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Presence of *Bradyrhizobia* under *Acacia albida*

N. Dupuy and B. Dreyfus¹

Abstract

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Soil samples taken beneath adult *Acacia albida* in Senegal revealed that large populations of *Bradyrhizobium* exist at depths of 30-35 m i.e., at the water table level. Sixty-seven strains of *Bradyrhizobium* were isolated from both shallow and deep soils. A study of their symbiotic capability showed that several strains isolated from deep soils can actively fix nitrogen in association with *Acacia albida*.

Introduction

It is thought that soil improvement beneath *Acacia albida* is linked to its nitrogen-fixing ability. Abundant nodulation on roots of *A. albida* trees growing in rice fields of Casamance, Senegal (annual rainfall 1000 mm) has been observed. However, no nodules have been found on roots of adult trees in the Sahelian zone (annual rainfall <1000 mm). Thus, there is a question whether *A. albida* does indeed fix nitrogen in arid zones.

In order to answer this, two approaches were considered. The first consisted of determining the presence of nodules on root systems as well as the existence of specific *Rhizobium* populations in the rhizosphere. The second approach involves measuring nitrogen fixed by tree-host associations using the isotope labelling method. Studies using the first approach are presented here.

Two holes were drilled beneath adult *A. albida* in order to sample the distribution of specific *Rhizobium* populations throughout the soil profile. Subsequently, *Rhizobium* strains from upper and lower horizons were collected and their nitrogen-fixing capability determined.

Materials and Methods

Sampling Method

Borings were done using a light, hand-operated auger to which 1.5-m long aluminum shafts (Dormer's Engineering, South Murwillumbah, NSW 2484, Australia) were attached as the hole deepened. One shaft sunk in Louga (200 km north of Dakar), located 8 m from the trunk of an isolated *A. albida*, reached the water table at of 34 m depth. A second shaft, dug in the Groundnut Basin near Bambey, was located between two *A. albida* trees spaced 5 m apart. Here, the water table was reached at 16.5 m. Soil samples were taken at various depths (Table 1), chilled, and analyzed in the laboratory for rhizobia capable of nodulating *A. albida*.

Rhizobium Densities in Soil Samples

Rhizobium densities of the soil samples were estimated using the most probable number method (Brockwell 1980). *A. albida* seeds were sterilized in concentrated sulfuric acid for 1 h, then washed several

1. Département de microbiologie, Institut français de recherche scientifique pour le développement en coopération (ORSTOM), B.P. 1386, Dakar, Senegal.

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Table 1. Nitrogen-fixing capability of *Rhizobium* strains isolated from soil samples taken at different depths at two sites in Senegal, 1990.

Origin	Depth (m)	Nitrogen-fixing ability ¹				Total number of isolate groups
		E	VG	G	P	
Louga	0.5	1	3	2	2	8
	2.5	0	3	1	0	4
	5.0	0	0	1	1	2
	17.5	0	2	1	1	4
	24.0	0	1	0	1	2
	27.5	1	0	0	0	1
	28.5	0	0	1	1	2
	30.0	0	0	2	0	2
	32.0	1	0	0	1	2
	33.5	0	0	2	0	2
34.0	0	0	2	0	2	
	Total	2	9	12	7	31
Diokoul	0.5	0	1	0	0	1
	2.5	0	0	1	0	1
	4.0	0	0	1	0	1
	11.5	0	0	1	0	1
	14.0	0	0	2	0	2
	Total	0	1	5	0	6
Casamance	Surface	0	8	5	3	16
N. Senegal	Surface	1	4	8	1	14

1. Distribution of rhizobium groups as a function of nitrogen-fixing ability. E = excellent, >9 $\mu\text{moles hr}^{-1}$; VG = very good, 6-9 $\mu\text{moles hr}^{-1}$; G = good, 3-6 $\mu\text{moles hr}^{-1}$; P = poor, <3 $\mu\text{moles hr}^{-1}$.

times in sterile water and finally germinated in petri dishes on agar-agar water (0.8%) for 48 h. Roots of the germinated seedlings were placed in test tubes containing Jensen medium; the shoots remained exposed to air. The seedlings were placed in a culture chamber and inoculated after 5 days with 1 mL of a suspended soil dilution. For each dilution, tubes showing nodulation after 4 weeks were counted. Non-inoculated controls did not nodulate.

Isolation of *Rhizobium* Strains

Nodules from inoculated tubes were sterilized in a 0.1% HgCl_2 solution for 3 min, washed in distilled water 6-7 times, then ground in a drop of water with a sterile glass rod. Strains of *Rhizobium* were isolated

on a YMA medium following the procedure of Vincent (1970).

Evaluation of Nitrogen-Fixing Capability

After removing the aerial parts, the liquid culture medium was discarded and the tubes were closed with stoppers. Acetylene was injected (10% of tube volume) into the tube, and acetylene reduction was measured by chromatography in the gaseous phase using the method of Hardy et al. (1968) after a 30-min incubation period at 28°C. *Rhizobium* activity remained linear for 90 min. The strains were divided into four classes according to the acetylene reduction assay results (Table 1).

Results

Rhizobium Distribution as a Function of Depth

Contrary to expectation, *Rhizobium* population densities were highest (Table 2) at the greatest depth measured (Louga). Also, certain intermediary zones (7-14

Table 2. Distribution of *Rhizobium* density (no. soil g^{-1}) associated with *Acacia albida* in two soil profiles in Senegal, 1990.

Louga		Diokoul	
Depth (m)	<i>Rhizobium</i> density	Depth (m)	<i>Rhizobium</i> density
Surface	68	Surface	<1
0.50	1271	0.50	22
2.55	91	2.50	22
5.05	159	4.00	51
7.55	<1	6.00	<1
11.05	<1	8.00	<1
14.05	<1	10.00	<1
17.65	91	11.50	27
21.05	<1	14.00	76
24.05	37	16.50	27
27.55	12	Water table	
28.65	119		
30.05	22		
32.05	24		
33.55	344		
34.00	1323		
Water table			

m at Louga, 6-10 m at Diokoul) contained less than 1 rhizobia per gram of soil throughout the profiles studied. *Rhizobium* populations were less numerous at the Diokoul site than the Louga site. This is probably due to differences in soil texture—the Diokoul soil is much more clayey and therefore probably not as favorable to *Rhizobium* multiplication and development as are the sandy dune soils of Louga.

Characteristics of *Rhizobium* Strains

Of the 67 strains isolated, all except two (ORS167, ORS173) demonstrated slow growth on the YMA medium and were thus considered as *Bradyrhizobium*. Eleven of the 31 Louga strains were classed as very good or excellent in terms of nitrogen-fixing capability. Two of the excellent strains (ORS130 and ORS136) were isolated at depths of 27.5 and 32 m. At Diokoul, however, no strains classed as very good or excellent were isolated below 0.5 m.

Discussion

Until now, it has been believed that *Rhizobium* populations reside only in the upper horizons of the soil; i.e., at depths not exceeding 1-2 m (Alexander 1977). Before this experiment, only the research team led by Virginia et al. (1986), following the work of Felker and Clark (1982), studied *Rhizobium* distribution in the soil. Their results showed that *Rhizobium* populations beneath *Prosopis glandulosa* in California could attain densities of $10 \times 10^3 \text{ g}^{-1}$ of soil, at depths reaching 4-6 m. The present study has demonstrated that *Rhizobium* can heavily populate soils (up to 1.3×10^3 per g of soil) beneath *A. albida* at depths never previously thought to have been inhabited by this bacterium.

Jenkins et al. (1987) showed that two distinct *Rhizobium* populations existed under *P. glandulosa*, according to depth. In the upper horizons, *Rhizobium* predominates, whereas deeper in the soil, *Bradyrhizobium* prevails. With the exception of two strains, all of the *Rhizobium* strains isolated in this experiment exhibited traits similar to those of *Bradyrhizobium* (Jordan 1984).

It is possible that the lower-horizon strains and those of the upper horizons form two distinct subgroups. Since environmental conditions at greater depths are relatively constant, the lower-horizon *Bradyrhizobium* may differ from upper-horizon strains

which are better adapted to wide environmental fluctuations. A taxonomic study based on the comparison of total protein profiles and of nutritional traits is under way to verify this hypothesis.

The presence of nodules on shallow roots of naturally occurring *A. albida* in the Sahelian zone is rare. This absence of nodules may result from the inhibitive effect of limiting factors of the environment, notably drought, and not genetic determinants linked to the plant. *A. albida* nodulates abundantly on continually moist soil, as in the rice fields at Casamance. The absence of large *Rhizobium* populations at greater depths at Casamance may have been due to a lack of oxygen or the difficulty of observing roots. Isotopic tracing may prove useful in determining whether *A. albida* fixes nitrogen in the Sahelian zone. Lower-horizon strains which proved to be very effective nitrogen-fixers could be used to colonize deep root systems of *A. albida*.

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