APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1981, p. 97-99 Vol. 41, No. 1
0099-2240/81/010097-03$02.00/0

Nodulation of *Acacia* Species by Fast- and Slow-Growing Tropical Strains of *Rhizobium*

B. L. DREYFUS AND Y. R. DOMMERGUES*

Laboratoire de Microbiologie des Sols, ORSTOM/Centre National de la Recherche Scientifique Dakar, Senegal, West Africa

Thirteen *Acacia* species were classified into three groups according to effective nodulation response patterns with fast- and slow-growing tropical strains of *Rhizobium*. The first group nodulated effectively with slow-growing, cowpea-type *Rhizobium* strains; the second, with fast-growing *Rhizobium* strains; and the third, with both fast- and slow-growing *Rhizobium* strains. The *Rhizobium* requirements of the *Acacia* species of the second group were similar to those of *Leucaena leucocephala*.

Shrubs and trees of the legume genus *Acacia* (*Mimosaceae*) are abundant in savannas and arid regions of Australia, Africa, South and North America, and India. In the Sahel region of Africa, *Acacia* is often the dominant tree species, where they grow in barren soils and dry sites unsuited for most crops. The *Acacia* species stabilize sandy and eroded soils and exploit deep underground water by virtue of their extensive root systems. They provide shade, forage for animals, firewood, charcoal, and gums. Most *Acacia* species nodulate with *Rhizobium* and fix N_2 (1, 2, 4, 6), but little is known about the specificity and the characteristics of *Rhizobium* symbionts (7, 8). It is known that *Rhizobium* requirements of some *Acacia* species seem to be specific and to involve nodulation by slow-growing, cowpea-type *Rhizobium* strains (3). However, one *Acacia* species, *Acacia farnesiana*, was shown to be nodulated by fast-growing strains of *Rhizobium* (10). In this paper, we report the result of a cross-inoculation study concerning the rhizobia associated with several native and introduced *Acacia* species usually grown in the Sahel region.

MATERIALS AND METHODS

Plant cultivation. To obtain fast and regular germination, the seeds were pretreated and surface sterilized with concentrated sulfuric acid. The times of treatment in H_2SO_4 were as follows, in minutes: *A. senegal*, 14; *A. bivenosa*, 20; *A. albida*, 30; *Leucaena leucocephala*, 30; *A. linaroides*, 30; *A. pyrifolia*, 30; *A. seyal*, 30; *A. tumida*, 30; *A. farnesiana*, 45; *A. holosericea*, 60; *A. raddiana*, 60; *A. mearnsii*, 120; *A. nilotica* var. *neb–neb*, 120; *A. nilotica* var. *tomentosa*, 120; *A. sieberiana*, 120.

After treatment, the seeds were washed with water until all traces of acid were removed. The seeds were germinated in sterile petri dishes of water agar and then transferred to tubes containing Jensen medium (11) or to polythene pouches containing sterilized soil. One drop of liquid *Rhizobium* culture, 10^9 cells per ml, was used for inoculation of tubes, and 1 ml of culture per plant was used for inoculation of pouches. Tubes were placed in a greenhouse, and pouches were incubated outside.

Bacterial growth medium. *Rhizobium* was grown on yeast extract-mannitol medium (11).

Total nitrogen. Plant shoots dried for 2 days at 60°C were weighed and finely ground, and total nitrogen was determined by the Kjeldahl method.

Acetylene reduction activity. The acetylene reduction activity of nodulated roots was measured by gas chromatography according to usual procedures (5).

Rhizobium strains. We isolated a large collection of tropical *Rhizobium* strains from different *Acacia* species and *L. leucocephala* growing in Senegal. Strains fell into two classes: fast- and slow-growing strains. Fast-growing strains had a generation time of 3 to 4 h; slow-growing strains had a generation time of 8 to 12 h. A taxonomic and cross-inoculation study to be published elsewhere (in preparation) indicated that slow-growing strains belonged to the cowpea miscellany and that fast-growing strains were distinctly different from slow-growing ones. Four fast-growing (ORS 901, ORS 902, ORS 908, and ORS 911) and four slow-growing (ORS 801, ORS 802, ORS 803, and ORS 806) *Rhizobium* strains were selected for study. ORS 901 and ORS 902 had been isolated from nodules of *A. senegal*; ORS 908 and ORS 803, from *A. bivenosa*; ORS 911, from *A. farnesiana*; ORS 801, from *A. holosericea*; ORS 802, from *A. sieberiana*; and ORS 806, from *L. leucocephala*. The slow-growing tropical cowpea strain CB 756 and the fast-growing *Leucaena* strain NGR 8 were obtained from Australia.

RESULTS AND DISCUSSION

The *Rhizobium* strains mentioned above were compared for their abilities to nodulate 13 *Acacia* species and *L. leucocephala*. Nodulation was observed in tubes 5 to 7 days after inoculation. Four to 5 weeks later, plants were scored for nodulation effectiveness by visual observation of plant vigor and nodule appearance. Uninoculated plants remained free of nodules.

O.R.S.T.O.M. Fonds Documentaire
N° : 81/81/b0416 ex1
Cote : B
Date : 23 JUIN 1981

shows that the different *Acacia* species studied fell into three inoculation groups according to the type of *Rhizobium* which nodulated them. (i) The first group, with *Acacia* species such as *A. albida*, nodulated only with slow-growing strains. (ii) A second group, with *Acacia* species such as *A. senegal*, nodulated only with fast-growing strains, and (iii) a third group, with *Acacia* species such as *A. seyal*, nodulated with both fast- and slow-growing strains.

L. leucocephala effectively nodulated with fast-growing strains isolated from *Acacia*. Strain NGR 8, which is usually recommended as an inoculant for *L. leucocephala*, effectively nodulated the *Acacia* species belonging to the second group. Consequently, we could conclude that *Acacia* species of the second group and *L. leucocephala* were members of the same inoculation group.

To evaluate the effectiveness of some fast- and slow-growing strains representative of each group, we inoculated three African *Acacia* species, *A. senegal*, *A. albida*, and *A. seyal*. Plants were grown in sterile soil placed in polythene pouches and harvested 2 months after inoculation.

Data in Table 2 support the conclusion of the first experiment. No nodules were found on *A. senegal* inoculated with slow-growing strains, and none were found on *A. albida* inoculated with fast-growing strains. Nodules were found on *A. seyal* when both fast- or slow-growing strains were used for inoculation, but effectiveness varied greatly with the strains. Cowpea strain CB 756 nodulated but was totally ineffective on *A. seyal*, whereas this strain effectively nodulated *A. albida*. Specific acetylene reduction activity measured on 10 of the 13 *Acacia* species studied was 30 to 90 μmol of C_2H_4 produced per h per g (dry weight) of nodule. This rate is comparable to that found in actively N_2-fixing legume crops such as soybeans.

Preliminary examination of nodule mass at age 2 months indicated that most of the species had a low nodule weight, ranging from 0.5 to 1.5 g (fresh weight) per plant. One species, *A. bivenosa*, was remarkable, however, for its much higher nodule weight: up to 4 g (fresh weight) per plant. Since the specific acetylene reduction activity of *A. bivenosa* nodules was 40 μmol of C_2H_4 per g (dry weight) of nodule, this plant may have a substantial potential for fixing N_2.

In spite of the fact that this study was limited to 13 *Acacia* species, we assume that the proposed classification could be applied to a number of other *Acacia* species. As far as the third group is concerned, mention should be made that some species of another genus, *Lotus*, have been reported to effectively nodulate with both fast- and slow-growing strains (9). This grouping of *Acacia* has practical implications. Since slow-growing strains of *Rhizobium* belonging to the unspecialized cowpea miscellany are common in many tropical soils, one could predict that the

TABLE 1. *Nodulation of 13 Acacia species and L. leucocephala by fast- and slow-growing strains of Rhizobium*

Species	Nodulation[a]									
	Fast-growing strains					Slow-growing strains				
	ORS 901	ORS 902	ORS 908	ORS 911	NGR 8	ORS 801	ORS 802	ORS 803	ORS 806	CB 756
Native African species										
A. albida	0	0	0	0	0	E	E	E	E	E
A. nilotica var. *nebneb*	E	E	E	E	E	0	0	0	0	0
A. nilotica var. *tomentosa*	E	E	E	E	E	0	0	0	0	0
A. raddiana	E	E	E	E	e	0	0	0	0	0
A. senegal	E	E	E	E	E	0	0	0	0	0
A. seyal	E	e	E	E	I	E	e	e	E	I
A. sieberiana	e	I	e	0	0	E	e	E	e	0
Introduced species										
A. bivenosa	I	I	E	I	I	E	e	E	I	e
A. farnesiana	E	E	E	E	E	0	I	0	0	I
A. holosericea	0	0	0	0	0	E	e	e	e	E
A. linaroides	0	0	0	0	0	E	e	e	I	I
A. mearnsii	0	0	0	0	0	E	E	e	E	e
A. tumida	0	0	I	I	0	e	e	I	0	I
L. leucocephala	E	e	E	e	E	0	0	0	e	0

[a] E, effective nodulation; e, partially effective nodulation; I, completely ineffective nodulation; 0, no nodules produced.

TABLE 2. *Effect of inoculation of three West African Acacia species in sterile soil by fast- and slow-growing strains of Rhizobium*[a]

Acacia species	*Rhizobium* strains[b]	Shoot			Nodules	
		Ht (cm)	Fresh wt (g)	Total N (%)	No.	Fresh wt (g)
A. senegal	ORS 901 (F)	21 ± 4.0	2.3 ± 0.5	2.1	13	0.2
	ORS 902 (F)	20 ± 3.2	2.1 ± 0.4	2.0	15	0.2
	ORS 801 (S)	7 ± 2.5	0.4 ± 0.2	1.5	0	0
	CB 756 (S)	6 ± 2.0	0.3 ± 0.2	1.5	0	0
	Uninoculated control	8 ± 3.1	0.4 ± 0.2	1.4	0	0
A. albida	ORS 901 (F)	13 ± 2.8	0.8 ± 0.2	1.4	0	0
	ORS 902 (F)	11 ± 2.3	0.8 ± 0.3	1.5	0	0
	ORS 801 (S)	23 ± 4.2	2.6 ± 0.5	2.3	6	0.1
	CB 756 (S)	28 ± 3.9	2.9 ± 0.3	2.2	11	0.2
	Uninoculated control	12 ± 3.3	0.7 ± 0.1	1.0	0	0
A. seyal	ORS 901 (F)	30 ± 5.0	3.1 ± 0.6	2.0	14	0.6
	ORS 902 (F)	16 ± 4.5	1.9 ± 0.3	1.7	7	0.2
	ORS 801 (S)	27 ± 3.6	3.0 ± 0.5	2.2	18	0.5
	CB 756 (S)	12 ± 2.2	1.2 ± 0.3	1.5	9	0.2
	Uninoculated control	13 ± 3.1	1.3 ± 0.2	1.4	0	0

[a] Plants were harvested when 2 months old. Results are given as mean of 15 replicates ± standard deviation.
[b] (F) Fast-growing strains of *Rhizobium*; (S) slow-growing strains of *Rhizobium*.

Acacia species nodulating with these strains would respond poorly to inoculation. By contrast, fast-growing *Rhizobium* strains are usually assumed to be more specific. Current experiments using nonsterile soils thus far confirm this hypothesis since inoculation appears to benefit *Acacia* species of the second, but not of the first, group. The situation for the second group may be comparable to that of *L. leucocephala* (8, 10). Finally, the experimental results reported here could reasonably be applied to nursery conditions, thus contributing to land reclamation and reforestation in the semiarid tropics.

ACKNOWLEDGMENT

We thank E. L. Schmidt for reviewing the manuscript.

LITERATURE CITED

1. **Beadle, N. C.** 1964. Nitrogen economy in arid and semiarid plant communities. 3. The symbiotic nitrogen-fixing organisms. Proc. Linn. Soc. N.S.W. **89**:273–286.
2. **Corby, H. D.** 1974. Systematic implications of nodulation among Rhodesian legumes. Kirkia **9**:301–329.
3. **Date, R. A.** 1977. Inoculation of tropical pasture legumes, p. 293–311. *In* J. M. Vincent, A. S. Whitney, and J. Bose (ed.), Exploiting the legume—*Rhizobium* symbiosis in tropical agriculture. University of Hawaii, Paia.
4. **Habish, H. A.** 1970. Effect of certain soil conditions on nodulation of *Acacia* spp. Plant Soil **33**:1–6.
5. **Hardy, R. W., R. D. Holsten, E. K. Jackson, and R. C. Burns.** 1968. The acetylene-ethylene assay for N_2-fixation: laboratory and field evaluation. Plant Physiol. **43**:1185–1207.
6. **Nakos, G.** 1977. Acetylene reduction (N_2-fixation) by nodules of *Acacia cyanophylla*. Soil Biol. Biochem. **9**: 131–133.
7. **National Academy of Sciences.** 1979. Forages, p. 123–163. *In* Tropical legumes: resources for the future. National Academy of Sciences, Washington, D.C.
8. **Norris, D. O.** 1973. Seed pelleting to improve nodulation of tropical and sub-tropical legumes. 5. The contrasting response to lime pelleting of two *Rhizobium* strains on *Leucaena leucocephala*. Aust. J. Exp. Agric. Anim. Husb. **13**:98–101.
9. **Pankhurst, C. E.** 1977. Symbiotic effectiveness of antibiotic resistant mutants of fast- and slow-growing strains of *Rhizobium* nodulating *Lotus* species. Can J. Microbiol. **23**:1026–1033.
10. **Trinick, M. J.** 1968. Nodulation of tropical legumes. I. Specificity in the *Rhizobium* symbiosis of *Leucaena leucocephala*. Exp. Agric. **4**:243–253.
11. **Vincent, J. M.** 1970. A manual for practical study of root-nodule bacteria, p. 164. IBP Handbook. Blackwell Scientific Publications, Oxford.