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EFFECT OF DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI AND *AZOSPIRILLUM* ON *EUCALYPTUS TERETICORNIS* SEEDLINGS

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SUGAVANAM, V., UDAIYAN, K. & DEVARAJ, P. 2000. Effect of different arbuscular mycorrhizal fungi and *Azospirillum on Eucalyptus tereticornis* seedlings. Transplanted seedlings of *Eucalyptus tereticornis* were inoculated with six arbuscular mycorrhizal fungi (*Gigaspora margarita, Glomus deserticola, G. fasciculatum, G. monosporum, G. mosseae* and *G. versiforme*) individually as well as in combinations with *Azospirillum*. The results indicated that inoculation of AM fungi or *Azospirillum* increased the growth, biomass, root colonisation and tissue N, P and K concentrations. Significant responses were observed in plants inoculated with *Glomus versiforme* or *Gigaspora margarita* in conjunction with *Azospirillum*.

Key words: *Eucalyptus tereticornis* - AM fungi - *Azospirillum* - growth - nutrient accumulation

SUGAVANAM, V., UDAIYAN, K. & DEVARAJ, P. 2000. Kesan kulat mikoriza arbuskular yang berbeza dan *Azospirillum* ke atas anak benih *Eucalyptus tereticornis*. Anak benih *Eucalyptus tereticornis* yang dialih diinokulat dengan enam kulat mikoriza arbuskular (*Gigaspora margarita, Glomus deserticola, G. fasciculatum, G. monosporum, G. mosseae* dan *G. versiforme*) secara individu serta secara kombinasi dengan *Azospirillum*. Keputusan menunjukkan bahawa penginokulatan kulat AM atau *Azospirillum* menambahkan pertumbuhan, biojisim, pengkolonian akar dan kepekatan tisu N, P dan K. Tindak balas bererti dicerap dalam tumbuhan yang diinokulat dengan *Glomus versiforme* atau *Gigaspora margarita* bersama-sama dengan *Azospirillum*.

Introduction

Eucalyptus is among the important trees of the world. A native of Australia and the East Indies, it was introduced to many parts of the world, and today ranks as one of the most ubiquitous trees. About 170 species, varieties and provenances of *Eucalyptus* have been tried all over India (Bhatia 1984), out of which the most outstanding is *Eucalyptus* hybrid, a form of *E. tereticornis* which has become the most popular and universal eucalypt in all the low rainfall areas of India, due to its unique adaptability to many of the agro-climatic situations, its ability to withstand drought over long periods, and its excellent coppicing power.

Eucalyptus tereticornis is used as raw material for rayon, paper and pulp industries. The new forest policy of the Government of India dictates that industries must meet their raw material demands from their own plantations by 2000 AD. For quick return fast growth is a desirable trait to be economically viable. Arbuscular mycorrhizal (AM) fungi greatly increase the ability of trees to recover phosphorus (P) from soil resulting in the stimulation of growth in many economically important plants including *Eucalyptus* (Boudarga & Dexheimer 1988, Reis & Krugner 1990, Adjoud *et al.* 1996). However, the efficacy of different AM fungi along with *Azospirillum* in improving *Eucalyptus tereticornis* seedling growth has not been investigated. Hence, an experiment was carried out to evaluate the response of *Eucalyptus tereticornis* seedlings inoculated with different AM fungi and *Azospirillum*.

Materials and methods

Soils

Sandy loam soil collected from the fields of the Bharathiar University, Coimbatore, India, was examined for its pH (1:1 soil:water) and electric conductivity. The total nitrogen (N) was determined by the micro-Kjeldahl's method of Jackson (1973). Available P was extracted from the soil in an ammonium phosphomolybdate solution and analysed using spectrophotometer (Jackson 1973).

Exchangeable potassium (K) was extracted from the soil in an ammonium acetate solution (pH=7.0) and measured with a digital flame photometer (Jackson 1973). The soil had a natural AM population of 72 spores per 100 g soil *(Acaulospora scrobiculata, Glomus fasciculatum, G. geosporum* and *G. mosseae).*

Plant material

Eucalyptus tereticornis seeds were procured from the Institute of Forest Genetics and Tree Breeding, Coimbatore, India, and sown for germination in autoclaved sand. Uniform, healthy 30-day-old seedlings were transplanted to 10x15 cm polythene bags containing 3 kg unsterile soil.

Inoculum

Arbuscular mycorrhizal fungi

Soil with infected root bits were collected from the pot cultures of AM fungi such as *Gigaspora margarita, Glomus deserticola, G. fasciculatum, G. monosporum, G. mosseae,* and *G. versiforme* maintained in sterilised sand: soil (1:1 by volume) mixture with *Sorghum vulgare* as the host plant in the greenhouse. The inoculum potential of the AM culture was determined by the probable number (MPN) method as outlined by Porter (1979). Inoculum was added at the rate of 24 000 infective propagules per polythene bag.

Azospirillum

Azospirillum sp. were isolated from the rhizosphere of *Eucalyptus tereticornis* cultured in a N-free bromothymol blue broth (Subba Rao 1986), and 5 ml of this culture were added to each polythene bag.

Harvesting and measurements

Growth parameters, viz. root length, shoot length, stem girth and plant dry weight were recorded after 150 days from the date of sowing. Root subsamples were processed for microscopic observations following the procedure of Phillips and Hayman (1970) and the percentage mycorrhizal infection was determined by the root slide technique of Read *et al.* (1976).

Spore population of each soil sample was estimated by the modified wet sieving and decanting technique of Gerdemann and Nicolson (1963). Total Kjeldahl N was analysed with a Kjeltec Auto Analyzer (1030). Phosphorus determination was performed with the vanadomolybdate phosphoric yellow colour method (Jackson 1973).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and the means were separated by Duncan's multiple range test ($p < 0.05$).

Results

Growth and biomass

The growth and dry weight of the *Eucalyptus tereticornis* seedlings were invariably enhanced by AM fungi and *Azospirillum* inoculation, though the increases were not always statistically significant. Among the different AM fungi, *Gigaspora margarita* significantly enhanced growth, stem girth and biomass compared to the control. Inoculation of *Glomus monosporum* significantly decreased root to shoot ratio. Seedlings co-inoculated with *Glomus versiforma* and *Azospirillum* sp. had significantly enhanced growth, stem girth and biomass compared to a single inoculation of either AM fungi or *Azospirillum* sp. (Table 1).

Table 1. Influence of different AM fungi and *Azospirillum* on the growth and biomass of *Eucalyptus tereticornis*

Treatment	Root length (cm)	Shoot length (cm)	Stem girth (mm)	Dry weight (mg plant⁻¹) Root (R)	Dry weight (mg plant⁻¹) Shoot (S)	R/S ratio
Control	16.2 e	37.6 f	15.0 e	204 g	1110 e	0.18 e
GIM	45.0 a	60.0 abcd	19.0 bcd	983 bcde	4112 abcd	0.23 bcd
GD	34.3 abc	57.2 abcde	16.5 cde	651 ef	2308 de	0.28 a
GF	31.0 bc	45.7 cdef	17.5 bcde	952 bcde	3918 bcd	0.24 abc
GM	29.1 bcde	48.0 cdef	19.0 bcd	721 de	2812 cde	0.25 ab
GMO	17.0 de	45.7 cdef	5.2 de	252 fg	2012 de	0.12 f
GV	21.5 cde	55.4 bcde	18.3 bcde	903 bcde	3620 bcd	0.24 abc
GIM + *Azospirillum*	38.1 ab	70.0 a	23.0 a	1320 ab	6264 a	0.21 bcde
GD + *Azospirillum*	41.8 ab	63.1 ab	22.2 ab	1154 abc	5612 ab	0.20 cde
GF + *Azospirillum*	38.3 ab	66.3 ab	21.8 ab	1172 abc	6126 a	0.19 de
GM + *Azospirillum*	36.4 ab	62.4 abc	21.3 ab	1124 abcd	4814 abc	0.23 bcd
GMO + *Azospirillum*	30.7 bcd	62.3 abc	19.2 abc	1100 abcd	236 abcd	0.25 ab
GV + *Azospirillum*	46.2 a	70.2 a	21.5 ab	1428 a	6322 a	0.22 bcde
Azospirillum	30.2 bcd	48.4 ef	18.2 bcde	804 cde	3610 bcd	0.22 bcde

GIM-*Gigaspora margarita*; GD-*Glomus deserticola*; GF-*Glomus fasciculatum*; GM-*Glomus mosseae*; GMO-*Glomus monosporum*; GV-*Glomus versiforme*.
Means within a parameter followed by the same letters are not significantly different according to Duncan's multiple range test ($p < 0.05$).

AM root colonisation and spore number

Seedlings inoculated with *Gigaspora margarita* and *Glomus fasciculatum* increased mycorrhizal infection and number of spores respectively in the soil. Co-inoculation with *Glomus versiforme* and *Azospirillum* sp. significantly increased root colonisation and sporulation compared to their single inoculations (Table 2).

Table 2. Influence of different AM fungi and *Azospirillum* on the spore count and root colonisation of *Eucalyptus tereticornis*

Treatment	Spore count per 10 g soil	Root colonisation (%)
Control	43 f	7.5 g
GIM	162 cd	42.4 bcde
GD	130 de	27.3 ef
GF	175 bcd	40.0 cdef
GM	146 de	35.6 def
GMO	137 de	33.0 def
GV	133 de	30.3 ef
GIM + *Azospirillum*	235 ab	63.2 ab
GD + *Azospirillum*	225 ab	60.2 abc
GF + *Azospirillum*	234 ab	63.2 ab
GM + *Azospirillum*	222 abc	52.0 abcd
GMO + *Azospirillum*	200 abcd	47.5 abcde
GV + *Azospirillum*	247 a	64.5 a
Azospirillum	84 ef	22.0 fg

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Means within a parameter followed by the same letters are not significantly different according to Duncan's multiple range test ($p < 0.05$).

Tissue N, P and K concentrations

Inoculation with the AM fungus *Glomus versiforme* increased tissue N, P and K concentrations in the plants. Combined inoculation of the seedlings with *Gigaspora margarita* and *Azospirillum* significantly increased tissue N and P concentration. Potassium concentration increased with *Glomus versiforme* and *Azospirillum* inoculation (Table 3).

Table 3. Influence of different AM fungi and *Azospirillum* on the nutrient concentrations of *Eucalyptus tereticornis*

Treatment	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	0.53 e	0.61 e	0.05 d	0.10 d	0.24 c	0.26 e
GIM	0.78 bcde	1.51 bcd	0.09 cd	0.18 bcd	0.28 c	0.35 bcde
GD	0.73 cde	1.13 cde	0.08 cd	0.17 cd	0.27 c	0.31 cde
GF	0.75 cde	1.42 bcd	0.08 cd	0.18 bcd	0.28 c	0.33 bcde
GM	0.68 cde	0.98 de	0.07 cd	0.13 d	0.26 c	0.29 de
GMO	0.65 de	0.91 de	0.06 d	0.13 d	0.25 c	0.28 de
GV	1.10 abcd	1.63 abcd	0.10 a	0.19 bcd	0.30 c	0.36 bcde
GIM + *Azospirillum*	1.45 a	2.26 a	0.20 a	0.36 a	0.38 abc	0.42 abc
GD + *Azospirillum*	1.21 abcd	1.98 ab	0.13 abcd	0.21 bcd	0.38 abc	0.43 abc
GF + *Azospirillum*	1.32 ab	2.08 ab	0.16 abc	0.26 abc	0.46 abc	0.46 ab
GM + *Azospirillum*	1.15 abcd	1.72 abc	0.11 bcd	0.20 bcd	0.33 abc	0.38 bcde
GMO + *Azospirillum*	1.16 abcd	1.83 abc	0.15 abc	0.26 abc	0.37 abc	0.41 abcd
GV + *Azospirillum*	1.23 abc	2.11 ab	0.18 ab	0.30 ab	0.48 a	0.52 a
Azospirillum	1.13 abcd	1.63 abcd	0.06 d	0.11 d	0.31 bc	0.37 bcde

GIM-*Gigaspora margarita*; GD-*Glomus deserticola*; GF-*Glomus fasciculatum*; GM-*Glomus mosseae*; GMO-*Glomus monosporum*; GV-*Glomus versiforme*.
Means within a parameter followed by the same letters are not significantly different according to Duncan's multiple range test ($p < 0.05$).

Discussion

Dual inoculation of AM fungi and *Azospirillum* increased the growth of *Eucalyptus tereticornis* seedlings. Though practically no research has been reported on forest tree species with reference to dual inoculation of AM fungi and *Azospirillum* in unsterile soil, a significant response to AM fungi and *Azospirillum* was recorded in annual crops (Barea *et al.* 1983). *Eucalyptus tereticornis* seedlings varied in their response to inoculation with different AM fungi, confirming earlier reports of host preferences towards AM fungi (Bagyaraj *et al.* 1989). Among the different AM fungi, *Glomus versiforme* in the presence of *Azospirillum* produced significantly higher growth, biomass, root colonisation and tissue nutrient concentration. The observed responses to inoculation with *G. versiforme* can be ascribed to better compatibility and establishment of the endophyte with *Eucalyptus tereticornis.* Mycorrhizal inoculation generally decreases the root to shoot ratio (Mathew & Johril 1989). Similarly, in the present study, inoculation of AM fungi either singly or in combination with *Azospirillum* sp. had significantly lowered the root to shoot ratios of inoculated seedlings.

Root colonisation and spore number were considerably higher in AM fungi or *Azospirillum* sp. inoculation than the uninoculated control. The extent of colonisation and spore number varied with different AM fungi. Seedlings inoculated with *Gigaspora margarita* and *Azospirillum* sp. had the highest root colonisation and spore number. The extent of infection is affected by many soil factors and one particularly important factor is soil phosphate level, which can reduce infection at high P by many strains of AM fungi considerably, the extent depending on the plant species and the fungus (Haselwandter & Bowen 1996).

Nitrogen, P and K concentrations were improved in *Gigaspora margarita* and *Azospirillum* inoculated plants. This can be attributed to the increased absorbing surface area of the extensive external network of mycelium produced by *Gigaspora margarita* in association with the host root system (Howler *et al.* 1981). Barea and Gonzalez (1986) reported that AM fungi are able to increase N concentration in plants by one or more mechanisms such as indirect enhancement of symbiotic N_2-fixation as a consequence of increase P supply by AM or direct uptake of N compounds by AM hyphae.

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