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# REDUCING NURSERY PERIOD IN FOUR TROPICAL HARDWOODS BY *RHIZOBIUM* INOCULATION

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**SWAMINATHAN, C. & SURENDRAN, C. 2001. Reducing nursery period in four tropical hardwoods by *Rhizobium* inoculation.** The age of tree seedlings at the time of planting varies from six months to one year and even two years in some species. Such use of nursery areas for protracted periods considerably escalates the cost of seedling production. With a view, therefore, to assessing whether nursery period could be reduced by the use of micro-organisms, four tree species, *Acacia auriculiformis, A. nilotica, Albizia lebbeck* and *Hardwickia binata*, were each inoculated with three different rhizobial strains and a non-specific rhizobium in conjunction with arbuscular mycorrhiza (AM). The results showed that with suitable strains it is possible to reduce the nursery age of the seedlings from six months to four months without compromising seedling vigour or survival rate. The growth of four-month-old inoculated seedlings was comparable with that of six-month-old normal (uninoculated) seedlings, and the survival rate of the former was higher when compared with uninoculated seedlings in all species.

Key words: Inoculation - rhizobial strains - seedlings - survival

**SWAMINATHAN, C. & SURENDRAN, C. 2001. Mengurangkan tempoh tapak semaian di dalam empat kayu keras tropika melalui penginokulatan *Rhizobium*.** Umur anak benih pokok pada masa penanaman berubah-ubah daripada enam bulan kepada satu tahun, malah dua tahun dalam sesetengah spesies. Penggunaan kawasan tapak semaian seperti ini untuk tempoh yang panjang ternyata meningkatkan kos pengeluaran anak benih. Oleh yang demikian, bagi menilai sama ada tempoh di tapak semaian dapat dikurangkan dengan menggunakan mikroorganisma, empat spesies pokok iaitu *Acacia auriculiformis, A. nilotica, Albizia lebbeck* dan *Hardwickia binata* diinokulat dengan tiga keterikan rizobia dan satu rhizobium tak spesifik berkaitan dengan mikoriza arbuskular (AM). Keputusan menunjukkan bahawa dengan keterikan yang sesuai, umur anak benih di tapak semaian dapat dikurangkan daripada enam bulan kepada empat bulan tanpa mengambil kira kecergasan anak benih atau kadar kemandirian. Pertumbuhan anak benih berumur empat bulan yang diinokulat adalah setanding dengan anak benih biasa berumur enam bulan (tidak diinokulat), dan kadar kemandirian anak benih yang berumur empat bulan adalah lebih tinggi jika dibandingkan dengan anak benih yang tidak dinokulat di dalam kesemua spesies.

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## Introduction

The age of tree seedlings for afforestation and tree planting programmes in different parts of South India varies from six months (e.g. acacias, albizias) to one year (e.g. teak, bamboo) and even two years in some species (e.g. tamarind). Seedlings of most tropical hardwoods are raised in a nursery for this period before they are out-planted. Such use of nursery areas for protracted periods considerably escalates the cost of seedlings production. To reduce this, seedlings can be inoculated with microbial synergestic associates without compromising seedling vigour and growth rate. The response of nitrogen fixers to artificial inoculation of rhizobia has been very well documented (Basak & Goyal 1975, Dreyfus & Dommergues 1981, Lawrie 1983). The use of rhizobia as a microbial inoculant for leguminous species is common in crops. Sprent (1985, 1986) and her associates in Scotland and Brazil carried out extensive studies on nodulation of woody species and updated our knowledge on nodulation in legumes. However, in tropical hardwoods, information on the influence of rhizobia on the growth of seedlings, the consequent reduction of time in the nursery, and post planting performance is scant. Four multipurpose tree legumes, viz. *Acacia auriculiformis, A. nilotica, Albizia lebbeck* and *Hardwickia binata,* which are promising species in different parts of Tamil Nadu, were selected to assess whether the nursery period can be reduced through the use of synergestic associates of micro-organisms. The selected tree species were each inoculated with different rhizobial strains and their response studied in the nursery and the field.

## Materials and methods

The study was conducted during 1993–95 at the Forest College and Research Institute, Mettupalayam, India (11° 19 'N, 76° 56 'E; 300 m above sea-level), which receives an annual rainfall of 830 mm. Mature pods were collected from 10-y-old woodlots of *Acacia nilotica* and *A. auriculiformis* and from 15-y-old trees of *Albizia lebbeck* and *Hardwickia binata* growing in the Alfisol of the College campus and the seeds extracted manually. Rhizobial strains, viz. ALMK, ANM 12, SSM 22 (strains isolated from the nodules of *A. nilotica, A. leucophloea* and *Albizia saman* respectively), a non-specific rhizobial strain commonly used for crops and arbuscular mycorrhiza (AM) were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. Seeds of the four tree species were acid scarified, washed and then inoculated using the following treatments:

- Inoculated with ALMK
- Inoculated with ANM 12
- Inoculated with SSM 22
- Inoculated with non-specific strain + AM (which is added to the soil mixture)
- Uninoculated control

Seeds were sown in polybags of 20 × 10 cm size with two holes at the bottom, filled with red earth, sand and farmyard manure at the ratio of 3:1:1. Inoculated seeds were sown two months later from the date of sowing of uninoculated seeds. One hundred seeds represented a replication and there were four replicates. After four months (i.e. six months for control seedlings) measurements of plant height, collar diameter, root length, nodule number and dry matter production (DMP) were recorded. As a continuation of the nursery study, a field study was also conducted using the same seedlings to assess the survival rate of treated seedlings. The seedlings were out-planted at a spacing of 2 × 2 m in a factorial randomised block design with four replications and their growth performance studied for 12 months. The data collected from both the studies were subjected to ANOVA after Panse and Sukhatme (1967).

## Results and discussion

Six-month-old containerised seedlings are used in different regions of Southern India for afforestation and various tree planting programmes, because the survival of planted seedlings is largely dictated by the below-ground root biomass which is optimal in seedlings of six months age. It is well known that inoculation with rhizobium ensures better root development and shoot growth. The present study focused on comparing the performance, at seedling stage and in the field, of six-month-old normal seedlings and four-month-old inoculated seedlings.

### *Growth attributes*

Above-ground and below-ground biomass data at the time of planting (four months for inoculated and six months for normal seedlings) of the four species are presented in Tables 1 and 2. Plant height was influenced by rhizobial inoculation in all species. The height of four-month-old inoculated seedlings was either similar to or greater than that of six-month-old normal seedlings indicating the feasibility of reducing time in the nursery by two months. This is ascribed to the synergestic association between rhizobium and legumes which has been well documented (Subbarao 1967, 1976, 1981, Hamidi 1976, Balaji 1985). The same trend was observed in all other growth parameters measured, i.e. diameter, root length, nodule number, fresh root and shoot weights and DMP. These observations conformed with those of Sharma *et al.* (1990).

### *Species*

The mean height at planting of the four species was 26.6 cm (*A. nilotica*), 22.1 cm (*A. lebbeck*), 11.1 cm (*A. auriculiformis*) and 8.6 cm (*H. binata*). *Albizia lebbeck* had the greatest diameter and number of nodules. *Hardiwickia binata* had the highest DMP (2.2 g) despite its small height and diameter. This indicates that *H. binata* produces heavier seedlings. The variations observed among species were in accordance with the findings of Pancholy (1991).

**Table 1.** Effect of microbial inoculate on above-ground biomass of seedling

| Treatment | Plant height (cm) | | | | Diameter (cm) | | | | Fresh shoot weight (g) | | | | Dry matter production (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA | AN | AL | HB | AA | AN | AL | HB | AA | AN | AL | HB | AA | AN | AL | HB |
| ALMK | 9.9 | 28.3 | 23.8 | 8.6 | 0.22 | 0.44 | 0.99 | 0.29 | 0.72 | 1.47 | 0.74 | 2.4 | 0.88 | 1.23 | 0.93 | 2.2 |
| ANM 12 | 10.7 | 29.8 | 20.4 | 6.4 | 0.21 | 0.42 | 0.88 | 0.23 | 0.89 | 1.76 | 0.79 | 2.2 | 1.03 | 1.26 | 1.09 | 2.0 |
| SSM 22 | 12.8 | 26.2 | 24.2 | 6.9 | 0.28 | 0.39 | 0.97 | 0.21 | 1.30 | 1.27 | 1.52 | 2.5 | 2.85 | 1.31 | 1.11 | 2.0 |
| R+AM | 12.2 | 26.0 | 20.1 | 6.0 | 0.25 | 0.42 | 1.04 | 0.19 | 1.24 | 1.66 | 0.94 | 2.6 | 1.03 | 1.28 | 1.08 | 2.2 |
| Control | 10.8 | 23.0 | 22.0 | 7.7 | 0.24 | 0.38 | 0.85 | 0.18 | 1.04 | 0.94 | 0.95 | 3.2 | 1.14 | 0.91 | 1.04 | 2.8 |
| SEd | 1.37 | 2.19 | 0.67 | 0.83 | 0.06 | 0.05 | 0.11 | 0.03 | 0.19 | 0.13 | 0.12 | 0.7 | 0.12 | 0.07 | 0.10 | 0.5 |
| CD | ns | 4.78 | ns | 1.74 | ns | ns | ns | 0.07 | 0.39 | 0.22 | ns | ns | 0.25 | 0.15 | ns | ns |

AA - *Acacia auriculiformis*, AN - *Acacia nilotica*, AL - *Albizia lebbeck*, HB - *Hardwickia binata*.
Control seedlings were six months old and inoculated seedlings were four months old.

**Table 2.** Effect of microbial inoculate on below-ground biomass of seedlings

| Treatment | Root length | | | | Nodule number | | | | Fresh root weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA | AN | AL | HB | AA | AN | AL | HB | AA | AN | AL | HB |
| ALMK | 18.9 | 28.1 | 15.1 | 34.8 | 1.2 | 2.5 | 4.2 | - | 0.68 | 1.09 | 1.33 | 2.76 |
| ANM 12 | 20.0 | 27.0 | 18.2 | 24.2 | 4.3 | 6.2 | 6.6 | - | 0.96 | 0.86 | 1.19 | 2.41 |
| SSM 22 | 23.7 | 23.5 | 18.4 | 24.7 | 6.9 | 3.9 | 4.8 | - | 0.98 | 1.27 | 1.52 | 2.19 |
| R+AM | 26.6 | 30.7 | 17.1 | 27.8 | 2.7 | 4.6 | 4.2 | - | 1.12 | 2.04 | 1.49 | 2.49 |
| Control | 25.2 | 21.5 | 17.4 | 29.7 | 2.8 | 2.3 | 5.6 | - | 0.86 | 0.94 | 1.30 | 3.28 |
| SEd | 1.9 | 1.1 | 0.7 | 0.5 | 0.8 | 0.2 | 1.1 | - | 0.06 | 0.17 | 0.14 | 0.15 |
| CD | 3.9 | 2.3 | 1.3 | 1.0 | 1.8 | 0.5 | ns | - | 0.12 | 0.38 | ns | 0.29 |

AA - *Acacia auriculiformis*, AN - *Acacia nilotica*, AL - *Albizia lebbeck*, HB - *Hardwickia binata*.
Control seedlings were six months old and inoculated seedlings were four months old.

### *Rhizobial strains*

Among the strains tested, inoculation with SSM22 produced the greatest response in *A. auriculiformis* for parameters such as plant height (12.8 cm), collar diameter (0.28 cm), nodule number (6.9), green shoot weight (1.30 g) and DMP (2.85 g). However, the longest and heaviest root of *A. auriculiformis* was produced in plants inoculated with the non-specific strain + AM, indicating that AM is important for root production and proliferation.

ANM 12 produced the greatest response in *A. nilotica* for all growth parameters except root length and root weight, which were also greatest in plants inoculated with R + AM. The response of *A. lebbeck* to the rhizobial treatments was different. SSM 22 strain had the greatest effect for all growth parameters except diameter and nodule number, which were most influenced by R + AM and ANM 12 respectively. The effect of age of the control seedlings was seen in the fresh root and shoot weights and DMP of *H. binata*. Although the longest root was observed in the ALMK inoculated seedlings of this species, the control gave longer root length than the other three treatments. Thus, for *H. binata* the age of seedlings is more important than height. The results also indicate that it is imperative to select rhizobial strains which are geographically and genetically suited to the legume species (Pancholy 1991). Rhizobium isolates from different tree species show wide variation in rhizobial specificity (Dobereinor 1984). Some isolates exhibit cross inoculation features and some do not. However, the observations made here with the limited tree species are neither exhaustive nor conclusive. A careful culture collection of rhizobia from a large number of tree species and cross inoculation merit consideration.

### *Field performance*

Plant height and survival data for the four species after 12 months in the field are presented in Figures 1 and 2. In the two acacias the inoculated seedlings generally grew better than the control seedlings. In *A. auriculiformis* the rhizobial strain SSM 22 was 26% taller than the control. Survival was comparable between inoculated and uninoculated control seedlings for all four species, although the ANM 12 rhizobial strain was effective at improving survival. Microbial inoculates and phosphate solubilising micro-organisms may help in the establishment of crops (Subbarao 1977). Besides ensuring better seedling growth and vigour (Pokhriyal *et al.* 1987), inoculants also improve the host plant's nutrient uptake and drought tolerance (Verma *et al.* 1994). Jamaladdin *et al.* (1995) found that the most effective rhizobium species was different for each tree species.

## Conclusion

Seedling growth and post planting survival of leguminous tree species are improved by inoculation with compatible rhizobial strains. Different species show differential responses to inoculations by a range of rhizobial strains. For

*A. auriculiformis*, SSM 22 strain is most suitable, for *A. nilotica*, ANM 12, for *A. lebbeck* SSM 22 and ANM 12, and for *H. binata* all strains produced a significant response. This study clearly demonstrates that it is possible to reduce the nursery period of tree seedlings without compromising seedling vigour or survival. This will reduce the cost of seedling production by saving expenditure on water, chemicals and labour for maintenance for two months.

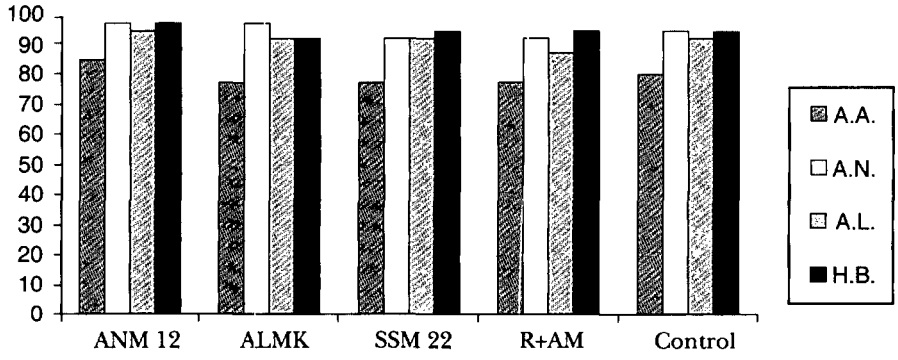


**Figure 1.** Field performance (survival %) of inoculated seedlings

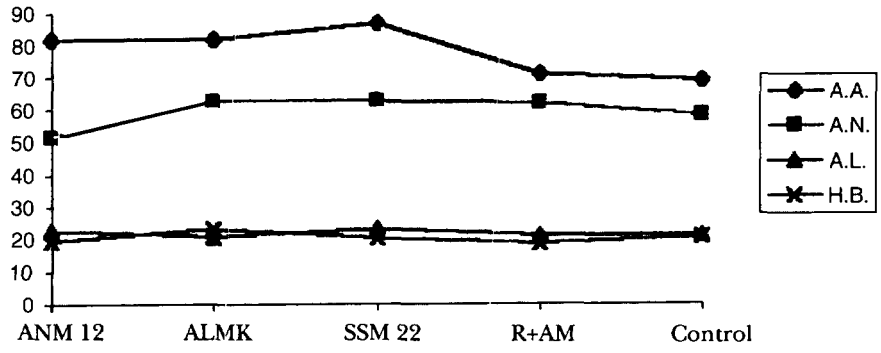


**Figure 2.** Field performance (height, cm) of inoculated seedlings

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## References

BALAJI, S. 1985. Studies on Symbiotic Nitrogen Fixation by Certain Tree Legume Rhizobia. M.Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore. 85 pp.

BASAK, M. K. & GOYAL, S. K. 1975. Studies on tree legumes I: nodulation pattern and characterisation of the symbiont. *Annals of Arid Zone* 14(4):367–370.

DOBEREINOR, J. 1984. *Nodulaco a fixacao de nitrogenic em Leguminosas florestains.* Pesq Agropec Bras. 19 S/n. 83 pp.

DREYFUS, B. L. & DOMMERGUES, Y. R. 1981. Nodulation of *Acacia* species by fast and slow growing tropical strains of rhizobium. *Applied Environmental Microbiology* 41:97–99.

HAMIDI, V. P. 1976. Field and glass house experiments on the response of legumes in Egypt to inoculation and fertilizers. Pp. 289–298 in Nutman, P. S. (Ed.) *Symbiotic Nitrogen Fixation in Plants.* Cambridge University Press, Cambridge

JAMALUDDIN, DADWAL, V. S. & CHOUHAN, J. S. 1995. Efficacy of different *Rhizobium* strains of forest tree species on *Albizia lebbeck. Indian Forester* 121(7):647–650.

LAWRIE, A. C. 1983. Relationships among rhizobia from native Australian legume. *Applied Environmental Microbiology* 45 (6):1822–1828.

PANCHOLY, A. 1991. Studies on the status of indegenous soil rhizobia associated with certain arid zone legume trees. *Nitrogen Fixing Tree Research Reports* (9):9–10.

PANSE, P. G. & SUKHATME, P.V. 1967. *Statistical Methods for Agricultural Workers.* Indian Council of Agricultural Research, New Delhi, India. 380 pp.

POKHRIYAL, T. C., RATURAI, A. S., PANT, S. P., PANDE, S. K. & BHATNAGAR, S. K. 1987. Nitrogen fixation in *Albizia, Acacia, Dalbergia* and *Leucaena. Indian Forester* 13(5):366–369.

SHARMA, P. R., NIRANJAN, B. & RAO, U. A. 1990. *Interaction between Rhizobium and Mycorrhizal Fungi and their Stimulatory Effect on Acacia nilotica.* Current Trends in Mycorrhizal Research, Haryana Agricultural University, Hisar. 180 pp.

SPRENT, J. I. 1985. Nitrogen fixation in arid environments. Pp. 215–229 in Wickens, G. E., Goodin, J. R. & Field, J. R. (Eds.) *Plants for Acid Lands.* George Allen and Unwin, London.

SPRENT, J. I. 1986. Potentials for nitrogen fixing legumes in the tropics. *International Tree Crops Jounral* 4:47–51.

SUBBARAO, N. S. 1967. Mechanism of infection of legume roots by *Rhizobium. Journal of Scientific and Industrial Research* 26:24–37.

SUBBARAO, N. S. 1976. Field response of legumes in India to inoculation and fertilizer application. Pp. 255–268 in Nutman, P. S. (Ed.) *Symbiotic Nitrogen Fixation in Plants.* Cambridge University Press, Cambridge.

SUBBARAO, N. S. 1977. *Soil Micro-organisms and Plant Growth.* Oxford and IBH, Publishing Co. New Delhi. 289 pp.

SUBBARAO, N. S. 1981. *Biofertilizers in Agriculture and Forestry.* Oxford and IBH Publications, New Delhi. 240 pp.

VERMA, R. K., JAMALUDDIN & GUTTA, B. N. 1994. Effect of inoculation of VAM fungi and rhizobium on growth and biomass production in *Acacia nilotica. Indian Forester* 120(12):1089–1094.