

RESPONSE OF SOME NITROGEN FIXING TREE SEEDLINGS TO *RHIZOBIUM* CROSS-INOCULATION

BEKUNDA, M.A.

Department of Soil Science, Makerere University, P.O. Box 7062, Kampala, Uganda

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ABSTRACT

Six introduced and one native nitrogen fixing tree species were grown in a potted non-sterilized Inceptisol inoculated with: species-specific rhizobia strains; *Rhizobium* strain TAL 1145, a *Leucaena* isolate; *Bradyrhizobium japonicum* strain TAL 169, and without inoculation. Twelve weeks later, significant species differences in dry matter yield, total N yield and nitrogen derived from the atmosphere (Ndfa) were obtained. Unlike earlier reports, *Acacia albida* and *Acacia senegal* were better N₂ fixers than *Leucaena leucocephala*. Two species did not respond to any of the inoculation treatments and none responded to inoculation with TAL 169. In species belonging to one cross-inoculation group, TAL 1145 had similar Ndfa values as those of species-specific rhizobia, implying that it is sufficient as the inoculum for this group.

Key Words : Cross-inoculation, *Rhizobium* strains, reference crop, isotope dilution.

INTRODUCTION

In earlier studies of the *Rhizobium*/legume association, "cross-inoculation" as a grouping of leguminous plants and the designation of *Rhizobium* species was based entirely on nodulation without reference to host benefit (7). Later reports indicate that a *Rhizobium* strain that is nitrogen-fixation effective in association with one plant species may also be effective with certain other species (1). Thus, leguminous species which nodulate with a range of rhizobia may establish reasonably well over large areas without inoculation due to the presence of indigenous *Rhizobium* strains. Similarly, they can prosper after inoculation with only one *Rhizobium* strain. However, it has been shown that considerable differences can occur between rhizobia in their

effectiveness on particular nitrogen fixing tree (NFT) hosts (6). It is, therefore, important that in selecting rhizobia for cross-inoculation, symbionts are prudently matched for maximum nitrogen fixation.

Nitrogen fixation abilities of a range of NFTs inoculated with rhizobia isolated from commonly grown legumes were evaluated in order to select a strain that could be used as a common inoculant.

MATERIALS AND METHODS

The research was conducted at the International Atomic Energy Agency (IAEA) Laboratory, Austria, in a glasshouse under natural light and internal air day and night temperatures of 28°C and 20°C respectively and relative humidity between 60% and 70%.

TABLE 1. Species-specific *Rhizobium* strains used to inoculate the various nitrogen fixing trees

| NFT species/genotype ^a | Rhizobium ^b | Parent host |
|-------------------------------------|------------------------|---------------------------|
| <i>Acacia albida</i> | TAL 1881 | <i>A. decurrens</i> |
| <i>Acacia senegal</i> | TAL 1428 | <i>A. senegal</i> |
| <i>Acacia auriculaeformis</i> | TAL 1446 | <i>A. auriculaeformis</i> |
| <i>Calliandra calothyrsus</i> | TAL 1806 | <i>C. calothyrsus</i> |
| <i>Prosopis juliflora</i> | TAL 600 | <i>P. chilensis</i> |
| <i>L. leucocephala</i> (Hengchun) | TAL 1887 | <i>L. leucocephala</i> |
| <i>L. leucocephala</i> (Cunningham) | TAL 1887 | <i>L. leucocephala</i> |
| <i>Gliricidia sepium</i> (84/14) | TAL 1779 | <i>G. sepium</i> |
| <i>Gliricidia sepium</i> (85/33) | TAL 1770 | <i>G. sepium</i> |

^a Apart from *A. albida*, the NFT species are not indigenous to the Lake Basin Region of Uganda from where the soils were collected.

^b *Rhizobium* strains were obtained from the NifTAL Project.

The soil used in the study was from Kabanyolo, Uganda (00°-28'-10"-N; 032°-36'-48"-E) representing some soils of the Lake Victoria Basin. The soil was Aeris Tropaquept (14) with the following characteristics: sand 50.5%; silt 17.1%; clay 32.4%; pH (water) 4.7; organic C 1.86%; total N 0.14%; CEC 17.5 me 100g⁻¹. The soil was air dried, sieved (<2 mm), mixed with quartz sand in a ratio of 1:1 and transferred to plastic pots (3 kg/pot).

Nine NFT species or genotypes were grown in soil inoculated with the following:

- their species-specific *Rhizobium* strains (Table 1);
- Rhizobium* strain TAL 1145, a *Leucaena leucocephala* isolate;
- Bradyrhizobium japonicum* strain TAL 169; and
- without inoculation.

The strains were cultured separately in yeast mannitol broth (12) for 7 days. Twenty ml of each inoculum (about 10⁹ cells ml⁻¹) were diluted to 100 ml with distilled water and mixed with soil before planting. Four seeds were planted in pots after scarification in concentrated sulphuric acid for 30 minutes. Two weeks after germination, seedlings were thinned to two per pot and ¹⁵N labelled ammonium sulphate (20 atom % excess at 5 mg N kg⁻¹) was added in solution at 40 ml per pot. Treatments were replicated three times and arranged in a completely randomized design.

Hoagland nutrient solution, free of N, was applied in 20 ml aliquots to each pot, weekly. Moisture was maintained near field capacity by weighing and replacing the deficit with demineralized water.

Whole plants were harvested after twelve weeks of growth. Shoots were harvested by cutting 10 mm above the soil surface. Roots and nodules were carefully separated from the soil and the adhering soil washed off with a jet of water against a 1.0 mm sieve. Nodules were then picked off the roots. Samples were oven dried at 70°C for 48 hr, weighed and ground to pass a 0.5 mm sieve. Shoots and roots (roots + nodules) were analyzed separately and later integrated on a whole plant basis. Total N and isotopic N analyses were by the automatic nitrogen analyzer (1500 Carlo Erba) coupled to a SIRA mass spectrometer (8). The isotope dilution equation (9) was used to calculate the proportions of nitrogen derived from the atmosphere (Ndfa) by nodulated plants. *Cassia siamea* and *Cassia spectabilis* (mean values) were the reference plants.

RESULTS

Significant dry matter yield increases in response to inoculation with species-specific rhizobia were observed in *G. sepium* genotypes and in *A. senegal*, while increases in response to inoculation with TAL 1145 were obtained in *G. sepium* genotypes and in *C. calothyrsus* (Fig. 1a). There

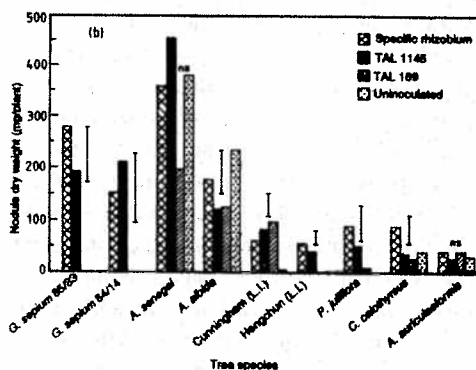
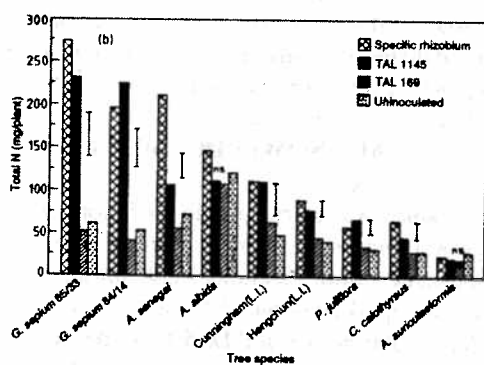
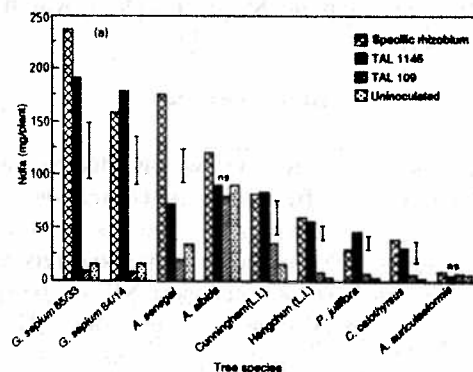
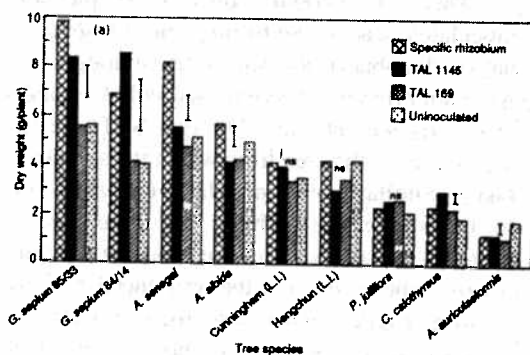


Fig. 1. Effect of *Rhizobium* cross-inoculation on dry matter yield (a) and total nitrogen yield (b) of seedlings of different NFT species and genotypes grown in pots. (L.I. = *Leucaena leucocephala*; Vertical bars = LSD, 5%).

Fig. 2. Effect of *Rhizobium* cross-inoculation on nitrogen fixation (a) and nodule mass of seedlings (b) of different NFT species and genotypes. (L.I. = *Leucaena leucocephala*; Vertical bars = LSD, 5%).

was no response to inoculation with TAL 169. Mean dry matter yield differed between species, with *Gliricidia sepium* genotypes yielding most and *Acacia auriculataeformis* yielding least.

All tree seedlings inoculated with species-specific rhizobia showed significant total N yield increases except *A. albida* and *A. auriculataeformis* (Fig. 1b). *G. sepium*, *L. leucocephala* genotypes and *P. juliflora* responded to inoculation with TAL 1145. Similarly, no significant response to inoculation with TAL 169 was observed. Where response to TAL 1145 and to the species-specific rhizobia occurred simultaneously, there were no

significant differences between the two treatments.

Inoculating the plants with their specific rhizobia or TAL 1145 increased nitrogen fixation in all species except *A. albida* and *A. auriculataeformis*. (Fig. 2a). The level of fixation was similar for both strains, except in *A. senegal* where TAL 1145 was inferior to TAL 1428. Again, no significant response to inoculation with TAL 169 was observed. In *A. senegal* and *C. calothyrsus* only species-specific rhizobia increased nitrogen fixation although nodulation was high in all treatments (Fig. 2b). As percent of total N yield, Ndfa, values ranged from 47.2% for

C. calothyrsus inoculated with TAL 1145 to 86.9% for *G. sepium* 85/33 inoculated with its specific *Rhizobium* TAL 1170.

DISCUSSION

Rhizobium strain TAL 1145 was as effective as the species-specific strains in *G. sepium*, *P. juliflora*, *C. calothyrsus* and its host *L. leucocephala*. This agrees with previous reports (1;11) which have indicated these NFTs as being in one cross-inoculation grouping. Some NFTs, including *A. albida*, *G. sepium* and *L. leucocephala*, are known to nodulate with *Bradyrhizobium* strains (5). In this study, however, *B. japonicum* did not nodulate any of the trees.

Ndfa values were obtained for the non-nodulated *G. sepium*, *L. leucocephala* and *P. juliflora*. These are residual values resulting from the unfulfilled assumption that NFTs and reference trees have similar growth characteristics (4), as confirmed by the absence of nodules on roots of the three NFT species (Fig. 2b). Nevertheless, the ^{15}N method for measuring biological nitrogen fixation is considered more accurate than other methodologies (2; 3; 13) although its application in NFTs has been mainly on young trees (4). In this study, it enabled detection of differences in N_2 fixation among various NFT species and genotypes, and in *Rhizobium* strain effectiveness. For example *G. sepium* was a better fixer than all the other tree species — a potential that has been shown in other studies only when using the ^{15}N methodology (3).

Dommergues (5) and Sanginga *et al.* (10) considered that there are high nitrogen fixing potential tree species (*Acacia mangium* and *Leucaena leucocephala*) and low nitrogen fixing potential trees (*A. albida* and *A. senegal*). This grouping was not reflected in the present studies where %Ndfa values for *A. senegal* (82.6) and *A. albida* (81.9) were significantly higher than those of *L. leucocephala* Cunningham (72.7) and Hengchun (66.4). The differences in these results may be due to soil pH. Trees were grown in an acid tropical soil (pH 4.8) to which the Acacias are naturally adapted while those in the study by Sanginga *et al.* (10) were grown in an alkaline soil (pH 8.3). This illustrates the importance of comparing NFTs of similar adaptation when

inoculated with their specific rhizobia.

There were nodules on roots of the non-inoculated acacias confirming the presence of native rhizobia in the soil used, although they were not effective in symbiosis with *A. senegal* and *A. auriculaeformis*. The two NFTs are not indigenous to the area from which the soils were taken. Similarly, non-inoculated *C. calothyrsus* formed ineffective nodules. In these species, therefore, competition for nodulation sites between the native and the introduced rhizobia could have reduced their response to inoculation.

This study confirms the need to inoculate introduced NFTs with appropriate *Rhizobium* strains if nitrogen fixation is to be enhanced. In this case, TAL 1145 is effective with NFT species belonging to one cross-inoculation group, and could therefore be produced as a single inoculant for these trees at introduction.

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