INFLUENCE OF VAM FUNGI ON GROWTH RESPONSE OF NEEM (*AZADIRACHTA INDICA*)

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SUMANA, D. A. & BAGYARAJ, D. J. 2003. Influence of VAM fungi on growth response of neem (*Azadirachta indica*). An investigation was carried out to screen and select an efficient vesicular arbuscular mycorrhizal (VAM) fungus for inoculating the forest tree species, neem (*Azadirachta indica*). The neem seedlings were inoculated with four different VAM fungi, two from a preliminary screening trial and the remaining two from the root zone soil of neem trees. VAM inoculated seedlings generally showed good response in having greater plant height, stem girth, biomass, P content and Zn concentration, biovolume index and quality index. The mycorrhizal root colonisation and spore numbers in the root zone soil was also more in VAM inoculated plants as compared with uninoculated plants. The best response was seen in neem plants inoculated with *Glomus mosseae.*

Key words: *Glomus mosseae* - symbiotic response - nursery inoculation - mycorrhizal technology

SUMANA, D. A. & BAGYARAJ, D. J. 2003. Kesan kulat mikoriza arbuskel vesikel terhadap gerak balas tumbesaran semambu (*Azadirachta indica*). Kajian dijalankan untuk menyaring dan memilih kulat mikoriza arbuskel vesikel yang berkesan bagi menginokulasi spesies pokok hutan iaitu semambu (*Azadirachta indica*). Anak benih semambu diinokulasi dengan empat jenis kulat mikoriza arbuskel versikel. Dua daripadanya diperoleh daripada ujian penyaringan awal dan dua lagi dipencilkan kepada tanah zon akar bagi pokok semambu. Anak benih yang diinokulasi dengan kulat tersebut menunjukkan ketinggian, ukur lilit, biojisim, kandungan P dan kepekatan Zn, indeks bioisi padu serta indeks kualiti yang lebih tinggi. Kolonisasi akar oleh mikoriza dan bilangan spora di dalam tanah zon akar adalah lebih tinggi dalam anak benih yang diinokulasi dengan kulat berbanding anak benih yang tidak diinokulasi. Gerak balas terbaik diperhatikan dalam anak benih semambu yang diinokulasi dengan *Glomus mosseae.*

Introduction

Neem (*Azadirachta indica*) is one of the most important multipurpose, evergreen forest tree species native to the Indian subcontinent. It is also referred to as global, ecofriendly, wonder tree as it is implicated to solve global problems such as deforestation, degradation of land, greenhouse effect and global warming (Tewari 1992). Neem seedlings in forest nurseries in India, as in many tropical countries,

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are raised in unsterile soil (with indigenous VAM fungi), either in polybags or in nursery beds. VAM fungi, a unique group of soil fungi forming symbiotic association with many of the tree species, facilitating their growth, are well documented (Cuenca *et al.* 1990, Rajan *et al.* 2000). There are several reports upholding the concept of host preference leading to the selection of efficient VAM fungi for inoculation of tree seedlings in the nursery (Reena & Bagyaraj 1990a, Sumana & Bagyaraj 1998). Growth stimulation and improved nutrient uptake in tree seedlings inoculated with efficient VAM fungi are also well documented (Rajan *et al.* 2000, Vasanthakrishna *et al.* 1995). Habte *et al.* (1993) reported that neem roots are colonised by VAM fungi and this species is dependent on mycorrhiza.

In a preliminary screening trial conducted in our laboratory, nine different VAM fungi obtained from different parts of the world which proved promising to be good for inoculating different tree species based on our earlier studies were screened for their symbiotic response with neem. The study suggested *Glomus mosseae* as the best fungus for inoculating neem followed by *G. fasciculatum.* The objective of the present study was to screen further the best two fungi obtained from preliminary screening trial, besides the local VAM fungi isolated from the root zone soil of neem trees in order to select an efficient strain of mycorrhizal fungus for inoculating neem in the nursery.

Materials and methods

Four different isolates of VAM fungi, two selected from a preliminary screening trial and two isolated from the root zone soil of neem trees (Table 1) were maintained as pot cultures in a green house using autoclaved sand:soil (1:1 v/v) mix as the substrate and *Chloris gayana* (Rhodes grass) as the host. The two indigenous isolates of VAM fungi isolated from the root zone soil of neem were identified using the manual by Schenck and Perez (1987).

Table 1 Influence of VAM fungi on plant height, number of leaves and stem girth of neem

VAM fungus	Plant height (cm)			No. of leaves	Stem girth (mm)
	90 DAT	135 DAT	180 DAT		
Glomus geosporum	16.60 b	17.30 b	18.60 b	6.70 b	4.70 b
Glomus deserticola	15.08 b	15.70 b	16.70 c	5.80 bc	3.30 c
Glomus fasciculatum	15.35 b	16.70 b	22.60 b	11.25 a	4.85 b
Glomus mosseae	20.60 a	21.70 a	24.70 a	11.90 a	5.08 a
Uninoculated	13.01 c	13.04 c	14.10 d	4.90 c	2.60 d

Means followed by the same letter in the same column do not differ significantly at p = 0.05
DAT = Days after transplanting

Neem seeds collected from a plus tree grown in the University of Agricultural Sciences, GKVK Campus, Bangalore were surface sterilised with 1% sodium hypochlorite for 30 min after removing the outer pulp from the fruits. Later, the seeds were washed thoroughly in sterile water, dried in shade and sown in boxes at

50 seeds per box (50 × 27 × 22 cm) holding autoclaved (121 °C at 1.1 kg cm^{-2} pressure for 1h) soil:sand (1:1 v/v) mix. Forty-five-day-old, healthy, uniform neem seedlings at four-leaved stage were transplanted into polybags (1 per poly bag) of size 25 × 15 cm holding 1.6 kg unsterilized sand:soil:compost (1:1:0.25) mix. The soil used was an alfisol of the type kaolinitic, isohypothermic typic Kanhaplustafs. The growth medium used had a pH of 5.7, available P of 2.6 ppm (NH_4F + HCl extractable) and an indigenous mycorrhizal population of 65 spores per 50 ml soil. Root pieces (cut to about 1 cm length) and soil mixture from pot cultures of Rhodes grass infected separately with the four test fungi were air dried and used as the mycorrhizal inocula. To each planting hole, 12 500 infective propagules (IP) of different fungi, based on most probable number estimation (Porter 1979) were added. One plant per bag was maintained in a completely randomised design with 20 replicates in the glasshouse for 180 days.

The plant height was recorded at 90, 135 and 180 days after transplanting. The number of leaves and stem girth at collar region were recorded 180 days after transplanting. The plants were harvested 180 days after transplanting. Root and shoot dry biomass were determined after drying (for 4 days) at 60 °C until constant weight. P content of shoot and root was determined by vanadomolybdate phosphoric yellow colour method (Olsen & Sommers 1982). The intensity of yellow colour due to phosphovanadomolybdate complex was read at 425 nm in a spectronic 20 spectrophotometer. The P concentration was determined by comparing the absorbance value with P standard curve. Zn concentration after wet oxidation was estimated by Atomic Absorption Spectrophotometer with Zn hollow cathode lamp set to a wavelength of 214 nm (Jackson 1973). Biovolume index, which is a non-destructive quick method to calculate the above-ground portion of the tree seedlings was calculated using the formula suggested by Hatchell (1985): Biovolume index = plant height (cm) x stem diameter (mm). Quality index which is a measure to assess the quality of seedling based on the height, stem diameter and dry biomass was also calculated using the following formula (Hatchell 1985).

$$\text{Quality index} = \frac{\text{Seedling dry biomass (g)}}{\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \text{Top dry biomass (g)}}$$

Roots were stained with trypan blue (Phillips & Hayman 1970) and the per cent mycorrhizal colonisation was determined by grid line intersect method (Giovannetti & Mosse 1980). Mycorrhizal spore number in the root zone soil was determined by wet sieving and decanting method (Gerdemann & Nicolson 1963).

The statistical analysis was done for all plant and mycorrhizal parameters using one-way analysis of variance and the means were separated by Duncan's multiple range test at 5 per cent level of significance (Little & Hills 1978).

Results

The two VAM fungi isolated from the root zone soil of neem were identified as *G. geosporum* and *G. deserticola.* The characters of two isolates were as follows:

Glomus geosporum

Sporocarps absent, spores formed singly in soil, globose to subglobose, ellipsoid, 98 × 115 µm in size at maturity. Number of walls three with 8 µm thickness. Spore colour yellowish brown with contents intact, sporogenous hyphae formed. Hyphal shape often cylindric with 16 µm diameter at the point of attachment (Figure 1).

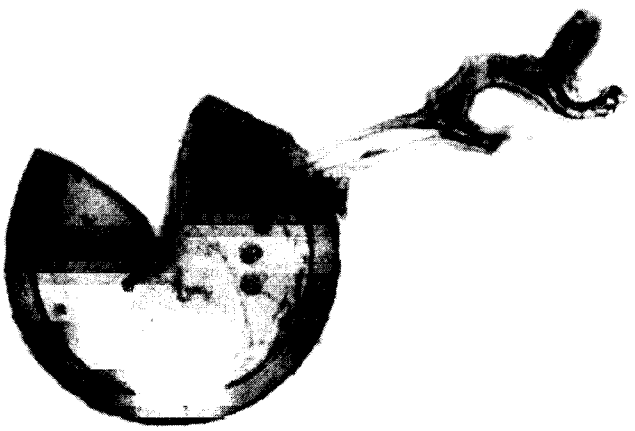

Figure 1 *Glomus geosporum* (400 ×)

Glomus deserticola

Sporocarps absent, spores borne singly in soil or within roots, globose to subglobose, 86 × 65 µm in size, shiny smooth, reddish brown in colour, with single wall of thickness 2.3 µm. Attached hypha cylindric, reddish brown in colour, adjacent to the spore but not occluding the hypha. Interior of the spore wall at the point of hyphal attachment thickened at maturity to form an inner mounded collar that appears to be closed by a membranous septum (Figure 2).

There was significant increase in plant height, number of leaves and stem girth in plants inoculated with VAM fungi as compared with uninoculated plants (Table 1). Significantly higher plant height, number of leaves and stem girth were observed in plants inoculated with *G. mosseae.* This was followed by *G. fasciculatum, G. geosporum* and *G. deserticola* treated plants. The lowest value was observed in uninoculated plants.

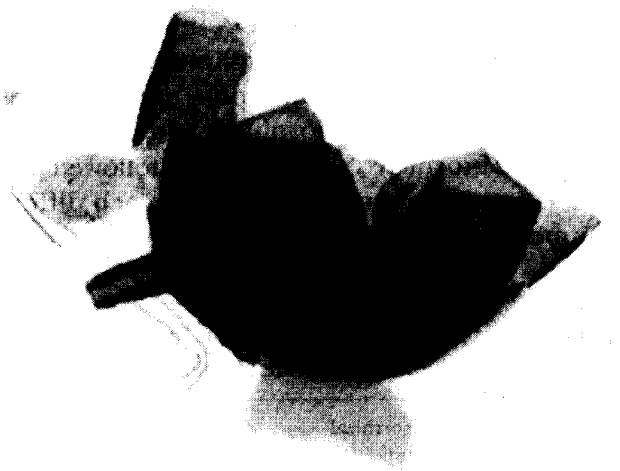

Figure 2 *Glomus deserticola* (400 ×)

Shoot and root biomasses were also significantly higher in plants inoculated with *G. mosseae*. The lowest biomass was observed in uninoculated plants (Table 2). The increase in shoot and root phosphorus content was significantly higher in plants inoculated with *G. mosseae*, followed by *G. fasciculatum*, *G. geosporum* and *G. deserticola* and the lowest value was seen in uninoculated plants (Table 2). The Zn concentration of shoot was maximum in plants inoculated with *G. mosseae* followed by *G. fasciculatum* and *G. geosporum*. Minimum Zn concentration was seen in uninoculated plants (Table 2). The root Zn concentration was highest in plants inoculated with *G .fasciculatum*, which was statistically similar to *G. mosseae*. This was followed by plants treated with *G. geosporum*. Uninoculated plants had the lowest Zn concentration.

Table 2 Influence of VAM fungi on shoot and root biomass, P content and Zn concentration of neem

VAM fungi	Biomass (g plant^{-1})		P content (mg plant^{-1})		Zn concentration (μg g^{-1})	
	Shoot	Root	Shoot	Root	Shoot	Root
Glomus geosporum	2.59 b	1.54 c	6.38 c	4.45 c	90.40 b	109.60 b
Glomus deserticola	1.85 b	1.23 c	3.79 c	2.40 c	67.20 c	82.40 c
Glomus fasciculatum	2.93 b	3.39 b	8.68 b	10.64 b	84.20 b	127.80 a
Glomus mosseae	4.26 a	4.53 a	11.83 a	17.44 a	186.00 a	126.00 a
Uninoculated	1.56 b	1.08 c	3.48 c	1.78 c	43.60 d	31.10 d

Means followed by the same letter in the same column do not differ significantly at p = 0.05

Regarding mycorrhizal parameters, maximum mycorrhizal root colonisation and spore numbers were observed in plants inoculated with *G. mosseae*. *G. fasciculatum*-inoculated plants had the next highest mycorrhizal colonisation and spore numbers. The lowest colonisation and spore numbers were experienced by uninoculated plants (Table 3). The biovolume index and quality index were significantly more in plants inoculated with *G. mosseae*, followed by plants inoculated with *G. fasciculatum*, *G. geosporum* and *G. deserticola*, each differing significantly from the other. Uninoculated plants had the lowest biovolume and quality indices (Table 3).

Table 3 Influence of VAM fungi on mycorrhizal colonization, spore number, biovolume index and quality index of neem

VAM fungi	Mycorrhizal colonisation (%)[a]	Spore number (50 ml soil^{-1})	Biovolume index	Quality index
Glomus geosporum	42.44 c	328.00 c	410.87 c	0.53 c
Glomus deserticola	34.65 d	248.60 d	175.32 d	0.46 d
Glomus fasciculatum	58.22 b	395.50 b	478.21 b	0.64 b
Glomus mosseae	63.10 a	433.20 a	611.61 a	0.85 a
Uninoculated	29.65 e	118.00 e	95.31 e	0.29 e

Means followed by the same letter in each column do not differ significantly at p = 0.05
[a]Values are angular transformed

Discussion

Through a preliminary screening trial conducted with VAM fungi available from our culture collection, *G. mosseae* and *G. fasciculatum* proved to be the promising fungi for increasing the growth of neem in the nursery.

Plant height, number of leaves and stem girth were significantly greater in plants inoculated with *G. mosseae* when compared with uninoculated plants. Plant biomass (shoot plus root) was enhanced by about 70% due to *G. mosseae* inoculation compared with uninoculated plants. The increases in plant biomass because of inoculation with *G . fasciculatum*, *G. geosporum* and *G.deserticola* were 58, 36 and 14% respectively compared with uninoculated plants. Such an increase in biomass was reported by Vasanthakrishna *et al.* (1995) in *Casuarina equisetifolia* and Rajan *et al.* (2000) in *Tectona grandis* when inoculated with efficient VAM fungi. Biovolume index and quality index were observed to be high in *G. mosseae*-inoculated plants, suggesting that they are of better quality.

Increased plant growth due to VAM inoculation is mainly through improved uptake of diffusion-limited nutrients such as P, Zn and Cu (Lambert *et al.* 1979, Manjunath *et al.* 1989). VAM fungi improving plant biomass were also good in increasing the P content of the host. The increase in shoot P content was 70% and root P content was 90% respectively in *G. mosseae*-inoculated plants. Similar observations were reported in *Dalbergia sissoo* which showed highest biomass and P

content because of inoculation with *G. fasciculatum* (Sumana & Bagyaraj 1996). Such higher P content in VAM inoculated plants is attributed to higher influx of P into the plant system through VAM fungi which explores the soil volume beyond P depletion zone (Bagyaraj & Varma 1995). Zn concentration in the plant (shoot plus root) was also maximum in *G. mosseae*-inoculated plants. Similar observations have been reported by Reena and Bagyaraj (1990b) in *Acacia nilotica* and *Calliandra calothyrsus,* and by Vasanthakrishna *et al.* (1995) in *Casuarina equisetifolia* seedlings.

In the present investigation, *G. mosseae* produced significantly higher per cent mycorrhizal root colonisation and spore numbers compared with other treatments, both being positively correlated. Higher colonisation allows more fungal host contact and more exchange of nutrients, hence better plant growth (Daft & Nicolson 1972).

The results of this study clearly brings out the poor performance of *G. geosporum* and *G. deserticola,* the two VAM fungi isolated from the root zone soil of neem (six years old) in enhancing the growth of neem seedlings raised in polybags in the nursery. Vasanthakrishna *et al.* (1994) reported the occurrence of mycorrhizal succession in *Casuarina.* They observed that *Glomus* was a dominant genus associated with *Casuarina* younger seedlings. However, the species of VAM fungi occurring in young seedlings were different from older trees. In the present study indigenous fungi associated with neem were isolated from four- to six-year-old trees. Perhaps, in neem also there exists the phenomenon of mycorrhizal succession. Hence, the fungi which occurred later in succession were not the best symbiont to young neem seedlings (one to six months old) in the nursery. Hence, it can be suggested that for selecting efficient VAM fungi to be used for inoculating nursery, isolation must be done from the young host plants of less than a year.

The present study showed that *G. mosseae* is the most promising and the best VAM symbiont for inoculating neem seedlings in the nursery. The next best fungus is *G. fasciculatum.* This technology being simple can easily be adapted by forest nursery men.

References

BAGYARAJ, D. J. & VARMA, A. 1995. Interactions between arbuscular mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid tropics. *Advances in Microbial Ecology* 14: 119–142.

CUENCA, G., HERRERA, R. & MENESES, E. 1990. Effects of VA mycorrhiza on the growth of cacao seedlings under nursery conditions in Venezuela. *Plant Soil* 116: 71–78.

DAFT, M. J. & NICOLSON, T. H. 1972. Effect of endogone mycorrhiza on plant growth IV. Quantitative relationship between the growth of the host and the development of endophyte in tomato and maize. *New Phytologist* 7: 287–295.

GERDEMANN, J. W. & NICOLSON, T. H. 1963. Spores of mycorrhizal endogone species extracted from the soil by wet sieving and decanting. *Transactions of the British Mycological Society* 46: 235–244.

GIOVANNETTI, M. & MOSSE, B. 1980. An evaluation of techniques to measure vesicular-arbuscular infection in roots. *New Phytologist* 4: 489–500.

HABTE, M., MURULEEDHARA, B. N. & IKAWA, H. 1993. Response of neem (*Azadirachta indica*) to soil P concentration and mycorrhizal colonization. *Arid Soil Research and Rehabilitation* 7: 327–333.

HATCHELL, G. E. 1985. Production of bare root seedlings. Pp. 395–402 in Shoulde, E. (Ed.) *Proceedings of Third Biennial Southern Silviculture Research Conference.* 7–9 November 1984. Altanta.

JACKSON, M. L. 1973. *Soil Chemical Analysis.* Third edition. Prentice Hall, New Delhi. 498 pp.

LAMBERT, D. H., BAKER, D. E. & COLE, H. 1979. The role of mycorrhizae in the interaction of phosphorous with zinc, copper and other elements. *Soil Science Society American Journal* 43: 976–980.

LITTLE, T. M. & HILLS, F. J. 1978. *Agricultural Experimentation, Design and Analysis.* John Wiley and Sons, New York. 63 pp.

MANJUNATH, A., HUE, N. V. & HABTE, M. 1989. Response of *Leucaena leucocephala* to vesicular arbuscular colonization and rock phosphate fertilization in an oxisol. *Plant and Soil* 114: 127–133.

OLSEN, S. R. & SOMMERS, L. E. 1982. Phosphorus. Pp. 403–430 in Page, A. L. *et al.* (Eds.) *Methods of Soil Analysis. Part 2: Chemical and Microbiology Properties.* Second edition. American Society of Agronomy, Madison.

PHILLIPS, J. H. & HAYMAN, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55: 158–161.

PORTER, W. H. 1979. The most probable number method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research* 17: 515–519.

RAJAN, S. K., REDDY, B. J. D. & BAGYARAJ, D. J. 2000. Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis. Forest Ecology and Management* 126: 91–95.

REENA, J. & BAGYARAJ, D. J. 1990a. Growth stimulation in *Tamarindus indica* by selected VA mycorrhizal fungi. *World Journal of Applied Microbiology and Biotechnology* 6: 59–63.

REENA, J. & BAGYARAJ, D. J. 1990b. Response of *Acacia nilotica* and *Calliandra calothyrsus* to different VA mycorrhizal fungi. *Arid Soil Research and Rehabilitation* 4: 261–268.

SCHENCK, N. C. & PEREZ, Y. 1987. *Manual for the Identification of VA Mycorrhizal Fungi.* Univeristy of Florida, Gainseville. 245 pp.

SUMANA, D. A. & BAGYARAJ, D. J. 1996. Growth stimulation of *Dalbergia sissoo* by selected VA mycorrhizal fungi. Pp. 246–251 in *Proceedings of the IUFRO Symposium on Impact of Diseases and Insect Pests in Tropical Forests.* Kerala Forest Research Institute, Peechi.

SUMANA, D. A. & BAGYARAJ, D. J. 1998. Effect of VAM fungi on the growth of *Acacia auriculiformis.* Pp. 39–44 in Prakash, A. (Ed.) *Fungi in Biotechnology.* First Edition. CBS Publishers & Distributors, New Delhi.

TEWARI, D. N. 1992. *Monograph on Neem* (Azadirachta indica *A. Juss*). International Book Distributors, Dehra Dun. 279 pp.

VASANTHAKRISHNA, M., BAGYARAJ, D. J. & MUTHANNA, M. B. 1994. VA mycorrhizal fungi associated with *Casuarina equisetifolia. Microbiological Research* 149: 23–25.

VASANTHAKRISHNA, M., BAGYARAJ, D. J. & NIRMALNATH, J. P. 1995. Selection of efficient VA mycorrhizal fungi for *Casuarina equisetifolia*—second screening. *New Forests* 9: 157–162.