J.M. 1978. Effects of interactions between different culture fractions of phosphate bacteria and Rhizobium on mycorrhizal infection, growth and nodulation of *Medicago sativa. Canadian Journal of Microbiology* **40**: 520–524.

Barea J.M. Azcon-Aguilar C. and Azcon R. 1987. Vesicular-arbuscular mycorrhiza improve both symbiotic N_2 fixation and N uptake from soil as assessed with a 15 N technique under field conditions. *New Phytologist* **106**: 717–725

Biermann B. and Lindermann R.G. 1983. Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extra radical vesicles as inoculam. *New Phytologist* **22**: 121–140

Braudeau J. 1970. El Cacao: *Tecnicas agricolas* y *producciones tropicales.* Editorial Blume. Barcelona, Espana, 297 p.

Daft M.J and El Giahmi A.A. 1975. Effects of *Glomus* infection on three legumes. Pp. 581–592 in Sanders F.E. and Tinker P.B. (eds.). *Endomycorrhiza.* Academic Press, London.

Dehne H.W. 1982. Interactions between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72**: 1115–1119.

Dickson A. Leaf A.L. and Hosner J.F. 1960. Quality appraisal of white spruce and white pine seedling stock in nurseries. *Forestry Chronicle* **36**: 10–13.

Gerdemann J.W. 1975. Vesicular-arbuscular mycorrhizae Pp. 575–561 in Torrey J.G. and Clarkson D. (eds.). *The development of function of roots.* Academic Press, New York.

Harley J.L. 1973. Symbiosis in eco system. *Federation of Natural Science Council Sri Lanka* 1: 31–48

Harley J.L. and Smith S.E. 1983. *Mycorrhizal Symbiosis*. Academic Press, London.

Ikram A. Mahmud A.W. Ghari M.N. Ibrahim M.T and Zainal A. B. 1992. Field nursery inoculation of *Hevea brazilensis* Muell. Arg. Seedling root stock with vesicular arbuscular mycorrhizal fungi. *Plant and Soil* **145**: 231–236.

IRRISTAT. 1993. Biometrics Unit, Ver 3/93. International Rice Research Institute, Manila, Philippines.

Jackson M.L. 1973. *Soil chemical analysis*. Prentice-Hall, New Delhi.

Jayachandran K. Schwab A.P. and Hetrick B.A.D. 1992. Mineralization of organic phosphorus by vesicular-arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* **24**: 897–903.

Johansen A. Jakobsen I. and Jensen E.S. 1993. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 3. Hyphal transport of 32 P and 15 N. *New Phytologist* **124**: 61–68.

Kucey R.M.N and Paul E.A. 1983. Vesicular-arbuscular mycorrhizal spore populations in various Saskatchewan soils and the effect of inoculation with *Glomus mosseae* on faba bean growth in green house and field trials. *Canadian Journal of Soil Science* **63**: 87–95.

Michelsen A. and Rosendahl S. 1990. The effect of VA mycorrhizae fungi, Phosphorus and drought stress on growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **124**: 7–14.

Michelsen A. 1992. Mycorrhizae and root nodulation in three seedlings from five nurseries in Ethiopia and Somalia. *Forest Ecology and Management* **48**: 335–344.

Patterson N.A. Chet I. and Kapulniky I. 1990. Effect of mycorrhizal inoculation on nodule initiation activity and contribution to legume productivity. *Symbiosis* **8**: 9–20.

Parke J.L. Linderman R.G. and Black C.H. 1983. The role of ectomycorrhizas in drought tolerance of Douglas fir seedlings. *New Phytologist* **95**: 83–95.

Phillips J.M. and Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and AM fungi for rapid assessment of infection. *Transactions of British Mycological Society* **55**: 158–161.

Read D.J. Koucheki H.K. and Hodgson J. 1976. Vesicular arbuscular mycorrhiza in natural vegetation systems. *New Phytologist* **77**: 641–653.

Redente E.F. and Reeves F.B. 1981. Interactions between vesicular-arbuscular mycorrhiza and Rhizobium and their effect on Sweetvetch growth. *Soil Science* 132: 410–415. Smith S.E. 1980. Mycorrhizas of autotrophic higher plants. *Biological Reviews* **55**: 475–510.

Udaiyan K. Karthikeyan A. and Muthukumar T. 1996. Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *Acacia farnesiana* willd. and *A. planifrons* W. *et* A. *Trees: structure and function* **11**: 65–71.

Udaiyan K. Sugavanam V. and Manian S. 1997. Growth response of wattle (*Acacia mearnsii*) seedlings to phosphorus fertilization and inoculation with *Glomus deserticola* and *Rhizobium* sp in non-sterile soil. *Journal of Tropical Forestry Science* **10**: 212–224.