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Nitrogen fixation in bambara groundnut, *Voandzeia subterranea* (L.) Thouars

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Introduction

Bambara groundnut (*Voandzeia subterranea* (L.) Thouars) is a desirable legume for Africa semi-arid tropical agriculture. Like cowpeas, the bambara groundnut nodulates freely in tropical soils. Although it produces a nutritious food, the bambara groundnut remains one of the crops most neglected by science (National Academy of Sciences, 1979).

The most important feature of legumes is their ability to fix atmospheric nitrogen and considerable efforts have been expended towards increasing the efficiency of its symbiotic association with *Rhizobium*. *Now*, it is accepted that seed inoculation with superior *Rhizobium* strains is necessary with most legumes. However, cowpea seed inoculation is usually unsuccessful because of competition between introduced and indigenous *Rhizobium* strains in the soil. Therefore, an important step towards improved nitrogen fixation is to isolate and identify competitive indigenous *Rhizobia* with a view to using elite strains as inoculants (Mulongoy *et al.*, 1980).

This paper presents data on screening field bambara groundnut varieties, nitrogen fixing ability of indigenous *Rhizobium* strains and evaluation of interactions between host plant and *Rhizobium* strain.

Materials and methods

Field varietal screening. Trials were done at two sites in Senegal during the 1985 rainy season. The sites differed in both rainfall (Bambey, 377 mm $annum^{-1}$; Nioro. 532 mm $annum^{-1}$) and in soil type (Table 1), Twenty four cultivars of bambara groundnut (listed in Fig. 1) were hand sown in a randomized block design replicated four times. The cultivars were sown in two treatments: without (-N) and with (+N) nitrogen fertilization (50 kg urea/ha). All plots received 60 kg P_2O_5/ha and 120 kg KCl/ha. Six weeks after planting, the relative effectiveness (RE) of each host plant/ indigenous *Rhizobium* strain combination was evaluated as follows (Mulongoy *et al.*, 1980):

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Table 1 Characteristics of soil at Bambey and Nioro experimental stations

Soil	ppm			Composition (%)			pH (H_2O)
	Total C	Total N	Available P (Olsen*)	Clay	Loam	Sand	
Bambey	2970	290	121	4	2	96	7.8
Nioro	4160	310	137	6	3	90	5.8

*Olsen *et al.* (1954).

$$RE = \frac{\text{'weight' of ten samples in the -N block}}{\text{'weight' of ten samples in the +N block}} \times 100$$

In this formula, 'weight' is either of shoot, root or nodule dry weight or shoot nitrogen content.

Nitrogen fixing ability of indigenous Rhizobium strains. The samples were taken in each treatment from five well-developed plants and still having immature pods and 45 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 yeast extract/mannitol medium. Nitrogen fixing ability of the isolates were compared in terms of shoot dry weight of bambara groundnut cultivar 85001 used as test plant and cultivated in Leonard jar assemblies (Leonard, 1943). Seeds were sterilized using 0.01% (w/v) $HgCl_2$ for 3 min and germinated in sterile sand for 3 d; each Seedling was then transplanted into a jar assembly. One ml suspension of rhizobial isolate broth (approx. 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 v/v) was added once a week during the growing period. After 35 d growth. plant tops were clipped off, dried and weighed for dry matter production.

Greenhouse evaluation of host plant-Rhizobium strain interactions. Both the indigenous and Niftal *Rhizohium* strains (listed in Table 3) were selected on the basis of their effectiveness. The Niftal strains – TAI, 22, TAL 169 and TAL 569 – were supplied by the NifTAL project, Hawaii, USA and served as standards for comparison against the indigenous ones. Twelve bambara groundnut cultivars also listed in Table 3 were used as host plants. Seed sterilization of each genotype, germination, transplantation, inoculation and watering were conducted as described above and seeds were planted in Leonard jars. Treatments were arranged in a randomized block design replicated five times. After SO days of growth, the plants were harvested. Plant 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. 1.) was calculated as follows:

$$\text{Nod. 1.} = N \times C \times S$$

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).

Results

Field varietal screening

At Bambey station the cultivars 83-127, Sud Cameroun, Sarakawa 3, Sarakawa 10 and Lassa 1 had a relative effectiveness (RE), based on shoot dry weight, below 40% (Fig. 1) while at Nioro station, all cultivars had the same RE of over 40% (Fig. 2). At Bambey station, only cultivar Sarakawa 10 had an RE, based on root dry weight, below SO%. At Nioro station, all cultivars had the same RE of over 50%. Relative Effectiveness, based on nodule dry weight, was much lower at Bambey than at Nioro: it was in the range of 80 to 120% at Bambey for cultivars 83-131, Ketao 3, Alheride 1, Lassa 1 and Lassa 4 and in the range of 360 to 480% at Nioro for cultivars 83-129. X3-131. Sud Cameroun, Sarakawa 2. Ketao 9. V2 and 85002. Relative Effectiveness, based on shoot nitrogen content, was over 50% for all cultivars except for 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 2) indicating their ability to set an effective symbiosis in the two different field conditions.

Table 2 Shoot dry weight (g/10 plants) of the performed cultivars of bambara groundnut in field at Bamhey and Nioro experimental stations

Bambara Cultivars	Bambey	Nioro
79-1	55.5^{b}	30.4^{b}
83-126	62.2”	33.6”
x3-129	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”
Lassa 3	78.5^{a}	38.6^{ab}
v2	61.5^{b}	41.3””
85001	56.5^{b}	42.0”

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).

Effectiveness of indigenous Rhizohium strains isolated from bamhara 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 interior colour. Thirty one isolates were so obtained: (I_1 to I_{31}, Fig. 3). The isolates were slow-growing and formed small (diam. < 1 mm), dry colonies on yeast mannitol agar. Four isolates (I_2, I_{13}, I_{22} and I_{30}) were lodged in West Africa MIRCEN (MAO) *Rhizobium* culture collection because of their high effectiveness with cultivar 85001 which was from Senegal and had good RE values at both Bambey and Nioro experimental

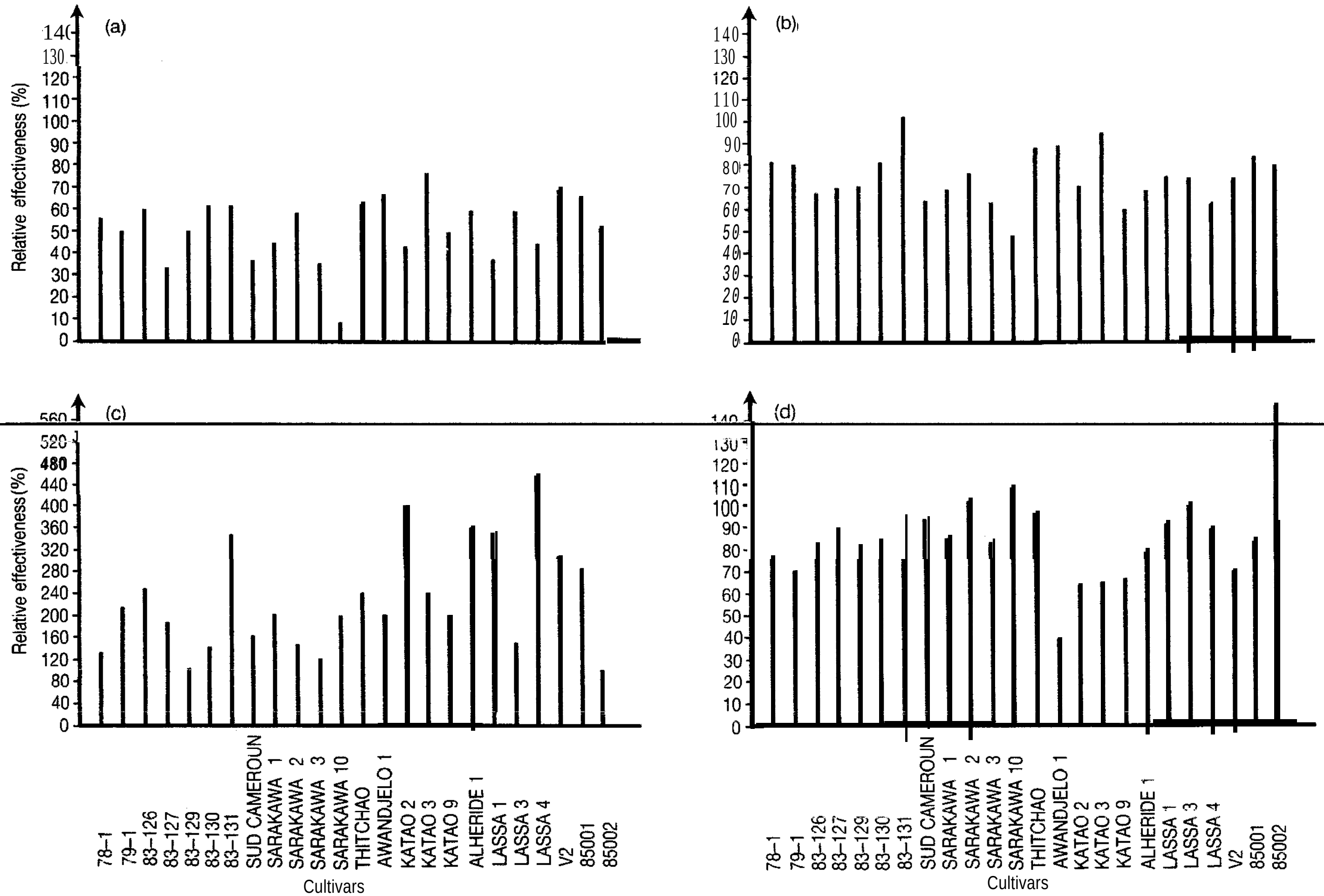

Fig. 1 Distribution of the Relative Effectiveness (RE) of 24 cultivars of *Voandzeia subterranea* at Bambey experimental station. A: RE based on shoot dry weight; B: RE based on root dry weight; C: RE hased on nodule dry weight; D: RE based on shoot nitrogen content.

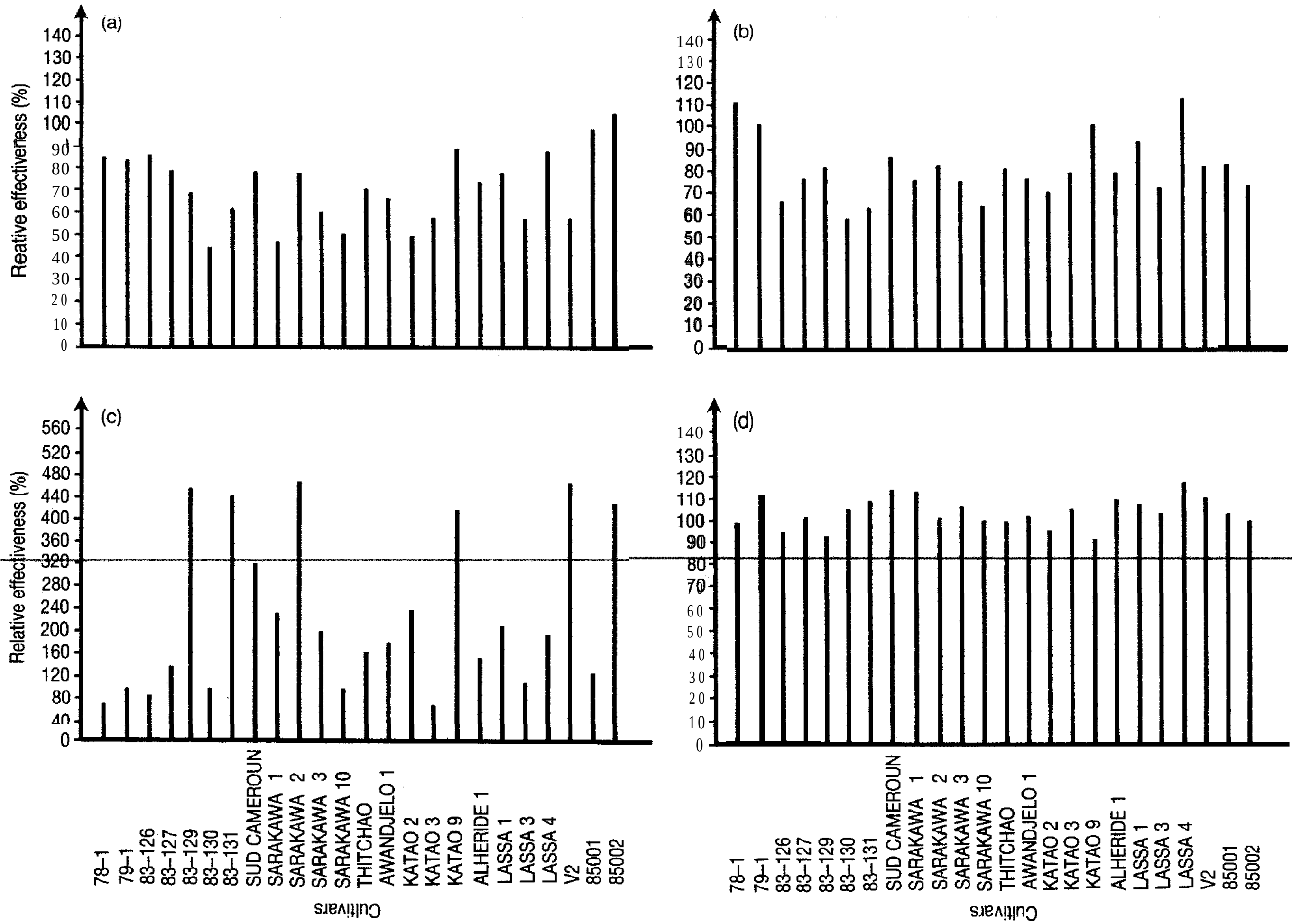

Fig. 2 Distribution of the Relative Effectiveness (RE) of 24 cultivars of *Voandzeia subterranea* at Nioro experimental station. A: RE based on shoot dry weight: B: RE based on root dry weight; C: RE based on nodule dry weight: D: RE based on shoot nitrogen content.

stations. These isolates were named MAO 113, MAO 118. MAO 121 and MAO 126, respectively, and are indicated by an asterisk in Fig. 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 5 cm of root system on all plants except controls which were all nodule free. Nodulation index and the effectiveness varied greatly with the strains (Table 3). 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 cultivar 83-131 except strain TAL 169 which exhibited partial nodulation. All *Rhizobium* strains were partially effective on cultivar Ketao 3. Similarly, *Rhizobium* strains MAO 113 and MAO 118 were ineffective on cultivar 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 cultivars Awandjelo 1 and 83-129 respectively; strain MAO 118 was ineffective on cultivars Awandjelo 1, Lassa 3 and V2; strain TAL 569 was ineffective on cultivars 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 .

Although Corbin *et al.*, (1977) had proposed a scoring system to classify nodulation and Bordeleau *et al.,* (1981) had reported a method for scoring nodulation, we adopted another method. The reliability of this method was indicated by the fact that nodulation index and shoot dry weight (Table 3) showed the same trend of effectiveness in host plant-Rhizobium strain combinations.

Discussion

Many studies have reported an improvement of biological nitrogen fixation resulting from legume cultivar selection (Heichel, 1982; Minchin *et al.*, 1978; Zary *et al.*, *1978).* However, selection for increased nitrogen fixation cannot be achieved by selection of the legume alone; both host genotype and rhizobial strain influence nitrogen fixation (Arrendell, *et al.*, 1985; Hohenberg *et al.*, *1982;* Isleib *et al.*, 1980; Zary *et al.*, 1978). Most of the studies reported on plant genotype *Rhizobium* strain interactions indicated differences between cultivars of the same species in ability 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 competitive and adapted strains, which can nodulate effectively most cultivars even strains are not widely distributed in the soil. Instead of introducing *new Rhizobium* strains, the isolated adapted *Rhizobium* strains may be re-introduced into the field so that they will compete against indigenous strains which are less numerous. Our results show that all *Rhizobium* strains including introduced (NifTAL) and indigenous strains nodulated all cultivars. When the hosts and rhizobial strains were arranged in an increasing order of effectiveness, one indigenous *Rhizobium* MAO 113 exhibited the highest effective association with the cultivars.

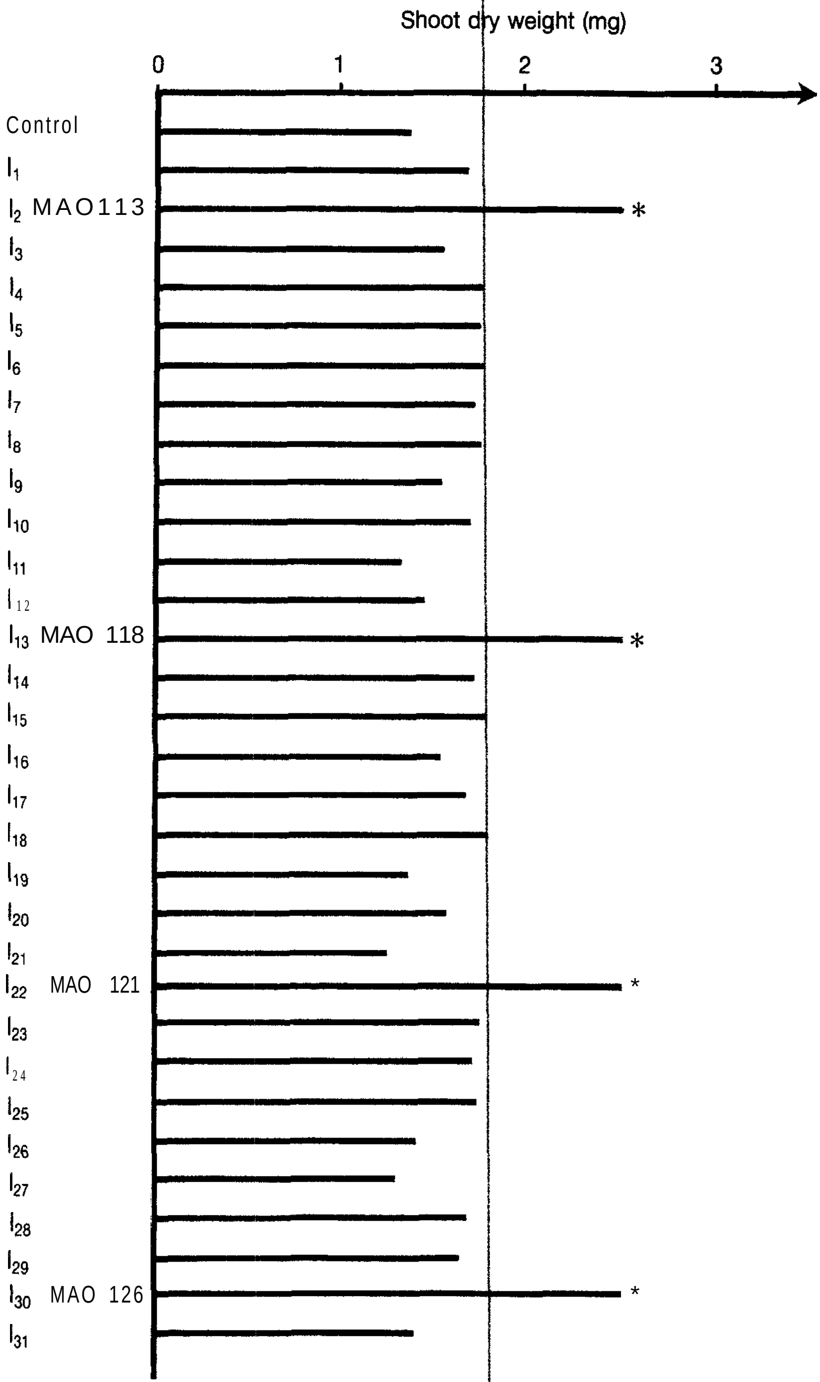

Fig. 3 Shoot dry weight of *Voandzeia subterranea* cultivated in Leonard jar assembly and inoculated with 31 rhizobial isolates. Treatments with * differ significantly with the control at the 0.05 level by Duncan's multiple range test (1955).

Table 3 Shoot dry weight (SDW) (g/plant) and nodulation index (Nod. I.) of 12 bambara groundnut cultivars inoculated by cowpea *Rhizobium* strains from West MIRCEN Africa (MAO) and NifTAL uroiect

Bambara Cultivars	Control		MAO 113		MAO 118		TAL 22		TAL 169		TAL 569	
	SDW[1]	Nod. I.[2]	SDW	Nod. I.	SDW	Nod. I.	SDW	Nod. I.	SDW	Nod. I.	SDW	Nod. I.
79-1	0.9[a]	nn	1.4[abc]		2.1[a]	E	7 0[a]	E	1.8[a]	E	1.8”	e
83-126	1.1[a]	nn	1.5[abc]	E	1.6[abc]	e	1.4[bc]	e	1 8[a]	E	1 6[ab]	e
X3-129	1.0”	nn	1.6[ab]	E	1.5[abc]	e	1 4[bc]	e	1.1[bc]	I	1.9[a]	E
83-131	1.0[a]	nn	1.6[ab]	E	1.8[ab]	E	1.8[ab]	E	1.5[ab]	e	1.8”	E
Sarakawa 1	1.2”	nn	1.6[ab]	E	1.6[abc]	e	1.8[ab]	E	1.5[ab]	e	1.5[ab]	e
Awandjelo 1	0.9”	nn	1.1[c]	I	1.2[bc]	I	1.5[abc]	e	1.9”	E	1.5[ab]	e
Ketao 2	0.9[a]	nn	1.6[ab]	E	1.8[ab]	e	1.5[abc]	e	1.5[ab]	e	1.1[b]	I
Ketao 3	,.2[a]	nn	1.3[abc]	e	1.6[abc]	e	1.4’”	e	1 .5[ab]	e	1.6[ab]	e
Alheride 1	1.2”	nn	1.3[abc]	e	1.6[abc]	e	1.8[ab]	E	1.4[abc]	e	1.1“	I
Lassa 3	1.0[a]	nn	1.7[a]	E	1.1[b]	I	1.9[ab]	E	1.1’”	e	1.1[b]	I
V2	1.0[a]	nn	1.5[abc]	I	1.1[b]	I	1.2[c]	e	0.9’	e	1.5[ab]	e
85001	0.9”	nn	1.4[abc]	e	2.0[a]	E	1.5[ab]	e	1.9[a]	E	1.8[a]	E

[1] Values followed by thc same letter in each column do not differ at the 0.05 level hy Duncan's multiple range test (1955). a: highly effective; ah: partially effective; b: ineffective.

[2] nn: non-nodulated; E: highly effective nodlation (≥ 4); e: partially effective nodulation (2 ≤ Nod. I. < 4); I: ineffective nodulation (Nod. I. < 2).

Therefore, strain MAO 113 is expected to give better nitrogen fixation under field conditions.

The strategy to improve nitrogen fixation by legumes in general is to first improve genotype and then select for best *Rhizobium* strains to increase nitrogen fixation. Selection of host plants with good general effectiveness in nitrogen fixing ability is an other avenue (Wynne *et al.* 1980). The most likely strategy is to select in each field condition the most effective nitrogen fixing host plant-*Rhizobium* strain combination. In our study, the higher relative effectiveness obtained with cultivars at Nioro experimental station might be due to better soil conditions (higher organic matter) that are favourable to this plant (Tardieux, 1975). However, the determination of the relative effectiveness of host plant/indigenous *Rhizobium* strains combinations permitted us to select the best combinations in each area. Greenhouse evaluation of these combinations is the initial phase of the process of increasing nitrogen fixation through *Rhizobium* strains selection. The second phase was to investigate field response of bambara groundnut to inoculation with the selected *Rhizobium* strains. Effect of inoculation with strains MAO 113, MAO 118, TAL 22 and TAL 569 on the grain yield of 79-1 and 83-131 was obtained in two Senegalese soil (Gueye 1987). However. long term efforts will be required to breed for improving nitrogen fixation in bambara groundnut.

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Summary

To improve biological nitrogen fixation in bambara groundut *(Voandzeiu subterranea* (L.) Thouars), the relative effectiveness of 24 cultivars was studied in the field at two experimental stations selected for their different ecological conditions. Thirty one *Rhizobium* strains were isolated and 12 cultivars were screened during this study. Both indigenous and introduced Niftal *Rhizobium* strains were used during a second study on host cultivars × *Rhizobium* strains interactions. Nodulation index and shoot dry weight were used to assess the efficiency. The widest effectiveness spectrum was observed with thc indigenous strain MAO 113 and the introduced strain TAL 22.

Résumé

Fixation d'azote dans lu noix de Bambara (Voandzeia subterrana *(L.) Thouars)*

Dans le but d'améliorer la fixation biologique d'azote dans In noix dc Bambara (*Voandzeia subterranea* (L.) Thouars), l'efficacité relative de 23 cultivars a été étudiée sur le terrain dans deux stations expérimentales choisies pour leurs conditions écologiques différentes. Trente-et-une souches de *Rhizobium* ont Cté isolées et 12 cultivars ont été testés au cours de cette étude. Tant les souches indigènes de *Rhizobium* que celles Niftal introduites ont été utilisées au cours d'une deuxième étude sur les interactions entre les souches de *Rhizobium* ct les cultivars-hôtes. L'indice de nodulation et le poids sec de la pousse ont été utilisés pour l'établissement de l'cffcience. Le spectre d'efficience le plus large a été observé avec la souche indigène MAO 113 et la souche introduite TAL 22.

Resumen

Afin de mejorar la fijación biológica **de** nitrogeno en el cacahuete de Bambara (*Voandzeia subterranea* (L.) Thouars), se realizó un estudio de su efectividad relativa en campo en dos estaciones experimentales seleccionadas por sus distintas condiciones ecologicas. Durante este estudio se aislaron treintaiuna cepas de *Rhizobium* y se probaron doce cultivares del huésped. En un segundo ensayo se estudiaron las interacciones huésped xcepa de *Rhizobium* para lo cual se utilizaron cepas de *Rhizobium* Niftal tanto indígenas como introducidas. La eficiencia se valoró determinando el indice de nodulación y el peso seco de la parte aerea. El espectro mas amplio de eficacia se observó con la cepa iindígena MAO 113 y la introducida TAL 22.