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IMPROVING THE YIELD AND BIOLOGICAL NITOGEN FIXATION OF BAMBARA GROUNDNUT

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FINAL REPORT

par

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1. INTRODUCTION.

In the developing countries, specially in the semi arid tropics in Africa, the food availability and quality is a social and political problem. It is very important for those countries to develop the research on the nutritional crops which are the resources for the future. On this point of view, bambara groundnut **(Voandzeia subterranea)** is a material of choice. Bambara groundnut is an underexploited legume and it is ground in Senegal in small scale traditional farms as a sole crop or in intercrop combinations with cereals.

Taking into account the fact that legume inoculation might serve as a economical means of increasing yields even in the case of small traditional farms, research on the Rhizubium technology for legume is needed in West Africa, specially in Senegal where legume inoculation with Rhizobium strains is not a common cultural practice, although its beneficial effects have been demonstrated by a number of field experiments carried out with soybean (Dommergues et al., 1979 ; Wey, pers. comm.). To our knowledge, no inoculation study has so far been carried out with bambara groundnut in Senegal. However, very few studies have been done on nodulation and nitrogen fixation by bambara groundnut (Denarie et al., 1968 ; Doku, 1969 ; Thompson and Devis, 1977).

In 1983, Senegal Institute of Agricultural Research (ISRA) had submitted a proposal to EOSTID-National Academy of Sciences (NAS) for studying the biological nitrogen fixation by bambara groundnut in the framework of the West Africa MIRCEN. In December 1983, Dr. Michael DOW had a long discussion with the MIRCEN staff on the objectives and methodology of the grant.

In May, 1984, the NAS approved to ISRA a two years grant to carry out a research project entitled "Improving the Yield and Biological Nitrogen Fixation of Bambara groundnut". This grant was prolonged until 1 September 1987.

2. OBJECTIVES.

The main objective of the project is to improve the yield of bambara groundnut through the nitrogen fixation. Because it is an underexploited legume, it is necessary to constitute first an important germplasm of bambara groundnut and a collection of effective Rhizobium strains. This is why the objectives of the first phase of this project are :

- Obtain promising bambara groundnut germplasm in local markets and from international collections.

- Determine variability and select pure strains based on phenotype and yield.

- Determine responsiveness to nitrogen and phosphorus, and determine wether further work on microbiological association will be fruitful.

- Collect **Rhizobium** strains from international collections including

Table 1. Soil characteristics of Bambey, Djibelor, Nioro and Sinthiou Maleme experimental stations.

Soils	ppm			Granulometry (%)			pH(H2O
	Total C	Total N	Avail.P°	Clay	Loam	Sand	
Bambey	2970	290	121.8	4.0	2.0	94.0	7.8
Djibelor	4220	440	10.1	9.5	4.8	87.5	4.5
Nioro	4160	310	137.0	6.4	3.6	90.0	5.s
Sinthiou Maleme	4380	450	156.5	4.8	2.5	92.7	6.2

° : Available P (Olsen, 1954).

Niftal, other MIRCENs, and the Wold Data Center.

- Screen **Rhizobium** strains, including some isolated locally, for competitiveness and effectiveness with identified plant lines.

- Observe possible disease.

2. MATERIALS AND METHODS.

2.1 - Soils.

Our experiments were carried out at four experimental stations in Senegal: Bambey, Djibelor, Nioro and Sinthiou Malème. Ecological conditions at these stations are different and the characteristics of the four types of soil are indicated in Table 1. All locations are indicated in Fig. 1.

2.2 - Bambara groundnut cultivars.

Seventy eight bambara groundnut cultivars obtained from different countries in Africa were listed in Table 2.

2.3 Rhizobium strains

Eleven strains listed in Table 3 were used in the main experiments we conducted during the grant period.

2.4 - Variety trials.

Variety trials for determining agronomic characteristics in correlation with the grain yield were conducted in 1985 at Bambey (rainfall : 380mm) and Nioro (rainfall : 532 mm) experimental stations. Cultivars obtained in 1984 such as 78-1 , 79-1 , 83-126, 83-127, 83-129, 83-130, 83-131 and Sud Cameroun were used for this study .

2.5 - Responsiveness of bambara groundnut to nitrogen and phosphorus applications.

Greenhouse experiment to determine the responsiveness of bambara groundnut to nitrogen and phosphorus application was conducted. Cultivars 79-1 and 83-131 were planted in a factorial complete randomized design consisting of two factors viz. nitrogen application as urea at three levels (0,20 and 100 ppm N) and phosphorus application as triple superphosphate at four levels (0,20, 40 and 80 ppm P) with five replications. Seeds were germinated in sand for 3 days and each seedling was then transplanted in a pot containing 3kg of unsterile Bambey Soil. Nitrogen and Phosphorus free Hewitt solution (Hewitt, 1966) diluted with water (1:3) was added once a week during the growing period.

After 55 days of growth, plant tops were clipeed off, dried, weighed for dry matter production, and grounded for nitrogen and phosphorus contents

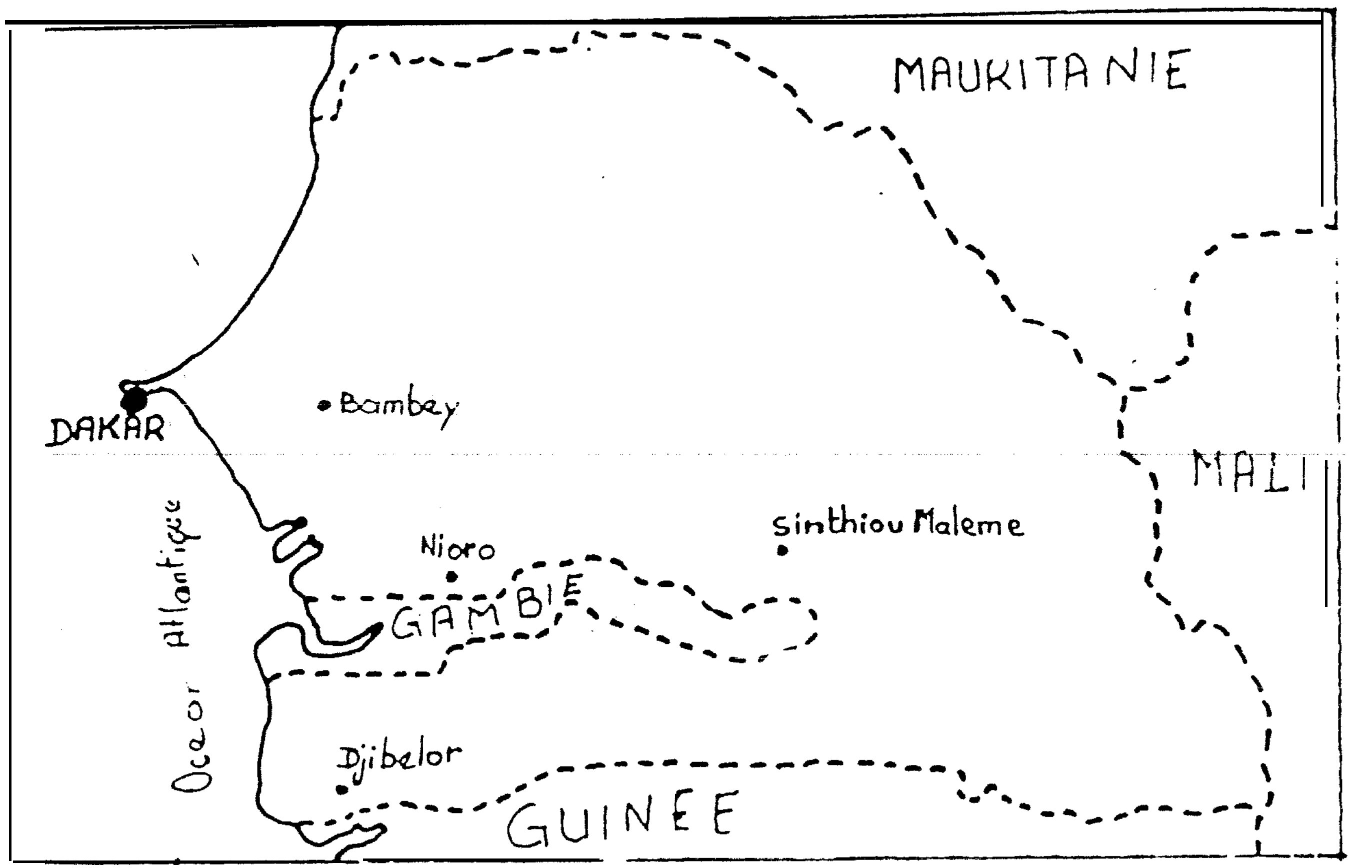

Fig 1 Republic of Senegal : ISRA's experimental stations.r

Germplasm [illegible]

Entries Number	Cultivars	Origin
	78-1	Senegal
	78-[illegible]	Senegal
	83-12[illegible]	Mali
	83-12[illegible]	Mali
	83-12[illegible]	Mali
	83-130	Mali
	83-131	Mali
	Sud Cameroun	Cameroun
	Sarakawa [illegible]	Togo (UMES)*
10	Sarakawa [illegible]	Togo (UMES)
11	Sarakawa [illegible]	Togo (UMES)
12	Sarakawa 1[illegible]	Togo (UMES)
13	Thitchao 8	Togo (UMES)
14	Awandjelo 1	Togo (UMES)
15	Ketao [illegible]	Togo (UMES)
16	Ketao [illegible]	Togo (UMES)
17	Ketao [illegible]	Togo (UMES)
18	Alheride 1	Togo (UMES)
19	Lassa [illegible]	Togo (UMES)
20	Lassa [illegible]	Togo (UMES)
21	Lassa [illegible]	Togo (UMES)
22	V2	Togo (UMES)
23	85001	Senegal
24	85002	Senegal
25	85003	Senegal
26	85004	Senegal
27	85005	Senegal
28	85006	Senegal
29	85007	Senegal
30	V1	Togo (UMES)
31	Mena [illegible]	Madagascar
32	Ma[illegible] [illegible]katra	Madagascar
33	Manga [illegible]	Madagascar
34	Atodi [illegible]	Madagascar
35	Madom [illegible]itatra	Madagascar
36	Kely Mana	Madagascar
37	Tsy Mandefitra	Madagascar
38	Manevaka	Madagascar
39	Voandelaka	Madagascar

Table 2 (continued)

S. No.	Cultivars	Origin
[illegible]	Manjela	Madagascar
[illegible]	Dona	Madagascar
42	Mamokatra	Madagascar
43	Bakoly	Madagascar
44	H.T.B.	Madagascar
45	SA.1	Madagascar
46	SA.2	Madagascar
47	SA.3	Madagascar
48	SA.4	Madagascar
49	SM.20	Togo
50	SM.6	Togo
51	SM.10B	Togo
52	SM.12	Togo
53	SM.13	Togo
54	SM.18 A	Togo
55	SM. 20 A	Togo
56	SM.34	Togo
57	SM.36	Togo
58	SM 37	Togo
59	SM.42	Togo
60	SM.52	Togo
61	SM.54	Tpgp
62	SM.58	Togo
63	SM.62	Togo
64	SM.64	Togo
65	SM.65	Tpgp
66	SM.67	Togo
67	SM.68	Togo
68	SM.69	Togo
69	SM.70	Togo
70	SM.71	Togo
71	SM.95	Togo
72	SM.102B	Togo
73	SM.108	Togo
74	SM.116 A	Togo
75	SM.117	Togo
76	SM.119	Togo
77	SM.122A	Togo
78	SM.127	Togo

*These cultivars were obtained with collaboration with the University of Maryland Eastern Shore (UMES).

Table 3. *Rhizobium* strains used during the grant period.

Strains	lantsp	Sources
MAO 11	Glycine max	West Africa MIRCEN
MAO 26	Glycine max	West Africa MIRCEN
MAO 113	Voandzeia subterranea	West Africa MIRCEN
MAO 118	Voandzeia subterranea	West Africa MIRCEN
BAM 618	Voandzeia subterranea	University Maryland East. Shore
TAL 22	Phaseolus lunatus	NifTAL project Hawaii, USA
TAL 169	Vigna unguiculata	NifTAL project Hawaii, USA
TAL 569	Desmodium uncinatum	NifTAL project Hawaii, USA
TAL 1380	unkwown	University Maryland East. Shore
MUNGO	unkown	University Maryland East. Shore

determination.

2.6 Field screening of bambara groundnut cultivars for biological nitrogen fixation.

During 1985 rainy season field screening experiments were carried out at Bambey (rainfall : 377mm) and Nioro (rainfall : 532mm) experimental stations . Twenty four bambara groundnut cultivars (entries from 1 to 24. Table 2) were hand sown in a randomized block design. The cultivars were sown in two treatments : without (-N) and with (+N) nitrogen fertilization (50 kg urea/ha). All plots received phosphorus and potassium fertilization (60 kg P_2O_5/ha and 120 kg KCl/ha respectively) .
Six weeks after planting, thr Relative Effectiveness (R.E.) of each host plant - indigenous Rhizobium strains combinatioil was evaluated by the following formula (Mulongoy et al., 1980):

$$R.E. = \frac{X \text{ of ten samples in the -N block}}{X \text{ of ten samples in the +N block}}$$

In this formula, x denotes either shoot , root or nodule dry weights or shoot nitrogen content.

2.7 - Isolation of native Rhizobium strains from bambara groundnut nodules.

2.7.1. Nitrogen fixing abiliting of indigenous Rhizobium strains.

The samples were taken in each treatment from five well developed plants and still having immature pods. Forty five well developed, pink nodules were sampled from the tap roots.
Rhizobium strains were isolated according to standard procedures (Vincent, 1970). Isolated rhizobia were cultured on agar slants made from YEM medium. Nitrogen fixing ability of the isolates were compared in terms of shoot dry weight of bambara groundnut cv. 85001 used as test plant and cultivated in Leonard jar assemblies (Leonard, 1943). Seeds were sterilized using 0.01% $HgCl_2$ for 3 mn, and germinated in sterile sand for 3 days ; each seedliny was then transplanted into a jar assembly. One ml suspension of rhizobial isolate broth (approximatively 10^9 cells) was added aseptically to the seedlings for all treatments except for an uninoculated control and plants were grown in a greenhouse. Nitrogen free Hewitt solution (Hewitt, 1966) diluted with water (1 :3) was added once a week during the growing period.
After 35 days of growth, plant tops were clipped off, dried and weighed fur dry matter production.

2.7.2. Greenhousse evaluation of host plant-Rhizobium strain interactions.
Both the MAO indigenous and introduced Niftal Rhizobium strains listed in Table 10 were selected on the basis of their effectiveness. The Niftal strains served as standars for comparison against the indigenous ones . Twelve bambara groundnut cultivars also listed in Table 10 were used as

host plants. Seed sterilization of each genotype, germination, transplantation, inoculation and watering were conducted as described above and then seedlings were transplanted in Leonard jars. Treatments were arranged in a randomized block design replicated five times.
After 50 days of growth, the plants were harvested. Plants tops were dried and weighed. Nodulation was scored taking into account the number (N), the internal colour (C) and the size (S) of nodules. The nodulation index (Nod. I.) was calculated as follows :

$$\text{Nod. I.} = N \times C \times S$$

Nodule number was rated on a scale from 0 (no nodule) to 3 (many nodules). Nodule internal colour was from 0 (white) to 1 (red) and nodule size was from 1 (small nodule) to 2 (big nodule).

2.9 - Field inoculation trials of bambara groundnut.

Tree experiments were carried out in 1985, 1986 and 1987.

2.8.1 - Experiment conducted in 1985.

The experiment was carried out at Bambey experimental station (rainfall : 377mm). The indigenous rhizobial population in the field selected for this work is 10 cowpea rhizobia/g of soil as determined by plant infection test using **Macroptilium atropurpureum**. The cultivar V2 obtained from Togo was used. Applied treatments were : uninoculated, no N ; uninoculated + 50 kg N (urea)/ha ; inoculated with 1g of six different peatbase **Rhizobium** inocula : Mungo, BAM 618, TAL 1380, AH 169, MAO 11 and MAO 26. Randomized block design with four replicates was used. The size of each plot was 3m and 2.1m and the plants were spaced by 15 and 30 cm. Application of fertilizers was 40 kg P_2O_5/ha and 120 kg KCl/ha.
Sixty days after planting, plants were sampled for nodulation, shoot and nodule dry weights and nitrogen content determination.

2.8.2 - Experiment conducted in 1986.

A cultivar x **Rhizobium** field experiment was initiated at two stations : Djibelor (rainfall : 934mm) and Sinthiou Malème (rainfall : 794mm). Two bambara groundnut cultivars (79-1 and 83-131) were inoculated with five **Rhizobium** strains : MAO 113, MAO 118, TAL 22, TAL 169 and TAL 569. In addition, there were two treatments without inoculation : one treatment including application of 23 kg N/ha as urea and the other no N fertilization. The experimental design was a split-plot with four replications. **Rhizobium** strains arranged in a randomized complete block were the main plots and cultivars the subplots. Each 2.4m x 1.5m experimental plot received a blanket application of 60 kg P_2O_5/ha as triple superphosphate and 72 kg K_2O/ha as muriate of potash. **Rhizobium** strains were used as laboratory-made peat base inoculants containing 10^8 cells/g. One gram of inoculant was placed in the seed-hole at planting.

Bambara groundnut cultivars were planted at a spacing of 30cm x 15cm. At harvest, grain yield was evaluated.

2.8.3 - Experiment conducted in 1987.

Field inoculation of bambara groundnut with **Rhizobium** strains was initiated at Sinthiou Maleme (rainfall : 662mm) in the aim to study the competitiveness of **Rhizobium** strains MAO 118, TAL 22, TAL 169 and TAL 569 against indigenous **Rhizobium** Immunofluorescence method was used for the competitiveness study. Fluorescent antibodies (FA) against TAL 22, TAL 169 and TAL 569 were supplied by Niftal project, and FR against MAO 118 was supplied by Dr. E.L. SCHMIDT from the university of Minnesota, at St. PAUL. All fluorescent antibodies were specific. The experimental design was a randomized complete block in which cvs. 79-1 and 83-131 were used. Each cultivar was inoculated either with a single strain or a mixture of strains. Each 3. 3om x 0.90m received a blanket application of 60 kg P_2O_5/ha as triple superphosphate and 72 kg K_2O/ha as muriate of potash. **Rhizobium** strains or a mixture of **Rhizobium** strains were used as laboratory made peat base inoculant containing 10^8 cells/g. The mixture contained the same amount of the different strains included the inoculum. One gram of inoculum was placed in the seed-hole at planting. Cultivars were planted at a spacing of 30cm x 15cm.
After 60 days of growth, nodule occupancy, shoot and nodule dry weights, nitrogen and phosphorus contents were determined. At harvert, grain yield was evaluated.

2.9 - Survey of diseases on bambara groundnut.

Bambey and Nioro experimental plots of bambara groundnut were visited periodically to see wether there is any disease. Farmers fields were also visited once just before flowering. Diseased samples of unknown disease were collected from experimental plot at Bambey. The premiliminary symptom of the disease is withering of leaves which is generaldryobserved after about one month from sowing. The leaves subsequently dry and finally resulting in complete death of the plant.
In order to find out the causal microorganism of the disease, isolations were made from the infected plant parts on Potato Dextrose Agar (PDA) medium as per standard procedure. The pathogenicity test of these isolates was carried out by inoculating 3 weeks old seedlings of two varieties viz, 83-129 and 83-130. Two pots of each variety were inoculated with the spore suspension of each isolate with the help of small atomizer. Uninoculated posts of each variety served as control. The pots were kept in the moist chamber for 48 hours and then transferred to greenhouse.

2.10 - Effect of pH on the growth of bambara.

Cultivar 79-1 was grown in pots containing 15 kg of unsterilized soil from Bambey irrigate zone (A) and non irrigate zone (B). The plants were watered with tap water (a) and distilled water (b). Thus there were four treatments viz. A + a (pH = 8.20), A + b (pH = 7.15), B + a (pH=7.50) and B + b (pH = 6.00) with four replicates arranged in completely randomized design. The pH of different treatments was determined before starting the experiment as well as after achievement of the experiment. Daily observations of the behaviour of plants were made. The plants were harvested 35 days after planting. The shoots and roots were separately dried and weighed.

3. RESULTS AND DISCUSSION.

3.1 -- Variety trials

Agronomic characteristics of the eight cultivars and their correlations with yield are shown in Tables 4 and 5. Variations were observed in responses of individual cultivar. Grain yields at Nioro were generally higher than at Bambey station. This is due to higher rainfall at Nioro (532mm) than at Bambey (380mm). However, grain yield was more than 1 t at both stations. At Bambey, the weight of 100 seeds only was significantly correlated with the yield while at Nioro, number and weight of pods and seeds were correlated with the yield. In conclusion, cv. 79-1 was the best one with 1419.40 kg/ha at Bambey and 1278.40 kg/ha at Nioro station. However, a second year test is needed at the same stations and/or other sites. After that, one or two cultivars will be proposed for vulgarisation. Therefore, it is necessary to initiate or to continue the study on population density or fertilization of bambara groundnut. Number and weight of thr pods and seeds and the weight of 100 seeds seemed determinant for yield evaluation. This would be important for a variety improvement program.

3.2 - Responsiveness of bambara groundnut to nitrogen and phosphorus.

3.2.1 - Effect of nitrogen. (Table 6)

Nitrogen supplied at 20 ppm N level increased shoot dry weight of cvs. 79-1 and 83-131 (+ 42% and 43% respectively). However, for both cultivars, there was no difference on shoot dry weights between 20 ppm and 100 ppm nitrogen application. Nitrogen content and total nitrogen were increased for both cultivars : supplies of 20 ppm N and 100 ppm N increased the nitrogen content of cv. 79-1 (19% and 58% respectively). For the cultivar 83-131, the corresponding increase was 14% and 73%. There was no effect of nitrogen fertilizer on phosphorus content for both cultivars.

3.2.2 - Effect of phosphorus. (Table 7)

Phosphorus supplied at 20 ppm P level increased shoot dry weight of cv. 79-1 (+ 25%) and there were no effect on shoot dry weight for cv. 83-131. No effect on nitrogen content was observed for both cultivars. However phosphorus content of both cultivars increased significantly with the increase of phosphorus application : supplies of 20 ppm P, 40 ppm P and

Table 4 : Agronomic characteristics of eight cultivars of bambara groundnut cultivated at Bambey experimental station.

	Number per plant				Weight (g) per plant					
Cultivars	Leaves	Stern	Pods	Seeds	Leaves	Stem	Pods	Seeds	Weight (g) of 100 seeds	Grain yield (kg/ha)
78-1	60.75 c	9.25 3	11.75 a	12.25 a	7.32 c	1.54 b	6.03 a	4.19 a	44.66 cd	843.00 b
79-1	57.25 c	3.25 a	15.00 a	15.25 a	10.67 bc	1.62 b	8.49 a	6.34 a	51.76 bc	1419.38 a
83-126	71.75 bc	9.25 a	13.25 a	14.50 a	9.66 bc	1.76 b	6.96 a	5.28 a	40.62 d	1094.02 ab
83-127	54.75 c	â.25 a	15.00 a	15.75 a	9.92 bc	1.72 b	9.60 a	7.25 a	47.87 bcd	891.22 b
83-129	92.00 a	13.50 a	iC.75 a	12.00 a	14.51 a	3.43 a	7.82 a	5.83 a	70.98 a	[illegible] b
83-130	79.25 ab	9.75 a	14.25 a	15.00 a	11.26 ab	2.12 b	7.53 a	5.83 a	47.39 bcd	1123.[illegible] ab
83-131	58.75 c	9.25 a	18.25 a	19.75 a	11.33 ab	1.91 b	11.16 a	8.51 a	53.00 b	[illegible] ab
Sud-Cameroun	62.00 bc	9.00 a	18.00 a	18.75 a	9.25 bc	2.08 b	8.39 a	6.68 a	45.32 bcd	102[illegible].25 b
Correlation with grain yield	0.07^{ns}	0.13^{ns}	0.03^{ns}	0.01^{ns}	0.22^{ns}	0.09^{ns}	0.11^{ns}	0.13^{ns}	0.44^{s}	

s : significant
ns : non significant
Values followed by the same letter in each column do not differ significantly at the 0.05 level by DUNCAN's multiple range test

Table 5 : Agronomic characteristics of eight cultivars of bambara groundnut at Nioro expérimental station.

	Number per plant				Weight (g) per plant					
Cultivars	Leaves	Stern	Pods	Seeds	Leaves	Stern	Pods	Seeds	Weight (g) of 100 seeds	Grain yield (kg/ha)
78-1	68.50 a	3.75 a	15.50 a	17.50 a	11.38 a	2.09 a	8.34 abc	6.19 abc	54.00 d	963.12 a
79-1	64.25 a	14.00 a	21.50 a	22.75 a	13.03 a	1.99 a	11.08 ab	8.76 ab	60.09 bc	1278.42 a
83-126	**64.50** a	10.25 a	11.75 a	10.75 a	11.39 a	1.67 a	7.43 abc	5.74 bc	63.98 ab	[illegible]
83-127	31.00 a	4.25 a	12.25 a	13.00 a	9.75 a	1.49 a	6.39 c	5.12 c	61.15 bc	[illegible]
83-129	59.75 a	3.75 a	17.00 a	ii.70 a	10.82 a	1.76 a	11.64 a	9.39 a	69.20 a	[illegible]
~~83-130~~	72.25 a	3.75 a	~~18.00 a~~	~~19.75 a~~	~~13.30 a~~	~~1.92 a~~	~~9.53 abc~~	~~7.76 abc~~	~~58.65 bcd~~	~~1247.42~~
83-131	[illegible]	14.25 a	13.00 a	15.00 a	12.47 a	2.34 a	7.17 bc	5.35 c	64.00 b	[illegible]
Sud-Cameroun	[illegible].50 a	9.50 a	16.25 a	19.25 a	11.54 a	1.75 a	8.26 abc	6.73 abc	55.65 cd	[illegible]
Correlation with grain yield	0.04 ns	0.03 ns	0.38 s	0.41 s	0.07 ns	0.15 ns	0.50 s	0.52 s	0.24 ns	

s : significant
ns : non significant
Values followed by the same letter in each column do not differ significantly at the 0.05 level by DUNCAN's multiple range [illegible]

Table 6. Effect of nitrogen on shoot dry weight (SDW), nitrogen (N%) and phosphorus (P%) content, total nitrogen and to tal phosphorus contents of bambara groundnut cvs. 79-1 and 83-131 cultivated in pots.

Cul tiv.	N appl. (ppm)	SDW (g/pl)	N%	P%	Total N (mg/pl.)	Total P (mg/pl.)
79-1	0	1.57 b	3.01 c	0.34 a	4'7 b	5 b
	20	2.23 a	3.59 b	0.34 a	8'7 a	8 a
	100	2.80 a	4.75 a	0.32 a	100 a	8 a
83-131	0	1.50 b	3.24 a	0.38 a	48 c	5 c
	20	2.14 a	3.70 b	0.34 a	79 b	? a
	100	2.1.2 a	5.60 a	0.30 a	117 a	6 b

Table 7. Effect of phosphorus on shoot dry weight (SDW), ni trogen (N%) and phosphorus (P%) contents, total ni trogen and total phosphorus of bambara groundnut cvs. 79-l and 83-131 cultivated in pots.

Culi v.	N appl. (ppm)	SDW (g/pl.)	N%	P%	Total N (mg/p 1.)	Total P (mg/pl.)
79-1	0	1.70 b	3.90 b	6.13 d	77 a	5 c
	20	2.12 a	3.64 a	0.26 c	79 a	5 c
	40	2.17 a	3.78 a	0.40 b	85 a	9 b
	80	2.11 a	3.81 a	0.54 a	81 a	4 a
83-131	0	1.86 ab	4.14 ab	0.13 d	78 c	2 b
	20	2.x a	4.27 ab	0.24 c	92 a	5 c
	40	2.02 a	4.00 a	0.39 b	83 b	8 b
	80	1.67 b	4.30 a	0.60 a	72 d	10 a

For Tables 6 and 7, in each column, values followed by the same letter for each cultivar, do not differ significantly at the 0.05 level by Duncan's multiple range test (1955).

80 ppm P increased the phosphorus content of cv. 79-1 of 100%, 208% and 315% respectively ; the corresponding increase for cv. 83-131 was 85%, 200% and 361%. Total phosphorus of both cultivars was increased with phosphorus application.

3.2.3 - Interaction between nitrogen and phosphorus. (Table 8)

There was a significative interaction between nitrogen and phosphorus on shoot dry weight , nitrogen and phosphorus contents, total nitrogen and total phosphorus of both cultivars. The increase of these parameters was more significative than that obtained with nitrogen or phosphorus used alone.

3.3 - Improving biological nitrogen fixation of bambara groundnut.

3.3.1 - Field varietal screening.

At Bambey station cvs. 83-127, Sud Cameroun , Sarakawa 3 , Sarakawa 10 and Lassa 1 had a R.E. based on shoot dry weight below 40% (Fig. 2) while at Nioro station, all cultivars had the same R.E. over 40% (Fig. 3). At Bambey station, only cultivar Sarakawa 10 had a R.E. based on root dry weight below 50% ; at Nioro station, all cultivars had the same R.E. over 50%. Relative Effectiveness based on nodule dry weight was much lower at Bambey than at Nioro : it was in the range of 80-120% at Bambey for cvs. 83-131, Ketao 3 , Alheride 1 , Lassa 1 and Lassa 4 and in the range of 360-480% at Nioro for cvs. 83-129, 83-131, Sud Cameroun, Sarakawa 2, Ketao 9, V 2 and 85002. Relative Effectiveness based on shoot nitrogen content was over 50% for all cultivars except for cv. Awandjelo 1 at Bambey station.
These results indicated that Bambey and Nioro rhizobial population established a moderately effective symbiosis with the cultivars of bambara groundnut used. Among these cultivars, 12 grew better at both the locations without nitrogen fertilizer (Table 9) indicating their ability to set an effective symbiosis in the two different field conditions.
These observations indicated a screening of our bambara groundnut germplasm for 12 lines capable of forming most effective symbiosis with suitable **Rhizobium** strains. They will be inoculated with strains isolated at Bambey and Nioro where they had an effective symbiosis as an attempt to improve their performance where they yield poorly.

3.3.2 -- Effectiveness of indigenous Rhizobium strains isolated from bambara groundnut nodules.

Bambara groundnut cultivars sampled were moderately nodulated on both the tap and lateral roots. Many nodules appeared to be effective as indicated by internal colour. Thirty one isolates were so obtained (I_1 to I_{31}, fig. 3). The isolates were slow growing and formed small (diameter <1mm). dry colonies on yeast mannitol agar. Four isolates (I_8, I_{13}, I_{22} and I_{30}) were included in West Africa MIRCEN (MAO) **Rhizobium** culture

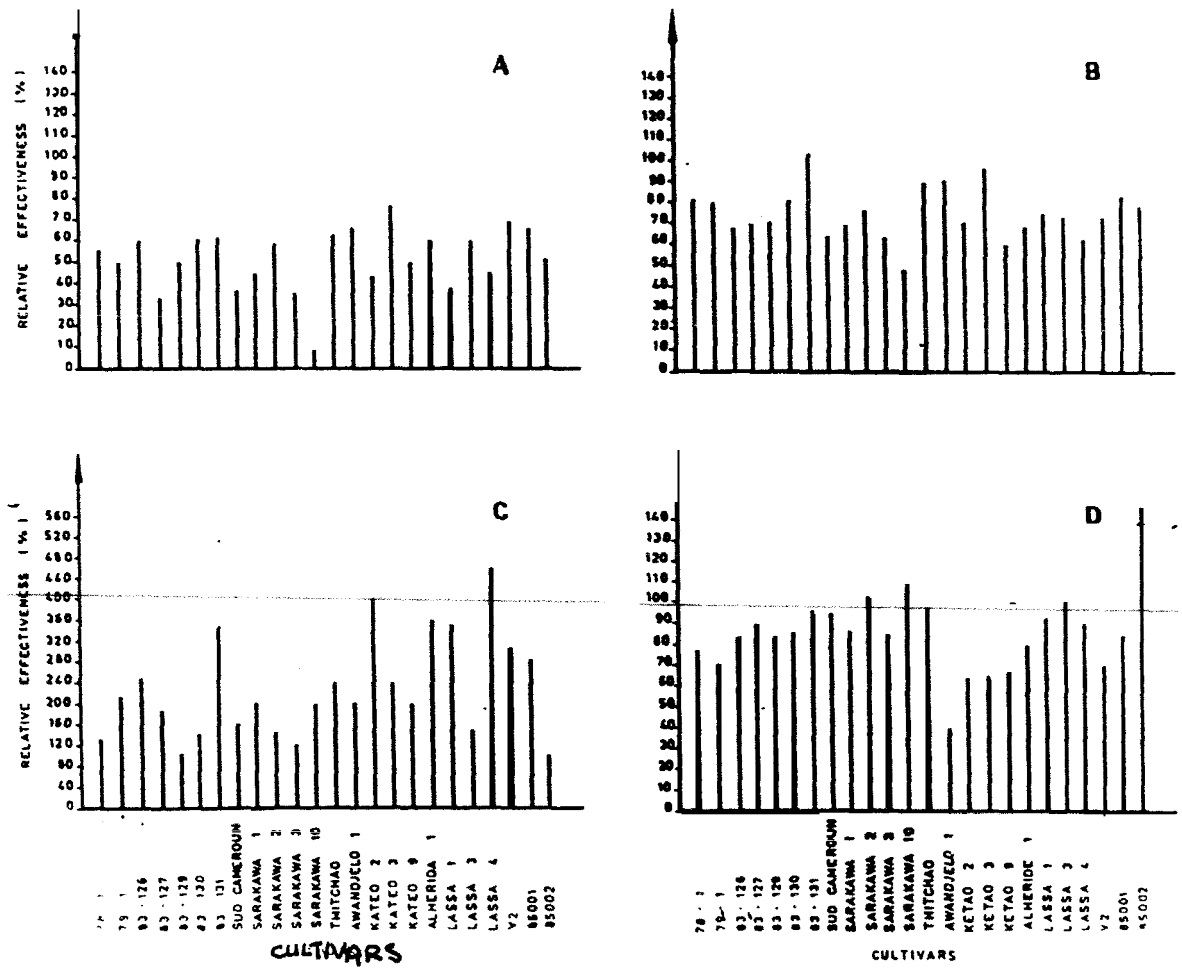

Fig. 2 Relative Effectiveness (R.E.) of 24 cultivars cultivated at Bambey experimental station
A : R.E. based on shoot dry weight. B : R.E. based on root dry weight./A : R.a
C : R.E. based on nodule dry weight. D : R.E. based on nitrogen content.

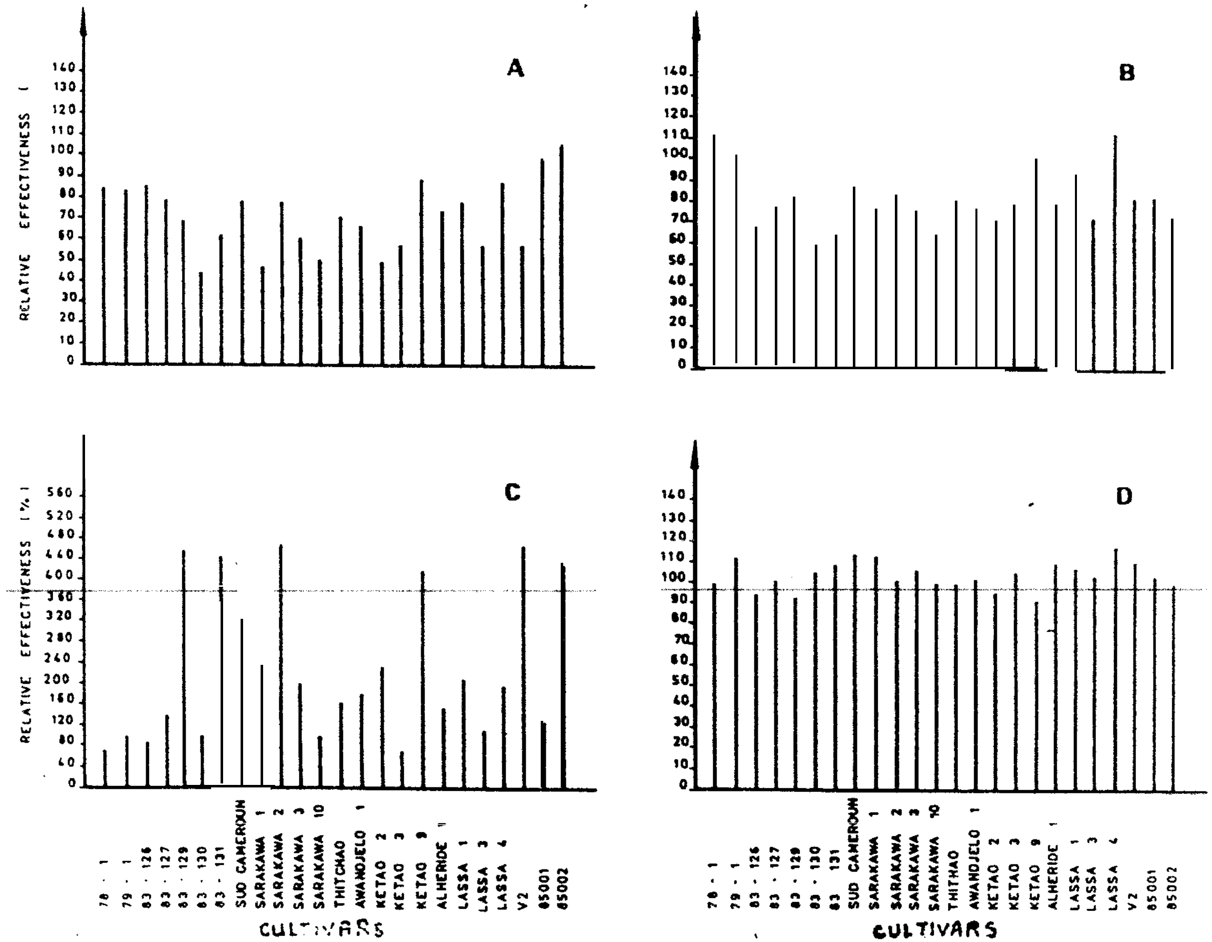

Fig. 3 Relative effectiveness (R.E.) of 24 cultivars cultivated at Nioro experimental stati

A : R.E. based on shoot dry weight. B : R.E. based on root dry weight.

C : R.E. based on nodule dry weight. D : R.E. based on nitrogen content.

Table [illegible] Effect of [illegible] and [illegible] on shoot dry weight [illegible], nitrogen (N%) and phosphorus (P%) contents, total [illegible] and phosphorus of bambara groundnut [illegible] 79 [illegible] and 87 131 [illegible] pots.

	N appl (ppm)	P appl (ppm)	SDW	N%		N total [illegible]	P total [illegible]
79 1	0	0	1.51 de	2.89 de	0.14 d	44 c	2 e
		20	1.73 bcde	2.68 e	0.26 c	46 c	4 d
		40	1.47 e	2.94 de	0.43 b	43 c	6 d
		80	1.58 de	3.52 bc	0.52 a	56 bc	8 c
	20	0	1.61 cde	3.92 b	0.12 d	60 bc	2 e
		20	2.26 abcd	3.60 bc	0.25 c	82 abc	5 d
		40	2.69 a	3.57 bc	0.41 b	98 ab	11 b
		80	2.34 abc	3.28 cd	0.56 a	77 abc	13 a
	100	0	1.97 abcde	4.90 a	0.12 d	96 ab	3 e
		20	2.37 abc	4.65 a	0.26 c	110 a	6 d
		40	2.36 abc	4.84 a	0.36 b	114 a	8 c
		80	2.40 ab	4.61 a	0.54 a	111 a	13 a
87 131	0	0	1.70 bc	3.05 f	0.15 d	51 f	3 c
		20	1.40 bc	3.51 cdef	0.28 de	50 f	4 c
		40	1.50 bc	3.30 def	0.46 b	49 fh	7 bc
		80	1.40 c	3.13 ef	0.63 a	43 h	8 b
	20	0	2.10 ab	3.63 cde	0.12 g	76 g	2 c
		20	2.50 a	3.73 cd	0.22 ef	90 e	6 b
		40	2.10 abc	3.58 cdef	0.37 c	74 g	8 b
		80	2.00 bc	3.90 c	0.66 a	76 g	13 a
	100	0	1.84 abc	5.76 a	0.17 g	107 c	2 c
		20	2.50 a	5.56 ab	0.21 ef	137 a	5 b
		40	2.50 a	5.20 b	0.33 cd	128 b	8 b
		80	1.70 bc	5.90 a	0.50 b	98 d	8 b

In each column, for each cultivar, values followed by the same letter do not differ significantly at the 0.05 level by Duncan's multiple range test (1955).

Table 9 : Shoot dry weight (g/10 plants) of the performed cultivars of bambara groundnut. in field at Bambey and Nioro experimental stations.

Cultivars	Bambey	Nioro
79-1	55.5 b	30.4 b
83-126	62.2 b	33.6 b
83-129	55.8 b	39.0 ab
83-131	56.5 b	30.2 b
Sarakawa 1	52.8 b	31.1 b
Awandjelo 1	64.2 b	40.5 ab
Ketao 2	63.8 b	31.7 b
Ketao 3	66.2 ab	32.9 b
Alheride 1	68.2 ab	43.9 a
Lassa 3	78.5 a	38.6 ab
V2	61.5 b	41.3 ab
85001	56.5 b	42.0 a

Values followed by the same letter in each column do not differ significantly at the 0.05 level by DUNCAN's multiple range test (1955).

collection because of their high effectiveness evaluated in terms of shoot dry weight with cv. 85001. This cultivar was selected as plant test because it is from Senegal and it had a good R.E. at both Bambey and Nioro experimental stations. These isolates were named MAO 113, MAO 118, MAO 121 and MAO 126 respectively (Fig. 4).

3.3.3 - Evaluation of host cultivar-Rhizobium strains interactions.

Rhizobium strains used were compared for their effectiveness to nodulate the most promising cultivars of bambara groundnut selected in field varietal screening. Nodules were found in the top 5cm of root system on all plants except controls which are all nodule free. Nodulation index and the effectiveness varied greatly with the strains (Table 10). Strains MAO 113 and MAO 118 exhibited effective nodulation on six and three cultivars respectively while strains TAL 22, TAL 169 and TAL 569 were effective on five, four and three cultivars respectively. All Rhizobium strains were effective on cv. 83-131 except strain TAL 169 which exhibited partial nodulation. All Rhizobium strains were partially effective on cv. Ketao 3. Similarly, Rhizobium strains MAO 113 and MAO 118 were ineffective on cv. V2 while strains TAL 22, TAL 169 and TAL 569 were partially effective on this cultivar. Strains MAO 113 and TAL 169 were ineffective on cvs. Awandjelo 1, and 83-129 respectively; strain MAO 118 was ineffective on cvs. Awandjelo 1, Lassa 3 and V2; strain TAL 569 was ineffective on cvs. Ketao 2, Alheride 1 and Lassa 3. Strain TAL 22 presented the widest effectiveness spectrum: it was fully effective on 42% and partially effective on 58% of all cultivars studied, followed by strain MAO 113. These results indicated that plant genotype had an important effect on nodulation of bambara groundnut.

Many of studies reported on plant genotype x Rhizobium strain interactions indicated differences between cultivars of the same species in abilitry to fix nitrogen using introduced rhizobial strains. Because it is difficult to introduce and establish new Rhizobium strains into fields containing indigenous strains (Brockwell, 1980), it is important to isolate competitative and adapted strains, which can nodulate effective: , most cultivars even these strains are not widely distributed in the soil. Instead of introducing new Rhizobium the isolated adapted Rhizobium strains may be re-introduced in mass into the field so they compete against indigenous strains which are less numerous. Our results show that all Rhizobium strains including introduced and indigenous strains nodulated all cultivars. When the host and rhizobial strains were arranged in an increasing order of effectiveness, one indigenous strain, MAO 113, exhibited the highest effective association with the cultivars. Therefore, strain MAO 113 is expected to give better nitrogen fixation under field conditions.

3.3.4. Field response of bambara groudnut to inoculation with Rhizobium strains.

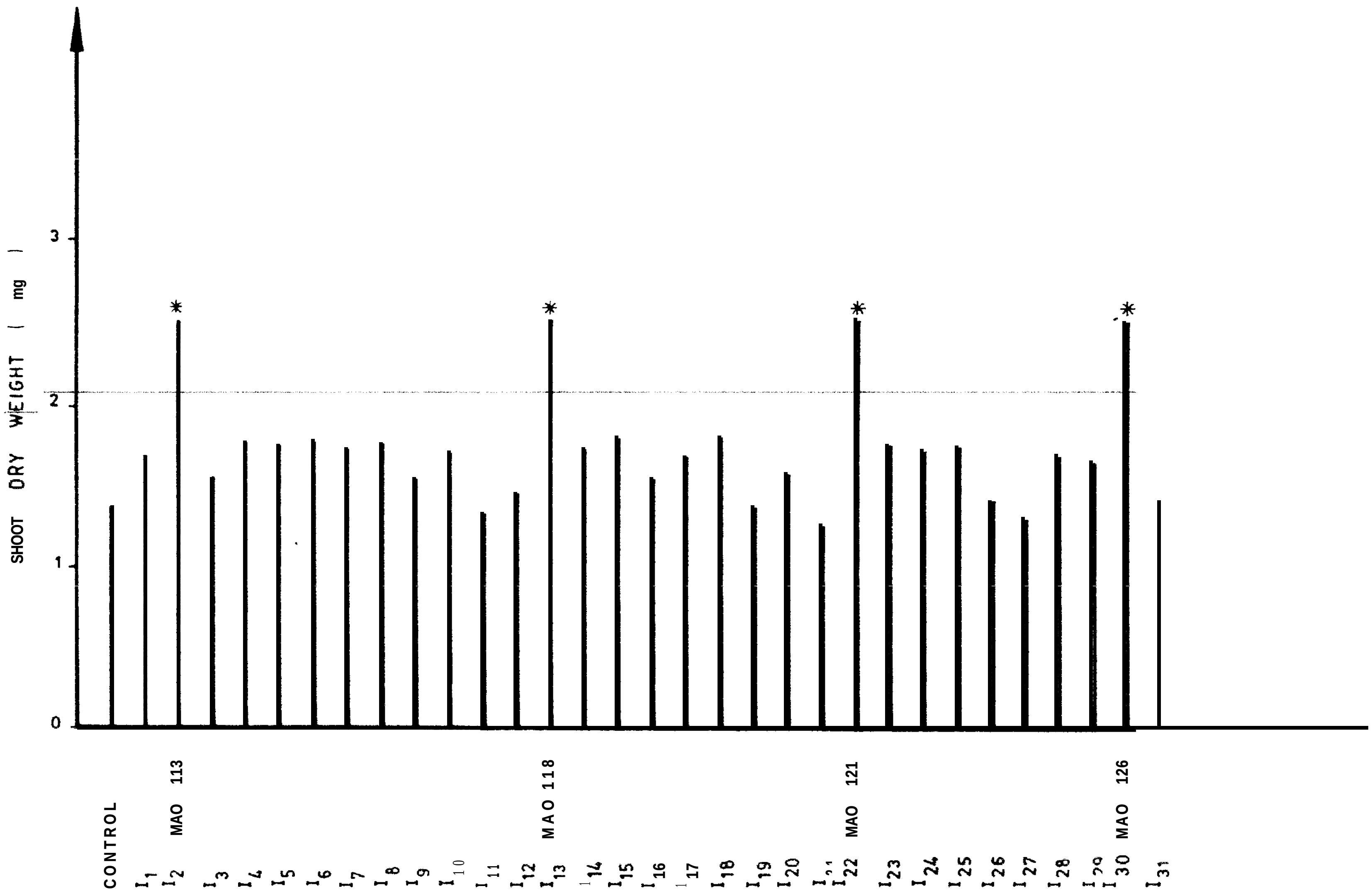

Fig. 4 Shoot dry weight of b. groundnut cv. 85001 cultivated in Leonard jar and inoculated with 31 *Rhizobium* strains.

Table 10 [illegible] weight (SDW) [illegible] plant and [illegible] index (Nod. I.) of 12 bambara groundnut cultivars inoculated [illegible] *Rhizobium* [illegible]
from [illegible] MIRCEN Africa (MAO) and NifTAL project.

Cultivars	CONTROL		MAO 1[illegible]		MAO 118		TAL 22		TAL [illegible]			
	SDW	Nod. I.	SDW	Nod. I.	SDW	Nod. I	SDW	Nod. I	SDW	Nod. I	SDW	Nod. I
[illegible]		nd			[illegible] a		[illegible] a	[illegible]				
[illegible]	[illegible] a	nd	[illegible] a		[illegible] abc	[illegible]	1.4 [illegible]	[illegible]				
[illegible]	[illegible] a	nd	[illegible] a		[illegible] abc	[illegible]	1.4 [illegible]	[illegible]			[illegible]	
[illegible]	[illegible] a	nd	[illegible]		[illegible] ab	[illegible]	1.8 ab	E	[illegible] a			
[illegible]	[illegible] a	nd	[illegible] a		[illegible] abc	[illegible]	1.8 ab	E		[illegible]	[illegible]	[illegible]
Kwandjel[illegible]	[illegible] a	nd	[illegible]		[illegible] bc	[illegible]	1.5 abc	[illegible]	[illegible]		[illegible]	
[illegible]	[illegible] a	nd	[illegible]		[illegible] ab	[illegible]	1.5 abc	[illegible]			[illegible]	
[illegible]	[illegible] a	nd	[illegible] ab	[illegible]	[illegible] abc	[illegible]	1 [illegible]	[illegible]	[illegible] a	[illegible]	[illegible] a	
	[illegible]	[illegible]			[illegible] abc		[illegible] a	[illegible]				
[illegible]	[illegible] a	[illegible]			[illegible]	I	[illegible] a	[illegible]				
[illegible]	[illegible] a	[illegible]	[illegible] a		[illegible]	[illegible]	1 [illegible]	[illegible]				
[illegible]	[illegible] a	nd	[illegible] a		[illegible] a	[illegible]	1.[illegible] a	[illegible]				

[illegible] - Values followed by the same letter in each column do not differ at the 0.05 level by DUNCAN's multiple range test ([illegible]). a : highly effective ; [illegible] : partially effective ; [illegible] : ineffective.

E : Highly effective nodulation (Nod.I.$\geq$[illegible]) ; [illegible] : partially effective nodulation (2$\leq$Nod. I.$<$4) ; I : ineffective nodulation (Nod.I.$<$[illegible]).

- nd : non determined.

3.3.4.1. Experiment conducted in 1985.

The results of this experiment are shown in Table 11.
Appreciable responses to inoculation were obtained in shoot dry weight with strain BAM 516 (increase of 144%), in nodule dry weight with strains Mungo, BAM 518 and MAO 26 (increase of 95%, 100%, 73% respectively) and in nitrogen content with strains BAM 618, TAL 1380, MAO 11 and MAO 25 (increase of 27%, 39%, 24% and 17% respectively). Increase in total nitrogen was obtained with strains BAM 618 (+ 209%) and MAO 11 (+ 135%).
From these results, it is apparent that bambara groundnut cv. V2 required an inoculation with suitable Rhizobium strain for adequate nodulation and nitrogen fixation in soil used.

3.3.4.2. Experiment conducted in 1986.

The results of this experiment are shown in Table 12.
Grain yields were always lower at Djibelor than at Sinthiou Maleme probably because of the lower fertility of Djibelor soil which is reflected by its low pH and low available P content. At Djibelor, inoculation of cv. 79-1 with strains MAO 113 and TAL 569 significantly resulted in 83 and 65 percent yield increase over the uninoculated treatment but the yield increase over the N-fertilized treatment was not significant. Inoculation did not affect the yield of cv. 83-131. At Sinthiou Maleme, inoculation of cultivars 79-1 and 83-131 with Rhizobium strain TAL 22 resulted in a 50 and 58 percent yield increase over the uninoculated treatment, whereas the corresponding yield increase over the N treatment was 50 and 27 percent respectively. Inoculation with strains MAO 113 and MAO 118 significantly increased (+ 51 and 42 percent) the yield of cv. 83-131 over the uninoculated treatment.

3.3.4.3. Experiment conducted in 1987.

Bambara groundnut nodule occupancy and grain yield for cvs. 79-1 and 83-131 are shown in Tables 13 and 14 respectively.
For both cultivars, nodules of non inoculated plants contained indigenous Rhizobium only. Nodules of plants inoculated with either strain MAO 116, strain TAL 22, strain TAL 169 or strain TAL 569 contained indigenous Rhizobium strains and/or the Rhizobium strain used as inoculant. In all cases, Rhizobium strains MAO 118, TAL 22 and TAL 569 were more competitive than the indigenous strains which were more competitive than strain TAL 169.
When the inoculant was a mixture of strains, two strains were found in the nodules only even if the inoculant contained more than two strains. For both cultivars, strain MAO 116 was more competitive followed by strains TAL 569 and TAL 22 respectively.
While inoculation did not affect the grain yield of cv. 83-131 (Table 14), cv. 79-1 significantly responded to inoculation with strain TAL 22: the yield increase was 160% over the uninoculated treatment. This data confirmed the results obtained in the experiment conducted in 1986 at

Table 11: Shoot and nodule dry weights, shoot nitrogen content and total shoot nitrogen of *Voandzeia subterranea* (cv. [illegible] cultivated at Bambey in field and inoculated with *Rhizobium* strains Mungo, Bam 618, TAL 1380, AH 169, MAO 1[illegible] and MA[illegible]

Treatments	Shoot dry weight (g/10 plants)	Nodule dry weight (mg/10 plants)	Shoot nitrogen content (N %)	Total shoot nitrogen (g/10 plants)
Uninoculated	10.75 d	55.00	1 81 g	1.95 e
Uninoculated + [illegible] kg N (urea)/ha	13.25 cd	40.00	1 88 f	2.50 de
Mungo	15.50 bcd	107.50	1 92 e	2.98 cde
Bam [illegible]	26.25 a	110.00 a	2.30 b	6.03 a
[illegible]	15.75 bcd	30.00 c	2.51 a	[illegible] bc
[illegible]	20.00 b	77.50 ab	1.87 f	3.7[illegible] bcd
[illegible]	20.50 ab	30.00 [illegible]	2.24 c	4.5[illegible] b
[illegible]	18.50 bc	95.00 a	2.12 d	3.90 bc

Values are averages of 10 plants.

-Values followed by the same letter in each column do not differ significantly at the 0.05 level by DUNCAN's multiple [illegible]

Table 12. Effect of N fertilization or inoculation with *Rhizobium* strains MAO 113, MAO 118, TAL 22, JAL 169 and TAL 569 on grain yield of field-grouwn bambara groundnut cvs. 79-1 and 83-131 at Djibelor and Sinthiou Maleme experimental stations.

Cultivars	Treatments	Grain yield (g/10 plants) Djibelor		Sinthiou Maleme	
79-1	Uninocul.	45.15	d	104.89	d
	N-Fertil.	60.2"	abcd	104.98	d
	MAO 113	74.34	ab	123.23	bcd
	MAO 118	65.0[illegible]	abcd	127.35	abcd
	TAL 22	57.20	bcd	157.62	abc
	TAL 169	60.18	abcd	118.07	cd
	TAL 569	82.58	a	131.11.	abcd
83-131	Uninocul.	60.49	abcd	108.08	d
	N-Fertil.	62.49	abcd	134.49	abcd
	MAO 113	68.89	abc	163.55	ab
	MAO 118	40.35	d	153.59	abc
	TAL 22	50.48	bcd	170.65	a
	TAL 169	51.79	bcd	144.07	abcd
	JAL 569	69.01,	abc	141.92	abcd

At each location, values followed by the same letter do not differ at the 0.05 level by Duncan's multiple range test (1955).

Table 13 : Nodule occupancy and grain yield of bambara groundnut cv 79-1 inoculeted with Rhizobium Strains MAO 118, TAL 22, TAL 169, TAL 569 at Sinthiou Malème experimental station.

Treatements	Single Occupancy of nodules (%)*					Multiple Occupancy of Nodules (%)	Grain Yield (kg/ha)
	Indigenous Strains (IS)	MAO 118	TAL 22	TAL 169	TAL 569		
Non inoculated	100	0	0	0	0	0	1654.20 c
MAO 118	15 (+ 25)	60 (+25)	0	0	0	25 (MAO 118 + IS)	3062.60 abc
TAL 22	20 (+ 35)	0	45 (+35)	0	0	35 (TAL 22 + IS)	4345.60 a
TAL 169	50 (+ 10)	0	0	40 (+10)	0	10 (TAL 169 + IS)	2766.40 abc
TAL 569	37	0	0	0	53	0	3392.10 abc
MAO 118 + TAL 22	0	50 (+30)	20 (+30)	0	0	30 (MAO 118 + TAL 22)	1494.00 c
MAO 118 + TAL 169	0	58 (+28)	0	14 (+28)	0	28 (MAO 118 + TAL 169)	2235.10 bc
MAO 118 . TAL 569	0	27 (+13)	0	0	60 (+13)	13 (MAO 118 + TAL 569)	3222.50 abc
TAL 22 + TAL 169	0	0	15 (+85)	cl	0	85 (TAL 22 + TAL 169)	2838.70 abc
TAL 22 + TAL 569	0	0	15 (+27)	0	58 (+27)	27 (TAL 22 + TAL 569	3022.40 abc
TAL 169 + TAL 569	0	0	18 (+21)	0	51 (+21)	21 (TAL 169 + TAL 569	2856.20 abc
MAO 118 + TAL 22 + TAL 169	0	40 (+40)	13 (+40)	7	0	40 (MAO 118 + TAL 22)	L886.30 abc
MAO 118 + TAL 22 + TAL 569	0	8 (+70)	2	0	20 (+70)	70 (MAO 118 + TAL 5691	2524.20 abc
MAO 118 + TAL 169 + TAL 569	0	10 (+67)	0	3	20 (+67)	67 (MAO il8 + TAL 569)	2252.40 bc
TAL 22 + TAL 169 + TAL 569	0	0	10 (+30)	7	53 (+30)	30 (TAL 22 + TAL 569)	4006.50 ab
MAO 118 + TAL 22 . TAL 169 + TAL 569	0	8 (+70)	0	0	22 (+70)	70 (MAO 118 + TAL 569)	4241.10 ab

* : In brackets and preceded by + sign : percentage of multiple occupancy of nodules which is equally reported in the last column with the responsable Rhizobium strains.

In "Grain yield column", values followed by the same letter do not differ significantly at the 0.05 level by Duncan's multiple range test (1955).

Table 14 : Nodule occupancy and grain yield of bambara groundnut cv 83-131 inoculated with *Rhizobium* MAO118, TAL 22, TAL 169, TAL 569 at Sinthiou Malème experimental station.

Treatments	Single Occupancy of nodules (%)*					Multiple Occupancy of Nodules (%)	Grain yield (kg/ha)
	Indigenous strains (IS)	MAO 118	TAL 22	TAL 169	TAL 569		
Non inoculated	100	0	0	0	0	0	1945.80 a
MAO 118	20 (+18)	62 (+18)	0	0	0	18 (MAO 118 + IS)	2899.20 a
TAL 22	15 (+41)	0	44 (+41)	0	0	41 (TAL 22 + IS)	1995.80 a
TAL 169	41 (+39)	0	0	20 (+39)	0	39 (TAL 169 + IS)	2126.90 a
TAL 569	38 (+20)	0	0	0	42 (+20)	20 (TAL 569 + IS)	1785.20 a
MAO 118 + TAL 22	0	54 (+23)	23 (+23)	0	0	23 (MAO 118 + TAL 22)	2685.60 a
MAO 118 + TAL 169	0	56 (+19)	0	25 (+19)	0	21 (MAO 118 + TAL 169)	2389.20 a
MAO 118 + TAL 569	0	50 (+10)	0	0	40 (+10)	10 (MAO 118 + TAL 569)	2902.40 a
TAL 22 + TAL 169	0	0	0	0	0	100 (TAL 22 + TAL 169)	3118.80 a
TAL 22 + TAL 569	0	0	20 (+29)	0	51 (+29)	29 (TAL 22 + TAL 569)	2554.00 a
TAL 169 + TAL 569	0	0	0	20 (+27)	53 (+27)	27 (TAL 169 + TAL 569)	2787.70 a
MAO 118 + TAL 22 + TAL 169	0	47 (+30)	20 (+30)	7	0	30 (MAO 118 + TAL 22)	2126.60 a
MAO 118 + TAL 22 + TAL 569	0	20 (+65)	5	0	10	65 (MAO 118 + TAL 569)	3105.80 a
MAO 118 + TAL 169 + TAL 569	0	18 (+70)	0	0	12 (+70)	70 (MAO 118 + TAL 569)	2347.90 a
TAL 22 + TAL 169 + TAL 569	0	0	13 (+40)	7	40 (+40)	40 (TAL 22 + TAL 569)	2741.20 a
MAO 118 + TAL 22 + TAL 169 + TAL 569	0	18 (+72)	0	0	10 (+72)	72 (MAO 118 . TAL 569;	3217.10 a

*: In brackets and preceded by . sign : percentage of multiple occupancy of nodulees which is equally reported in the last column with the responsable *Rhizobium* strains.

In "Grain yield column", values followed by the same letter do not differ significantly at the 0.05 level by Duncan's multiple range test (1955).

Sinthiou Maleme using the cultivar and the same **Rhizobium** strain.

3.4. Survey of disease on bambara groundnut.

No serious disease was noticed on bambara groundnut except one at Bambey experimental station. The microscopic observations of the isolates obtained from the diseased plants revalated the presence of **Alternaria sp.** However, in the pathogenicity test, all the isolates failed to produce the disease symptoms. But during the course of time, the symptoms were developed on both varieties including the uninoculated plants. This indicated that the **Alternaria** fungus was not the cause of the disease, but it was developed syprophytically on the dead tissues.

It was suspected that the death of bambara groundnut plants at Bambey was associated with the high pH of irrigation water which was later confirmed by another experiment for which results are presented under 3.5.

3.5. Response of bambara groundnut to different pH levels.

At pH 6.00, plant leaves were present and some leaves were yellow. At pH 7.20 and 7.50, the plants grew better : all leaves were still green and did not wither. At pH 8.20, all leaves withered, the plants were small and were dying. Fig.5 shows shoot and root dry weights of cv. 79-1 at different pH levels. Better dry matter production was obtained at pH 7.15. These results indicated that high pH levels stopped the growth of bambara groundnut. Probably unknown nutrient uptake was not possible at thes pH levels. Pollack (1973) reported nutrient defiency symptoms in bambara groundnut cultivated in water-culture or sand-culture lacking either nitrogen, phosphorus, potassium, sulfur or iron. However, the behaviour of the plant varied with the cultivars.

4. CONCLUS ION.

Although bambara groundnut ranks as the third most important legume crop in Africa, it remains an underexploited legume in Senegal. It is produced only in small traditional farms in the south-east of the country. There is now a germplasm collection at the National Center of Agronomic Research at Bambey including 78 cultivars obtained from local market and from international collections.

Bambara groundnut is ground generally without nitrogen fertilizer or other amendement such as phosphorus application. However, the growth of bambara groundnut could be significantly improved by nitrogen and/or phosphorus fertilizer and consequently its nitrogen and phosphorus contents could be increased, suggesting that microbiological associations will be fruitful. These associations could be with rhizobial strains for improving nitrogen fixation and vesicular and arbuscular mycorrhiza for improving phosphorus uptake.

The strategy adopted for improving biological nitrogen fixation of bambara groundnut is to select in each field condition the most effective nitrogen fixing host plant-**Rhizobium** strain combinations. The determination of

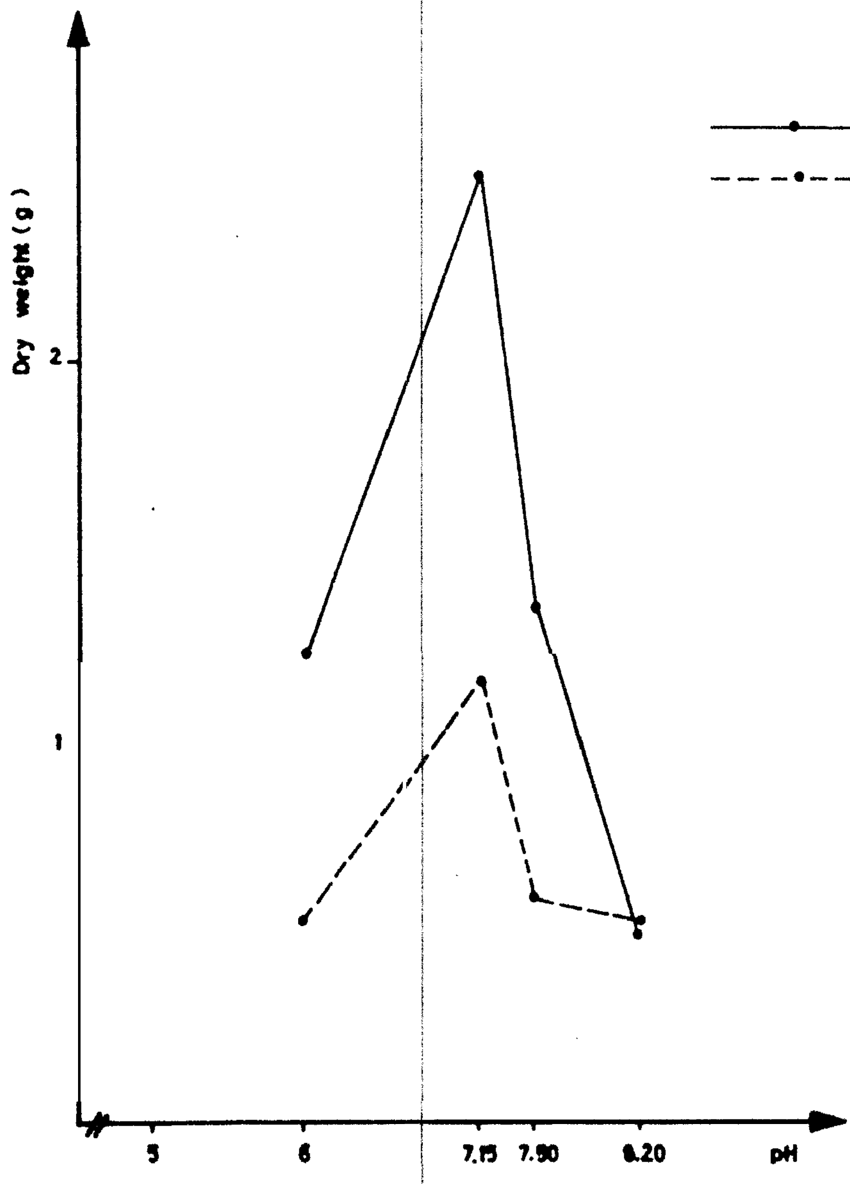

Fig. 5 Shoot and root dry weights of b. groundnut cv. 79-1 cultivated in pots at different pH levels.

the relative effectiveness of host plant-indigenous **Rhizobium** strain combinations permitted us to select the best associations.
Although the nitrogen fixed cannot be estimated accurately, the beneficial effect of inoculation on shoot dry weight, nodule development, shoot nitrogen content or grain yield showed in some cultivar x **Rhizobium** combinations could be expected because preliminary analysis had shown that the soil at Bambey, Djibelor and Sinthiou Maleme had low population of **Bradyrhizobium** (<200/g), in spite of the fact that in all sites, peanut has been grown for a very long time. Consequently, inoculation of bambara groundnut should be recommanded in senegalese soils with low population of **Bradyrhizobium** but one should keep in mind the fact that there is a significant cultivar x **Rhizobium** strain interaction which varies with sites.
However, long term efforts will be required to breed for improving nitrogen fixation in bambara groundnut in which no serious disease was observed in Senegal.

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REFERENCES.

BROCKWELL, J. 1980 xperiments with crop and pasture legumes. -Principles and practice. In **Methods for evaluating biological nitrogen fixation.** Edited by Bergensen, F.J. pp. 417-490. Chichester, New York, Brisbane Toronto : John Wiley and Sons.

DENARIE, J., ANDRIAMANATENA,S. and RAMONJY, J. 1968 L'inoculation des légumineuses à Madagascar. Résultats de l'expérimentation. **L'Agronomie Tropicale** (Paris) 23 (9), 925-966.

DOKU,E.V. 1969 Host specificity among five species in the cowpea cross-inoculation group. **Plant and Soil.** 30, 126-128.

DOMMERGUES, Y.R., DIEM, H.G. and DIVIES,C. 1979 Polyacrylamide-Entrapped Rhizobium as inoculant for legumes. **Applied Environmental Microbiology.** 37, 779-781.

DUNCAN, D.B. 1955 Multiple range and multiple F test. Biometrics. [illegible] 1-42.

HEWITT, E.J. 1966 Sanc and wa ter culture methods used in the study of plant nutrition. **Technical communication no. 22 (2nd ed.) 547pp. Commonwealth Agricultural Bureau. London**

LEONARD, L.T. 1943 A simple assembly for use in the testing of culture of rhizobia. **Journal of bactzeriology.** 45, 523-527.

MULONGOY, K., AYANABA, A. and PULVER, E. 1980 Exploiting the diversity in the cowpea-rhizobia symbiosis for increasing cowpea production. In **Proceeding of Global Impact of Applied Microbiology held at LAgos ; Part2. Rhizosphere biology. pp. 121-125.** Academic Press. Londobn.

OLSEN, S.R., COLE. L.v., WATANABE, F.S. and DEAN, L.A. 1954 Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dep. Agric. 939.

POLLACK,S.B. 1973 The bambara groundnut : its morphology. cu**1**tue and nutritional defiency symptoms. **M.S. Thesis, Universi ty of Florida, Gainsville, USA.**

THOMPSON. E.J. and DENNIS, E.A. 1977 Studies on nodulation and nitrogen fixation by selected legumes. In **Proceedings of the University of Ghana Council for Scientific and Idustrial Research Symposium on Grain LEgume in Ghana, 10-11 December, 1976.** LEgon, Ghana. Faculty of Agriculture, University of Ghana?, pp. 85-102.

VINCENT, J.M. A manual for practical study of the root nodule bacteria. **IBP Handbook no. 15** Blackwell Scientific Publications. Oxford and Edinburg. 164 pp.

APPENDIX 1 : Publications.

GUEYE, M. and BORDELEAU, L.M. 1987 Nitrogen fixation in bambara groundnut (Voandzeia subterranea (L.) Thouars). Mircen Journal of Aplied Microbiology and Biotechnology. In press

APPENDIX [illegible] : Equipment and supplies received during the grant period

Equipment and supplies	1985	1986	1987
Incubator	1		
Autostill	1		
pH meter	1		
Shaker (*)	1		
Centrifuge	1		
Freezer	1		
Refrigerator	1		
Kjeldahl digester		1	
Kjeldahl distilling unit		1	
Vacuum pump			1
Cemicals		Many	Many
Glassware	Many	Many	Many

(*) : The first shaker NAS sent was returned to TH4homas Scientific Co. Att. M. Mitchel MONICHINSKI.

APPENDIX 3 : Training and consultant visits.

1. Programme training was not included in the grant. But a consultant visit was held during the second year of the project. Consultant was Dr. BORDELEAU, L.M. from Canada Agriculture. He made some observations and suggestions as indicated by Dr. SOMASEGARAN, P. from NifTAL project in Hawaii, USA previously appointed as consultant for the grant.

2. NAS audit mission supervised by Ms. Joyce FREELAND inspected the project in 1986. Ms. FREELAND gave her conclusions to NAS.

APPENDIX 4 : Grant Staff

The following staff devoted the percentage of the work timr indicated below to research under the grant.

Ndiaga	CISSE	Breeder	30%
D.G.	GAIKWAD (*)	Pathologist	30%
Mamadou	GUEYE	Microbiologist	80%
Oumar	TOURE	Technician	100%

(*) : D.G. GAIKWAD replaced Demba F. MBAYE because D.G. GAIKWAD works for legume pathology programme whereas Demba F. MEAYE works for cereal pathology programme according to ISRA staff.