RHIZOBIUM SPECIFICITY IN *LEUCAENA*

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ABSTRACT

Twenty-seven accessions of *Leucaena*, representing 20 species, were assessed for effective N-fixing symbioses in association with 13 strains of *Rhizobium* in N-free conditions in a glasshouse. Fifteen accessions formed effective symbioses with most strains of *Rhizobium*. Only 4 accessions required specific strains. Conversely, 9 of the strains of *Rhizobium* formed effective symbioses with most accessions of *Leucaena*. Only one strain was ineffective. Strains CB3060 and CB3126 were effective with 21 and 22 accessions of *Leucaena*, respectively.

KEYWORDS

leucaena, rhizobium, N-fixation, agroforestry, genotypic variation

INTRODUCTION

Highly effective rhizobial inoculants for nitrogen fixation are available for commercial cultivars of *Leucaena leucocephala* but considerable intraspecific variation in *Rhizobium* association has been identified (Date, unpublished data). Effective nodulation by native rhizobia is reported in many countries but establishment and early growth of uninoculated plants is comparatively slow (Halliday and Somasegaran, 1983). Little is known of *Rhizobium* specificity for effective nitrogen fixation in other species of *Leucaena*. In view of the current interest in agronomic evaluation of accessions from diverse *Leucaena* taxa it is important to understand more of the specificity of the *Leucaena* x *Rhizobium* interactions for effective N-fixation, as ineffective associations may limit yield potential.

MATERIALS AND METHODS

Site and design: The experiment was conducted during the summer of 1996 at the CSIRO, Cunningham Laboratory, Brisbane, Australia. Twenty seven accessions of *Leucaena* representing 20 species, their sub-species and 2 inter-specific hybrids (Table 1) were grown in N-free sand-culture, using a modified Leonard-jar technique (Norris and Date 1976). Seedling plants were separately inoculated with thirteen strains of *Rhizobium* from the CSIRO ATFGRC collection. Current and previous commercially recommended inoculant strains for *Leucaena leucocephala* (Table 2) were included.

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 strains worthy of field testing. A completely randomised design with 2 replications was used. Sand-jars were re-randomised at two-weekly intervals during the growing period.

Data analysis: The N-fixing ability of *Rhizobium* strains nodulating plants grown in N-free media is directly related to the dry weight of the plant (Norris and Date, 1976). An effectiveness index (EI = (DW strain/DW +N control) x 100) was used to compare *Leucaena* accessions x *Rhizobium* strain interactions. From experience an effectiveness index of 80% indicates highly effective *Rhizobium* association.

RESULTS AND DISCUSSION

Accessions of *Leucaena*: Mean *Rhizobium* effectiveness indices for the 27 *Leucaena* accessions ranged from 109% for *L. esculenta* subsp. *esculenta* OFI 47/87 and *L. shannonii* subsp. *magnifica* OFI 19/84 to 49% for the *L. pallida* x *L. leucocephala* KX2 hybrid (Table 1). Fifteen accessions showed mean effectiveness indices of 80% or greater and a mean number of effective rhizobial strain associations of 9.9 (Table 1). These included the important accessions *L. leucocephala* subsp. *glabrata*, *L. pallida* and *L. diversifolia* x *L. leucocephala* KX3 hybrid. The remaining 12 accessions had mean effectiveness indices of 70% or less, with a mean number of effective *Rhizobium* strain associations of only 3.3. These included 2 accessions of potential commercial importance *L. trichandra* OFI 53/88 and the *L. pallida* x *L. leucocephala* KX2 hybrid. Further *Rhizobium* strain selection is required for development of these accessions.

Rhizobial strains: Effective strains of *Rhizobium* (EI>80%) were available for 26 of the 27 accessions of *Leucaena*, but no one strain was consistently effective for all accessions. *Rhizobium* strain CB3126 was the most effective strain (P>0.05%) and formed effective associations with 22 *Leucaena* accessions (Table 2). A further 4 accessions associated moderately well with CB3126 having effectiveness indices from 64 to 76%. Strain CB3126 has been selected for inoculation of *Desmanthus virgatus* cultivars in Australia, and is known to possess good soil and rhizosphere colonisation characteristics and tolerance of mildly acidic soils (Brandon *et al.*, 1997).

Strain CB3060 was highly effective with 21 of the 27 *Leucaena* accessions. The remaining 6 accessions showed moderately effective association with CB3060, with only 1 accession (OFI 43/85) giving an effectiveness index of <60%. This apparent promiscuity of CB3060 across the *Leucaena* genus is a good result as the strain is known to be highly competitive with both native and introduced *Rhizobium* strains, colonising comparatively rapidly in field situations. It is also tolerant of relatively low soil pH (to pH 5.0) and survives well in the soil in the absence of a host (Wong *et al.*, 1989).

Eight other rhizobial strains were also highly effective (effectiveness indices >80%) with an average 16 *Leucaena* accessions. Strains CB3298 and MS111 were ineffective in 17 and 25 accessions respectively, and strain CB3299 produced nodules but was ineffective in all accessions.

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REFERENCES

Brandon, N.J., R.A. Date, R.L. Clem, B.A. Robertson, and T.W.G. Graham. 1997. Growth responses of *Desmanthus virgatus* to inoculation with *Rhizobium* strain CB3126. II. A field trial at 4 sites in southern Queensland. Tropical Grasslands. in press.

Halliday, J. and P. Somasegaran. 1983. Nodulation, nitrogen fixation, and *Rhizobium* strain affinities in the genus *Leucaena*. Pages 27-32. Leucaena research in the Asian-Pacific Region. Proceedings of a workshop, Singapore 23-26 November 1982 Ottawa, Canada, IDRC.

Wong, C.C., J. Sundram, R.A. Date, and R.J. Roughley. 1989. Nodulation of *Leucaena leucocephala* in acid soils of peninsular Malaysia. Tropical Grasslands. **23:** 171-178.

Table 1
Mean effectiveness indices and number of effective associations of *Leucaena* accessions with 13 strains of *Rhizobium*

Species/subspecies	Accession ID.	Mean effectiveness index (%)	No. of effective associations (EI80%)
L.collinsii collinsii	OFI 52/88	70	7
L.collinsii zacapana	OFI 56/88	83	9
L.cuspidata	OFI 83/94	55	2
L.diversifolia	OFI 83/92	67	4
L.diversifolia x L.leucocephala[1]	K156xK8	99	12
L.trichandra	OFI 4/91	63	3
L.trichandra	OFI 53/88	61	1
L.esculenta esculenta	OFI 47/87	109	10
L.esculenta matudae	OFI 49/87	97	10
L.greggii	OFI 82/87	82	8
L.involucrata	OFI 87/92	86	9
L.lanceolata lanceolata	OFI 43/85	66	5
L.lanceolata sousae	OFI 50/87	66	3
L.lempirana	OFI 6/91	108	11
L.leucocephala glabrata	Cunningham	80	10
L.macrophylla macrophylla	OFI 55/88	70	5
L.macrophylla nelsonii	OFI 47/85	87	9
L.multicapitula	OFI 81/87	69	5
L.pallida	OFI 52/87	91	10
L.pallida x L.leucocephala[1]	K376xK8	49	0
L.pueblana	OFI 125/92	52	1
L.pulverulenta	OFI 83/87	81	9
L.retusa	OFI 23/86	83	9
L.salvadorensis	OFI 36/88	85	9
L.shannonii magnifica	OFI 19/84	109	11
L.shannonii shannonii	OFI 135/92	67	4
L.trichodes	OFI 61/88	99	12

[1] artificial hybrid

Table 2
Host of isolation, mean effectiveness indices and number of effective associations of strains of *Rhizobium* with 27 accessions of *Leucaena*

Strain ID.	Other identifier	Host of isolation	Mean effectiveness index (%)	No. of effective associations (EI80%)
NGR8	CB948	*L. leucocephala*	93	17
CB3060	TAL1145	*L. diversifolia*	92	21
TAL600	CB3108	*Prosopis chilensis*	83	16
CB3126		*L. leucocephala*	101	22
CB3128		*L. leucocephala*	89	19
CB3131		*L. trichodes*	83	13
MS111	CB3138	*L. leucocephala*	55	2
CB3298		*L. retusa*	70	10
CB3299		*L. greggii*	16	0
CB3361		*L. leucocephala*	81	16
CB3427		*L. pulverulenta*	17	89
CB3522		*L. cuspidata*	87	17
NifTAL		several	88	17