# Specificity of rhizobial strains for effective $N_2$ fixation in the genus *Leucaena*

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## Abstract

Twenty-seven accessions of *Leucaena*, representing 19 species, were assessed for effective N-fixing symbioses in association with 13 strains of *Rhizobium* (derived primarily from *Leucaena* spp. hosts) in N-free conditions in a glasshouse experiment. Two major leucaena accession groups were identified using pattern analysis. Fifteen of the 27 accessions of *Leucaena* associated with a wide range of rhizobial strains and only 4 accessions appeared to have specific rhizobial strain requirements for effective nitrogen fixation.

Nine of the 13 strains of *Rhizobium* formed highly effective associations with most accessions of *Leucaena* and only 1 strain (CB3299) was ineffective for $N_2$ fixation with all accessions. Rhizobial strains CB3126, NGR8 and CB3060 were the most broadly effective, associating highly effectively with 22, 17 and 21 accessions of *Leucaena,* respectively.

Accessions of *Leucaena* were grouped according to rhizobial strain effectiveness, but biological characters such as native range, ploidy level and chromosome number correlated poorly with accession groups.

## Introduction

With the exception of *Leucaena leucocephala*, little is known about rhizobial strain specificity for effective nitrogen fixation in the *Leucaena* genus. *L. leucocephala* is nodulated effectively by native rhizobia in Malaysia (Wong *et al.* 1989), Thailand (Homchan *et al.* 1989), Hawaii (Singleton and Tavares 1986), Australia (Bushby 1982; Jones and Date 1995) and India (Basavaraju and Hegde 1983), but the onset of nitrogen fixation and early growth of uninoculated plants is comparatively slow. Furthermore, considerable intraspecific variation in *Rhizobium* association has been identified within *L. leucocephala* (R.A. Date, unpublished data) and accession × *Rhizobium* strain testing may be necessary. Strain CB3060 is the current commercial inoculant for *L. leucocephala* in Australia and is widely used internationally. It was isolated from *L. diversifolia* growing at the CSIRO Lansdown Field Station in north Queensland, Australia, is effective with a range of tree legume species (Halliday and Somasegaran 1983), has good competitive ability and persistence in the presence of native and introduced rhizobia, and nodulates leucaena under acid-soil conditions (Moawad and Bohlool 1984; Wong *et al.* 1989).

Studies on specificity of *Rhizobium* for lesser-known species of *Leucaena* are conflicting. Early research by Halliday and Somasegaran (1983) suggested that large variation in response to strains of *Rhizobium* was likely both within and between species of *Leucaena* for ability to nodulate and establish effective symbiosis. Highly effective associations between CB3060 for *L. diversifolia* and its hybrid with *L. leucocephala* (KX3) (Somasegaran and Martin 1986), *L. greggii, L. pallida, L. pulverulenta* and *L. retusa* (Date *et al.* 1993), *L. lanceolata* and *L. shannonii* (Halliday and Somasegaran 1983) have been reported. However, Halliday and Somasegaran (1983) reported poor nodule formation on *L. pulverulenta* by CB3060 and no nodule formation on *L. retusa*.

In view of the current interest in agronomic evaluation of large numbers of accessions of *Leucaena* (Hughes 1993; Shelton *et al.* 1995), it

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is important to understand more of the specificity of the *Leucaena* x *Rhizobium* interactions for effective $N_2$ fixation, as ineffective associations may limit yield potential. Accordingly, the extent of this specificity was examined in a glasshouse experiment with 13 strains of *Rhizobium* and 27 accessions of *Leucaena*.

## Materials and methods

The experiment was conducted during the summer of 1996 at the CSIRO Cunningham Laboratory, Brisbane, Australia. Accessions of *Leucaena* were selected to represent 19 species and their subspecies and 2 inter-specific hybrids (Table 1). The accessions were grown in sand-culture, using a modified Leonard-jar technique (Norris and Date 1976). Jars, complete with sand and nutrient solution, were autoclaved to provide bacteria-free conditions prior to sowing and inoculation. Ultraviolet-sterilised, deionised water was used to replace water used by plants. Four pregerminated seeds were sown into each sand-jar and thinned to 3 after a 10-day establishment period.

Thirteen strains of *Rhizobium* were selected from the CSIRO Australian Tropical Forages Genetic Resource Centre collection. The strains originated predominantly from species of *Leucaena* and from diverse geographical locations. They included current and previous commercial *Leucaena* inoculant strains (Table 2). Cultures for inoculation of seedling plants were grown in yeast extract-mannitol broth. One ml of culture, containing $10^8$–$10^9$ colony forming units (cfu)/ml, was applied to each sand-jar following thinning of seedlings.

Two uninoculated control treatments were included: a zero nitrogen treatment to verify that *Rhizobium*-free conditions had been maintained (–N), and a luxury nitrogen fertiliser treatment (+N), to identify, by comparing growth, strains worthy of field testing. Nitrogen was applied to the luxury N treatment as $KNO_3$ in split applications (10 and 20 days after planting) at rates to support a predicted maximum total dry matter (DM) of 7.5 g DM/jar (B.F. Mullen, unpublished data).

A completely randomised design with 2 replications was used. Sand-jars were re-randomised at 2-weekly intervals during the growing period.

### *Sampling*

Change in leaf colour was monitored by rating against the Royal Society of London colour charts 143 and 144, at weekly intervals from the second week after planting, to ascertain the time to onset of effective nodulation. Plants were harvested 8 weeks after planting and plant tops were dehydrated for 48 hours at 60°C, separated into leaf and stem and weighed. Plant roots were washed clean of sand, dehydrated for 72 hours at 60°C and weighed. During harvesting, washed roots were scored for nodulation (0 = no nodules present; 5 = very heavily nodulated), nodule size (average nodule length) and nodule colour (1 = all white; 3 = brown/pink/white; 5 = all pink).

### *Data analysis*

Rhizobial strain effectiveness was examined within accessions of *Leucaena* by ANOVA using total plant dry weights/jar (Norris and Date 1976). Inherent differences in DM productivity prevent comparison between accessions of *Leucaena*, so each accession was analysed individually for dry weight response to strains of *Rhizobium*. To compare leucaena accession x rhizobial strain interactions, DM data were converted to an index of effectiveness (IE), expressed as a percentage of non-limiting nitrogen control (Haydock *et al.* 1980). The IE was used to examine strain x accession interactions. Categories of effectiveness were: 0–35% IE – ineffective; 35–49% IE – partially effective; 50–79% IE – effective; ≥80% IE – highly effective. Strains acheiving an IE of ≥80% are worthy of field testing (R.A. Date, personal experience).

Accessions of *Leucaena* were grouped according to their rhizobial strain responses using a hierarchical, agglomerative clustering technique, based on incremental sums of squares and using squared Euclidean distance as a dissimilarity measure (Belbin 1990). Data were standardised within accessions to remove accession DM productivity from the analysis, permitting direct evaluation of rhizobial strain effectiveness.

Explanations of leucaena accession group membership were attempted by examining similarities and dissimilarities in the endemic location (Hughes 1993) and taxonomy (Harris *et al.* 1994) of accessions within the groups.

**Table 1.** Summary information for accessions of *Leucaena* used in the study.

| Species/subspecies | Accession ID | Ploidy level | Country of origin | Latitude | Altitude range for accession | Annual rainfall | Mean annual temperature |
|---|---|---|---|---|---|---|---|
| | | | | | (m) | (mm) | (°C) |
| *L. collinsii* subsp. *collinsii* | OFI 52/88 | 2n=2x=52 | Mexico | 16°36′ | 400–550 | 948 | 24.7 |
| *L. collinsii* subsp. *zacapana* | OFI 56/88 | ? | Guatamala | 15°07′ | 100–200 | 723 | 27.2 |
| *L. cuspidata* | OFI 83/94 | 2n=4x=112 | Mexico | 20°41′ | 2040 | na[2] | na |
| *L. diversifolia* | OFI 83/92 | 2n=4x=104 | Mexico | 18°04′ | 350–500 | 3435 | 21.9 |
| *L. diversifolia* × *L. leucocephala*[1] | K156xK8 | 2n=4x=104 | | | | | |
| *L. trichandra* | OFI 4/91 | 2n=2x=52 | Honduras | 14°12′ | 1850–2000 | 1462 | 17.3 |
| *L. trichandra* | OFI 53/88 | 2n=2x=52 | Guatamala | 14°49′ | 1400–1450 | 1121 | 21.6 |
| *L. esculenta* subsp. *esculenta* | OFI 47/87 | 2n=2x=52 | Mexico | 18°18′ | 1400–1700 | 1264 | 21.9 |
| *L. esculenta* subsp. *matudae* | OFI 49/87 | ? | Mexico | 17°51′ | 550–750 | 730 | 27.7 |
| *L. greggii* | OFI 82/87 | 2n=2x=56 | Mexico | 24°50′ | 1450–1700 | 393 | 18.9 |
| *L. involucrata* | OFI 87/92 | ? | Mexico | 28°55′ | 700 | 614 | 18.6 |
| *L. lanceolata* subsp. *lanceolata* | OFI 43/85 | 2n=2x=52 | Mexico | 16°02′ | 10 | 1041 | 27.2 |
| *L. lanceolata* subsp. *sousae* | OFI 50/87 | 2n=2x=52 | Mexico | 16°02′ | 0–100 | 1039 | 28.2 |
| *L. lempirana* | OFI 6/91 | ? | Honduras | 15°17′ | 200 | 1116 | 24.5 |
| *L. leucocephala* subsp. *glabrata*[1] | Cunningham | 2n=4x=104 | | | | | |
| *L. macrophylla* subsp. *macrophylla* | OFI 55/88 | 2n=2x=52 | Mexico | 18°00′ | 1100–1200 | 622 | 28.8 |
| *L. macrophylla* subsp. *nelsonii* | OFI 47/85 | ? | Mexico | 15°59′ | 10 | 1039 | 28.2 |
| *L. multicapitula* | OFI 81/87 | 2n=2x=52 | Panama | 7°55′ | 10–50 | 1075 | 27.7 |
| *L. pallida* | OFI 52/87 | 2n=4x=104 | Mexico | 18°38′ | 2100 | 528 | 17.6 |
| *L. pallida* × *L. leucocephala*[1] | K376xK8 | 2n=4x=104 | | | | | |
| *L. pueblana* | OFI 125/92 | ? | Mexico | 17°53′ | 610 | 564 | 16.3 |
| *L. pulverulenta* | OFI 83/87 | 2n=2x=56 | Mexico | 23°36′ | 1000–1500 | 750 | 18.4 |
| *L. retusa* | OFI 23/86 | 2n=2x=56 | Mexico | 28°44′ | 800–1500 | 634 | 18.0 |
| *L. salvadorensis* | OFI 36/88 | 2n=2x=56 | Honduras | 13°26′ | 480–600 | 1120 | 26.5 |
| *L. shannonii* subsp. *magnifica* | OFI 19/84 | ? | Guatamala | 14°40′ | 900–950 | 928 | 24.0 |
| *L. shannonii* subsp. *shannonii* | OFI 135/92 | 2n=2x=52 | Mexico | 16°41′ | 475 | 948 | 24.7 |
| *L. trichodes* | OFI 61/88 | 2n=2x=52 | Equador | 1°21′ | 150–400 | 492 | 24.8 |

[1]Artificial hybrid/cultivar.
[2]Not available.

**Table 2.** Summary information for strains of *Rhizobium* used in the study.

| Strain ID | Other identifier | Country of origin | Host of isolation | Comments |
|---|---|---|---|---|
| CB3126 | | Mexico | *L. leucocephala* | Soil pH 6.2 at collection site — performed well on *Desmanthus virgatus* in low pH soils |
| NGR8 | CB948 | New Guinea | *L. leucocephala* | Former commercial strain — poor soil and root colonisation in acid soils |
| CB3060 | TAL1145 | Australia | *L. diversifolia* | Current commercial strain — good acid soil tolerance |
| CB3427 | | Australia | *L. pulverulenta* | From CSIRO Lansdown, north Queensland |
| CB3128 | | Mexico | *L. leucocephala* | Soil pH 8.5 at collection site |
| CB3522 | | Mexico | *L. cuspidata* | Only isolate specific to *L. cuspidata*, untested strain |
| NifTAL | | USA | several | Commercial composite peat inoculum comprising strains CB3060, MS111 and NifTAL 1770 |
| TAL600 | CB3108 | USA | *Prosopis chilensis* | One of the best NifTAL strains — less effective than CB3060 in acid soils |
| CB3131 | | Mexico | *L. trichodes* | Performed well with *L. leucocephala* in CSIRO glasshouse trials |
| CB3361 | | Mexico | *L. leucocephala* | From vigorous stand of *L. leucaena* on commercial dairy farm in Mexico |
| CB3298 | | Australia | *L. retusa* | Untested strain |
| MS111 | CB3138 | Malaysia | *L. leucocephala* | Effective in acid soils down to pH 4.2 |
| CB3299 | | Australia | *L. greggii* | Untested strain |

## Results

### *Total dry matter yields*

DM yields of +N treatments of 26 of the 27 accessions of *Leucaena* were not significantly different ($P>0.05$) from the best respective rhizobial strain treatments. Strain CB3126, the highest yielding strain, significantly outyielded the respective –N controls in all accessions of *Leucaena* (Table 3). In contrast, strain CB3299 produced the lowest DM yield responses in all accessions of *Leucaena* and yields were not significantly different from the respective –N treatments. Rhizobial strains interacted poorly with the *L. pallida* × *L. leucocephala* KX2 hybrid and the +N treatment outyielded all inoculated treatments.

**Table 3.** Total dry matter yields (g/jar) of accessions of *Leucaena* inoculated with 13 strains of *Rhizobium* including uninoculated (–N) and N-fertilised (+N) treatments.

| *Leucaena* accession ID | CB3126 | NGR8 | CB3060 | CB3427 | CB3128 | CB3522 | NifTAL | TAL600 | CB3131 | CB3361 | CB3298 | MS111 | CB3299 | –N | +N | LSD (P<0.05) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OFI 52/88 | 3.0 | 4.4 | 3.0 | 2.6 | 3.3 | 3.2 | 3.1 | 2.7 | 3.5 | 2.1 | 1.9 | 1.3 | 0.5 | 0.6 | 3.8 | 1.7 |
| OFI 56/88 | 2.4 | 3.8 | 2.2 | 2.5 | 2.2 | 3.4 | 2.7 | 2.6 | 2.1 | 2.4 | 1.6 | 1.3 | 0.3 | 0.5 | 2.8 | 1.7 |
| OFI 83/94 | 2.0 | 2.6 | 2.4 | 1.6 | 1.4 | 1.6 | 2.1 | 1.6 | 1.8 | 2.2 | 1.2 | 1.1 | 0.3 | 0.3 | 3.0 | 1.3 |
| OFI 4/91 | 2.2 | 2.2 | 2.9 | 1.8 | 2.8 | 1.7 | 2.3 | 2.4 | 1.8 | 2.8 | 1.2 | 1.0 | 0.7 | 1.0 | 3.2 | 1.1 |
| OFI 83/92 | 2.1 | 1.6 | 2.1 | 1.7 | 2.3 | 2.3 | 2.0 | 2.0 | 1.8 | 2.0 | 1.7 | 1.6 | 0.4 | 0.4 | 2.7 | 1.1 |
| OFI 53/88 | 2.1 | 2.0 | 2.1 | 2.0 | 1.7 | 1.7 | 2.1 | 1.4 | 1.7 | 2.2 | 1.6 | 0.8 | 0.4 | 0.3 | 2.8 | 0.8 |
| OFI 47/87 | 4.6 | 4.7 | 3.9 | 3.3 | 5.0 | 3.6 | 4.0 | 3.6 | 3.9 | 2.2 | 3.2 | 2.4 | 0.8 | 1.1 | 3.2 | 1.2 |
| OFI 82/87 | 2.6 | 2.4 | 3.3 | 1.9 | 3.3 | 1.8 | 2.3 | 2.7 | 2.5 | 2.4 | 1.2 | 1.6 | 0.4 | 0.4 | 2.7 | 1.6 |
| OFI 87/92 | 3.2 | 2.8 | 4.2 | 3.5 | 2.6 | 2.4 | 2.5 | 3.6 | 3.3 | 2.7 | 2.3 | 2.1 | 0.6 | 0.6 | 3.2 | 1.1 |
| OFI 43/85 | 3.5 | 2.1 | 1.7 | 3.6 | 1.7 | 2.9 | 3.6 | 1.8 | 2.3 | 3.3 | 1.9 | 1.4 | 0.5 | 0.5 | 3.6 | 1.1 |
| OFI 50/87 | 3.7 | 2.7 | 3.9 | 4.2 | 3.2 | 3.0 | 2.2 | 2.4 | 2.9 | 3.3 | 3.4 | 1.9 | 0.9 | 0.9 | 4.4 | 2.3 |
| OFI 6/91 | 3.9 | 3.0 | 3.3 | 3.0 | 2.6 | 3.3 | 3.1 | 2.5 | 2.9 | 3.2 | 2.6 | 1.8 | 0.5 | 0.4 | 2.5 | 1.5 |
| Cunningham | 3.3 | 3.6 | 3.5 | 3.0 | 3.5 | 2.9 | 2.8 | 2.9 | 2.8 | 2.1 | 2.8 | 2.2 | 0.3 | 0.6 | 3.4 | 1.2 |
| OFI 55/88 | 3.4 | 2.8 | 3.2 | 2.7 | 3.0 | 3.0 | 3.4 | 2.4 | 2.7 | 3.1 | 2.7 | 2.2 | 0.6 | 0.7 | 3.9 | 1.7 |
| OFI 47/85 | 3.4 | 3.0 | 1.6 | 2.6 | 2.6 | 2.7 | 2.6 | 2.4 | 1.9 | 2.3 | 2.1 | 1.8 | 0.3 | 0.4 | 2.6 | 1.4 |
| OFI 81/87 | 1.7 | 1.2 | 1.8 | 1.2 | 1.1 | 1.2 | 1.9 | 1.6 | 1.1 | 1.5 | 1.1 | 0.8 | 0.3 | 0.3 | 1.9 | 0.6 |
| OFI 52/87 | 5.6 | 5.4 | 5.5 | 5.6 | 5.3 | 5.8 | 5.6 | 6.0 | 4.9 | 3.8 | 5.3 | 3.9 | 0.7 | 0.7 | 5.3 | 2.2 |
| OFI 125/92 | 2.3 | 2.8 | 4.2 | 4.9 | 3.3 | 3.5 | 2.6 | 3.2 | 3.9 | 3.0 | 2.1 | 1.9 | 0.9 | 1.0 | 5.7 | 1.3 |
| OFI 83/87 | 2.7 | 1.6 | 2.4 | 2.3 | 1.9 | 2.3 | 2.1 | 1.8 | 2.2 | 2.0 | 1.5 | 1.2 | 0.4 | 0.3 | 2.3 | 1.0 |
| OFI 23/86 | 3.1 | 3.0 | 2.6 | 2.6 | 2.6 | 2.4 | 2.5 | 2.2 | 2.1 | 2.0 | 1.7 | 1.6 | 0.7 | 0.7 | 2.7 | 1.3 |
| OFI 36/88 | 6.8 | 5.0 | 3.4 | 5.3 | 4.5 | 4.1 | 5.4 | 5.2 | 3.9 | 4.9 | 4.5 | 3.5 | 1.0 | 1.2 | 5.2 | 1.8 |
| OFI 19/84 | 3.7 | 2.3 | 2.5 | 3.4 | 3.0 | 4.1 | 3.8 | 2.5 | 2.7 | 3.5 | 2.7 | 1.8 | 0.6 | 0.6 | 2.6 | 1.4 |
| OFI 135/92 | 3.6 | 3.2 | 3.4 | 2.5 | 3.2 | 2.7 | 2.3 | 2.6 | 2.6 | 2.9 | 2.6 | 1.6 | 0.5 | 0.5 | 3.8 | 1.2 |
| OFI 61/88 | 3.7 | 3.1 | 2.5 | 3.6 | 3.1 | 2.6 | 3.4 | 2.9 | 3.1 | 2.9 | 2.2 | 2.2 | 0.9 | 0.9 | 2.8 | 2.0 |
| KX2 | 3.5 | 3.9 | 3.2 | 3.4 | 3.2 | 2.9 | 2.6 | 2.2 | 2.6 | 1.9 | 2.4 | 2.0 | 0.4 | 0.6 | 5.4 | 1.2 |
| KX3 | 3.1 | 3.6 | 3.4 | 3.1 | 2.4 | 2.4 | 2.4 | 3.0 | 3.3 | 2.1 | 3.2 | 2.4 | 0.4 | 0.4 | 2.7 | 1.2 |
| OFI 49/87 | 5.9 | 5.8 | 5.0 | 4.6 | 6.6 | 5.8 | 3.7 | 5.6 | 5.5 | 4.2 | 4.2 | 3.0 | 1.1 | 1.2 | 4.8 | 2.8 |
| Mean | 3.4 | 3.1 | 3.1 | 3.0 | 3.0 | 2.9 | 2.9 | 2.8 | 2.8 | 2.7 | 2.4 | 1.9 | 0.6 | 0.6 | 3.4 | |

**Table 4.** Indices of effectiveness of symbioses (% of +N control) of 27 accessions of *Leucaena* with 13 strains of *Rhizobium*.

| *Leucaena* accession ID | CB3126 | NGR8 | CB3060 | CB3427 | CB3128 | CB3522 | NifTAL | TAL600 | CB3131 | CB3361 | CB3298 | MS111 | CB3299 | Mean | No. of effective associations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OFI 47/87 | 145 | 148 | 122 | 104 | 156 | 127 | 112 | 114 | 123 | 68 | 99 | 74 | 26 | 109 | 10 |
| OFI 19/84 | 143 | 89 | 95 | 132 | 115 | 146 | 160 | 98 | 104 | 135 | 106 | 71 | 23 | 109 | 11 |
| OFI 6/91 | 155 | 118 | 129 | 117 | 102 | 121 | 131 | 97 | 114 | 128 | 102 | 69 | 18 | 108 | 11 |
| KX3 | 116 | 134 | 125 | 115 | 91 | 91 | 88 | 110 | 121 | 80 | 118 | 88 | 14 | 99 | 12 |
| OFI 61/88 | 132 | 110 | 88 | 127 | 111 | 123 | 92 | 104 | 109 | 104 | 80 | 80 | 32 | 99 | 12 |
| OFI 49/87 | 123 | 120 | 104 | 95 | 138 | 76 | 121 | 116 | 113 | 88 | 88 | 62 | 23 | 97 | 10 |
| OFI 52/87 | 106 | 102 | 103 | 105 | 99 | 105 | 108 | 112 | 92 | 71 | 99 | 73 | 14 | 91 | 10 |
| OFI 47/85 | 133 | 116 | 60 | 100 | 99 | 100 | 105 | 94 | 74 | 90 | 81 | 70 | 12 | 87 | 9 |
| OFI 87/92 | 98 | 88 | 132 | 109 | 82 | 80 | 74 | 111 | 104 | 83 | 73 | 65 | 17 | 86 | 9 |
| OFI 36/88 | 131 | 96 | 64 | 101 | 87 | 102 | 80 | 100 | 75 | 93 | 86 | 67 | 19 | 85 | 9 |
| OFI 23/86 | 114 | 110 | 96 | 98 | 98 | 92 | 89 | 82 | 80 | 74 | 65 | 61 | 24 | 83 | 9 |
| OFI 56/88 | 88 | 139 | 80 | 91 | 80 | 100 | 124 | 94 | 76 | 88 | 56 | 48 | 12 | 83 | 9 |
| OFI 82/87 | 97 | 91 | 124 | 71 | 124 | 85 | 68 | 101 | 93 | 89 | 45 | 61 | 15 | 82 | 8 |
| OFI 83/87 | 116 | 71 | 105 | 100 | 83 | 92 | 100 | 80 | 94 | 85 | 66 | 51 | 16 | 81 | 9 |
| Cunningham | 96 | 106 | 102 | 87 | 103 | 81 | 84 | 86 | 82 | 62 | 83 | 64 | 10 | 80 | 10 |
| OFI 52/88 | 80 | 116 | 80 | 70 | 86 | 83 | 84 | 72 | 92 | 55 | 51 | 34 | 13 | 70 | 7 |
| OFI 55/88 | 87 | 72 | 81 | 70 | 76 | 88 | 80 | 62 | 69 | 80 | 69 | 57 | 15 | 70 | 5 |
| OFI 81/87 | 93 | 62 | 98 | 65 | 60 | 101 | 66 | 88 | 61 | 80 | 58 | 44 | 16 | 69 | 5 |
| OFI 135/92 | 93 | 83 | 88 | 64 | 84 | 59 | 70 | 68 | 68 | 77 | 68 | 41 | 12 | 67 | 4 |
| OFI 83/92 | 80 | 58 | 80 | 65 | 84 | 74 | 86 | 75 | 68 | 74 | 62 | 58 | 14 | 67 | 4 |
| OFI 50/87 | 83 | 62 | 89 | 96 | 72 | 51 | 69 | 55 | 66 | 75 | 77 | 44 | 21 | 66 | 3 |
| OFI 43/85 | 99 | 59 | 46 | 102 | 48 | 101 | 83 | 50 | 64 | 94 | 54 | 40 | 14 | 66 | 5 |
| OFI 4/91 | 68 | 71 | 92 | 57 | 88 | 72 | 55 | 76 | 58 | 87 | 38 | 31 | 21 | 63 | 3 |
| OFI 53/88 | 76 | 73 | 76 | 73 | 62 | 76 | 60 | 49 | 61 | 81 | 57 | 30 | 13 | 61 | 1 |
| OFI 83/94 | 65 | 85 | 80 | 52 | 46 | 68 | 53 | 53 | 58 | 72 | 40 | 38 | 9 | 55 | 2 |
| OFI 125/92 | 41 | 50 | 74 | 86 | 59 | 46 | 62 | 55 | 69 | 52 | 36 | 33 | 15 | 52 | 1 |
| KX2 | 64 | 72 | 60 | 63 | 59 | 49 | 55 | 42 | 48 | 36 | 45 | 38 | 7 | 49 | 0 |
| Mean | 101 | 93 | 92 | 89 | 89 | 88 | 87 | 83 | 83 | 81 | 70 | 55 | 16 | 79 | |
| No. of effective associations | 22 | 17 | 21 | 17 | 19 | 17 | 17 | 16 | 13 | 16 | 10 | 2 | 0 | | |

LSD(P<0.05) *Leucaena* accession means = 12; *Rhizobium* strain means = 8; Accession × strain = 42.

The consistently low DM yields (Table 3) and a lack of nodules (data not presented) on the –N control plants indicated that the experimental system was free from external *Rhizobium* throughout the trial period. The +N control treatments achieved a mean growth of 3.4 g/jar which was similar to mean yields from the best rhizobial strain treatments. The yield range for +N treatments was 1.9 g/jar (OFI 81/87) to 5.4 g/jar (KX2).

*Nodulation ratings*

Inoculated accessions of *Leucaena* formed nodules varying in colour from white to pink. Strain CB3299 formed predominantly small white nodules (mean length 1.8mm) whereas other strains formed pink-brown nodules (data not presented). Nodule size ranged from 1–6 mm (mean 3.2 mm) in length and varied among accessions of *Leucaena. Leucaena greggii, L. macrophylla* subsp. *nelsonii, L. esculenta* subsp. *matudae* and *L. pueblana* had the largest nodules with mean lengths of 4.7, 4.4, 4.3 and 4.1 mm, respectively.

*Time to effective association*

The +N treatments attained a dark green colour by Week 2 while the –N treatments had yellowed by Week 2 and failed to regain a green leaf colour. Mean time from sowing to effective association with rhizobia, based on achievement of a dark-green leaf colour, was 4.1 weeks. Nine of the 13 rhizobial strain treatments greened within an average of 4 weeks (data not presented). Strain MS111 was slow to green while strain CB3299 failed to produce green leaves in 23 of the 27 accessions. Number of weeks to greening was significantly ($P<0.05$) but poorly correlated ($r^2 = 0.46$) with IE.

Rhizobium *effectiveness index*

Mean rhizobial strain IE for the 27 accessions of *Leucaena* ranged from 109% (*L. esculenta* subsp. *esculenta* OFI 47/87 and *L. shannonii* subsp. *magnifica* OFI 19/84) to 49% (*L. pallida* × *L. leucocephala* KX2 hybrid). Fifteen of the accessions formed symbioses with a mean IE of ≥80% and a mean number of highly effective rhizobial strain associations of 9.9 (Table 4). The remaining 12 accessions had mean IEs of ≤70%, with a mean number of highly effective rhizobial strain associations of only 3.3.

Pattern analysis indicated 2 major effectiveness response groups for accessions of leucaena: Group A, with 3 sub-groups; and Group B, with 2 sub-groups (Figure 1). All Group A accessions, except *L. collinsii* subsp. *collinsii,* had highly effective rhizobial associations, whereas Group B accessions were less effectively nodulated. Groups A1, A2 and A3 had mean IEs of 104, 86 and 77%, respectively, whereas Groups B1 and B2 had mean IEs of 65 and 51%, respectively.

Strain CB3126 was the most effective rhizobial treatment ($P<0.05$), forming highly effective symbioses with 22 accessions of *Leucaena* (Table 4). A further 4 accessions associated effectively with CB3126, having IEs of 64–76%. Only *L. pueblana* had an IE of <60% in association with this strain. Strain CB3060 was highly effective with 21 of the 27 accessions of *Leucaena* and effective (60–76%) with 5 of the remaining 6 accessions. Eight other rhizobial strains were also highly effective on an average 17 *Leucaena* accessions. Strain CB3298 was effective or highly effective with 13 and 9 accessions, respectively, but was only partially effective with 5 accessions. Strain MS111 was ineffective with 4 accessions and only partially effective with a further 6 accessions and CB3299 was ineffective with all accessions.

Three major rhizobial groups were identified by pattern analysis with Group 1 containing 10 highly effective strains (mean IE of 89%), Group 2 containing 2 effective strains (mean IE of 63%) and Group 3 containing 1 ineffective strain (mean IE of 16%).

*Climatic/taxonomic associations*

Climate and geographical distribution in the native range of accessions of *Leucaena* (Table 1) did not correlate strongly with the groups identified by pattern analysis, with the exception of the northern Mexican accessions *L. greggii, L. retusa, L. pulverulenta* and *L. involucrata*. These accessions were grouped together in A2 (Figure 1). *Leucaena greggii* and *L. retusa* also have close taxonomic association (Harris *et al.* 1994). The 2 *L. collinsii* subspecies were grouped into A3. Other taxonomic groupings were weakly related to the leucaena accession groups (data not presented). Four of the 6 large leaflet complex accessions (*L. macrophylla* subsp. *macrophylla, L. lanceolata* subspp. *lanceolata* and *sousae* and *L. multicapitula*) were grouped in B1. Ploidy level and chromosome number were unrelated to accession groups. Tetraploid accessions were represented in 4 of the 5 accession groups.

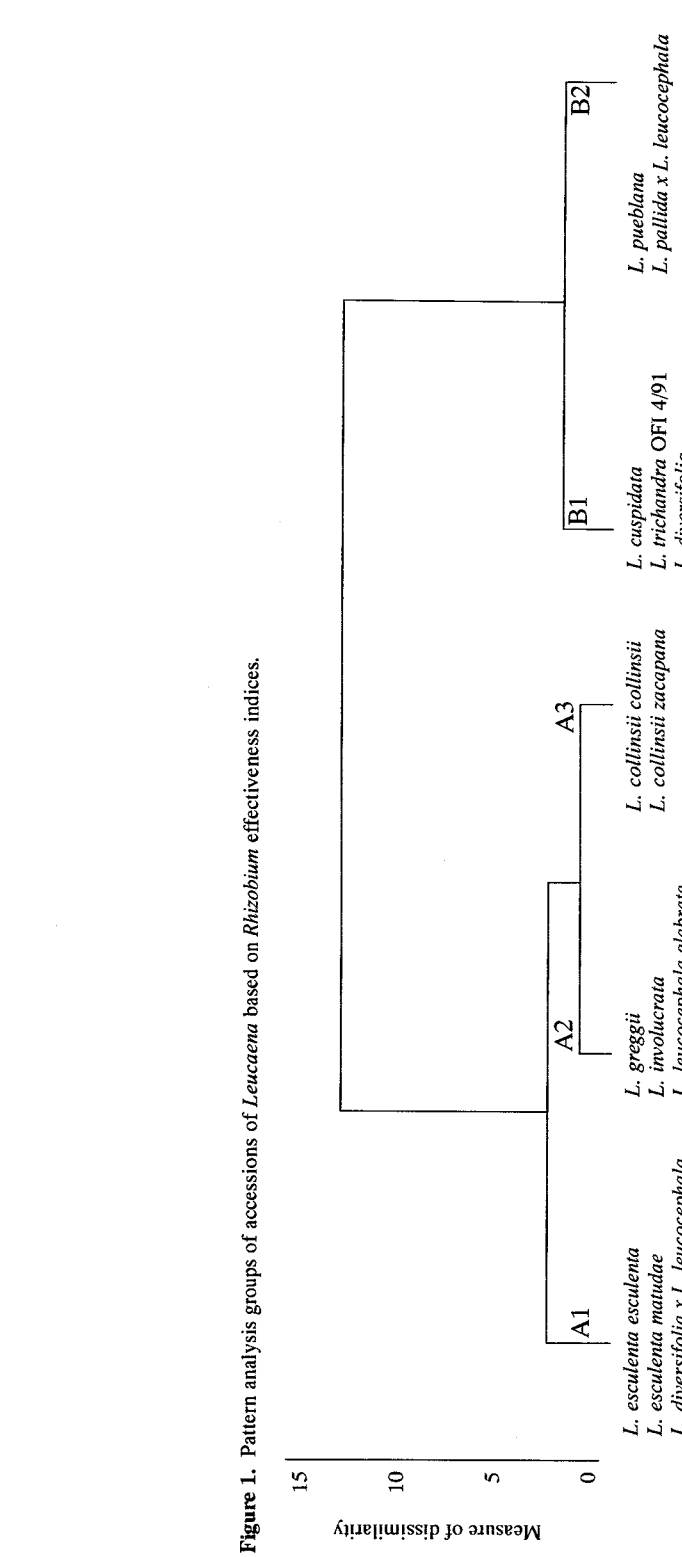


**Figure 1.** Pattern analysis groups of accessions of *Leucaena* based on *Rhizobium* effectiveness indices.

## Discussion

Based on their N-fixation response to 13 strains of *Rhizobium*, 27 accessions of *Leucaena* representing 19 species and 2 hybrids, separated into promiscuous (A) and moderately specific (B) groups (Figure 1).

### *Accessions of* Leucaena

The 15 accessions in Group A, with highly effective rhizobial associations, included the important accessions *L. leucocephala* subsp. *glabrata*, *L. pallida* and *L. diversifolia* × *L. leucocephala* KX3 hybrid. The apparent promiscuity of these accessions is an important result, particularly for their utilisation in developing countries where legume seed may not be inoculated. However, the 11 Group B accessions were more specific in their rhizobial associations and were highly effective with only a few strains of *Rhizobium*. Group B included 3 taxa of potential commercial importance: *L. trichandra, L. diversifolia* and the KX2 hybrid. R.A. Date (unpublished data) also found *L. diversifolia* (CPI33820) to be specific in its rhizobial requirements. In our experiment, strain CB3060 was more effective on *L. diversifolia* and *L. trichandra* accessions than TAL600, NGR8 and MS111. The apparent specificity of *L. trichandra, L. diversifolia* and the KX2 hybrid is of concern since these species are rated highly for cultivar development due to their inherent DM productivity, tolerance of psyllid insects and adaptation to low temperature. Further selection of rhizobia effective with these species will be necessary for commercial success. The poor association of accessions of *L. lanceolata* and *L. shannonii* in this experiment conflicts with other reports (Halliday and Somasegaran 1983). Our results may indicate the specificity for N-fixing *Rhizobium* at the accession level.

Time to effective association, based on leaf colour response, correlated poorly with IE. Variation in leaf hue between accessions reduced the objectivity of the colour ratings in some cases and this may have adversely affected the relationship. For example, the dull, pale green colour of *L. pallida* accessions did not correspond well with colours included on the selected colour charts.

### *Rhizobial strains*

Confirmation that strain CB3060 is highly effective on this wide range of species of *Leucaena* is important. When used as a seed inoculum, CB3060 is highly competitive with both native and introduced strains of *Rhizobium* (Moawad and Bohlool 1984), rapidly colonises the soil and rhizosphere (Homchan *et al.* 1989), tolerates relatively low soil pH (to pH 5.0) and survives well in the soil in the absence of a host (Wong *et al.* 1989). Highly effective associations of CB3060 with *L. retusa* and *L. pulverulenta* contrast with previous reports of no or poor nodulation, respectively, with these genotypes, although accessions/provenances were not specified (Halliday and Somasegaran 1983).

Strain CB3126 associated highly effectively with 22 accessions of *Leucaena* and potentially could be developed as an alternative to CB3060 for inoculation of leucaena. Strain CB3126 is recommended for inoculation of *Desmanthus virgatus* cultivars in Australia (Date 1991), and has good soil and rhizosphere colonising capacity and tolerance of mildly acidic soils (Brandon *et al.* 1998).

Strain NGR8, previously a commercial strain, also performed well in this experiment, associating highly effectively with 17 of the 28 accessions of *Leucaena*. However, NGR8 is less effective than CB3060 in low pH soils (Bushby 1982) and has poor competitive ability.

Strain MS111 was selected in Malaysia for its tolerance of acid soils. In field experiments with *L. leucocephala* at 4 sites on acid soils in Malaysia, Wong *et al.* (1989) reported MS111 to form more nodules and be more persistent than CB3060 and to produce similar DM responses in *L. leucocephala*. In the current experiment, under near optimal growth conditions, MS111 responded relatively poorly with most accessions of *Leucaena*. Field testing in acid soils of MS111 against strains CB3060 and CB3126 is required before it is used to inoculate other species of *Leucaena*.

Strain CB3299, isolated from *L. greggii*, was ineffective with all accessions of *Leucaena*. However, it formed medium sized, pink-brown nodules on *L. retusa* (closely related to *L. greggii*). Halliday and Somasegaran (1983) reported that *L. retusa* failed to nodulate with rhizobia which readily nodulated many other species of *Leucaena*.

The Leonard-jar technique provides controlled conditions for evaluation of rhizobial strain effectiveness, but gives no indication of a strain's ability to colonise and persist under field conditions.

### *Biological correlations with leucaena groups identified by rhizobial associations*

Factors such as climate in the native range, ploidy level and chromosome number were unrelated to leucaena groups identified by rhizobial association, and only weak taxonomic linkages were evident (Table 1). In contrast, the rhizobial responses of accessions of *Stylosanthes hamata* were highly correlated with ploidy level (Date and Norris 1979). Many species of *Leucaena* have been domesticated and disseminated throughout central America over the past 2000 years (Hughes 1993) and it is possible that closely related species have evolved subsequently in the presence of very different rhizobial populations. Furthermore, an understanding of biological correlations with *Leucaena/Rhizobium* effectiveness groups may have been achieved better by selecting rhizobia from regions within the indigenous range of each accession of *Leucaena*. Strain selection in this experiment was based on known effectiveness and/or field association, at least with particular species of *Leucaena*, and consequently strains came from diverse locations and hosts (Table 2).

## Acknowledgements

This work was financially supported by the Australian Centre for International Agricultural Research (Project 9433) and the Tropical Grassland Society. The authors thank Dr H.M. Shelton for his comments and editorial assistance during the drafting of this manuscript.

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*(Received for publication September 22, 1997; accepted December 23, 1997)*