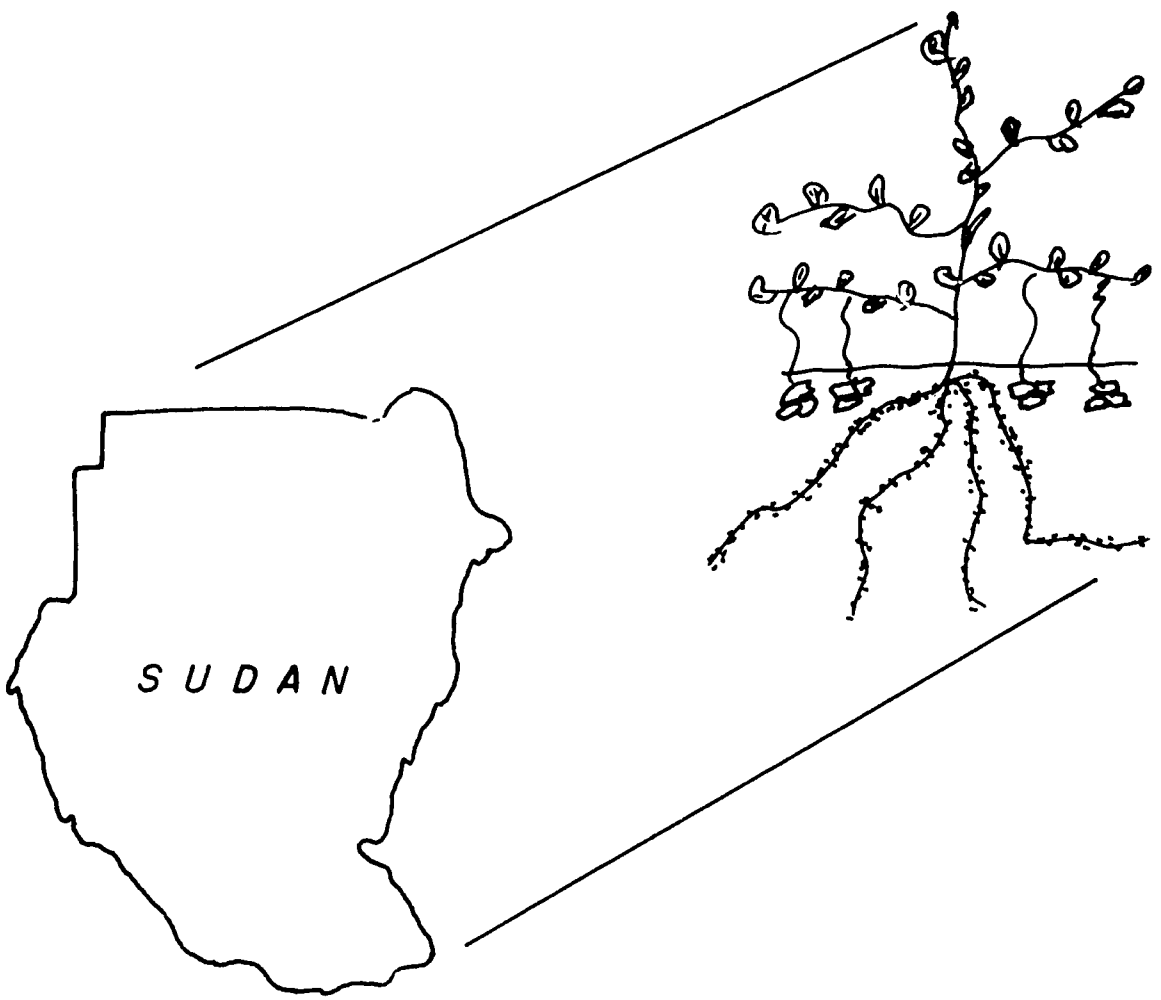
Completion Report

January 1980 - January 1984

Groundnut Inoculation In Sudan



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By

T.E. Loynachan and M.A. Hadad

Department of Agronomy

Iowa State University

Ames, Iowa 50011

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PREFACE

This completion report summarizes "Groundnut inoculation in Sudan", which was a cooperative research project between the Department of Agronomy, Iowa Agriculture and Home Economics Experiment Station, Iowa State University and the Soil Science Section, Wad Medani Research Station, Agricultural Research Corporation, Sudan.

Financial support for the project was provided, in part, by a competitive grant from the SEA/CR-AID Nitrogen Fixation Program. Project Manager for the grant during the first two years was Dr. Robert Miller and the Program Manager for the last two years was Dr. Gerald Elkan. The period of the overall contract was January 14, 1980 through January 31, 1984. The original termination date of the project was January 31, 1983, but was extended one year upon mutual agreement.

The main body of the completion report consists of four parts. Each part reports on a specific aspect of the project, and is written in publication format. Only part I currently has been published.

> Hadad, M. A., T. E. Loynachan, and M. M. Musa. 1982. Inoculation trials on groundnuts (Arachis hypogaea) in Sudan. pp. 249-256. In: Biological Nitrogen Fixation Technology for Tropical Agriculture. CIAT. Cali, Colombia.

All other data in this report should be considered unpublished information (personal communication) until such time as it appears in final print in a scientific journal.

The successful completion of this project would have been impossible without the helpful and thoughtful cooperation of many individuals. A few more directly involved with the project and their affiliations are indicated in the completion report distribution listing found at the end of this report. The authors wish to thank each for their specific contribution.

PROJECT SUMMARY

Sudan, as an unindustrialized nation with little export, can not afford the high costs of synthesized nitrogen fertilizers. An attractive alternative to commercial nitrogen fertilizers for crop production is the adoption of biological nitrogen fixation technology. Little was known, however, prior to this study about the factors concerning nitrogen fixation efficacy of groundnut (peanut), a major crop in rotations in Sudan.

Initial surveys were conducted the first year of the study (Part I) to gather basic information on factors affecting nitrogen fixation of groundnut. Factors surveyed included the role of nutrients (both macro- and micro-nutrients), the effect of commonly applied pesticides (both herbicides and fungicides), and an initial survey characterization of rhizobial populations of soils throughout the country. In general, groundnut was not responsive to fertilization with either macro- or micro-nutrients, with the exception of nitrogen fertilization, and the common pesticides had little effect on nodulation of groundnut in replicated studies at Wad Medani, Sudan. Of the numerous sites surveyed throughout Sudan, only one location, a sandy soil in Western Sudan, contained fewer than 1 $x10^2$ cowpea-type rhizobia g^{-1} soil capable of forming nodules on siratro. A further evaluation of soil properties and the duration since groundnuts were last planted in rotations (Part II) revealed no significant correlation between the number of rhizobia present in soils and measured parameters. Observation plots were established at five locations the first year. Since the plots were not replicated, no statistical analyses could be made, but the average top dry weight at each location was greater with inoculation than without inoculation, suggesting promise for future studies.

Field isolates were collected from Sudan the first year and returned to

Iowa State for greenhouse evaluation. Initial screenings indicated that the field isolates were not as efficient in nitrogen fixation as strains commercially available. In the second and third years of study, strain 8A11 from the Nitragin Company and TAL 309 from North American Plant Breeders were evaluated in the field. Inoculation methods that were evaluated included strains added singly or in mixture, formulated in peat or oil base carriers, and placed with the seed or at 5 and 10 cm depths below the seed. In these replicated trials, inoculation in general resulted in little improvement in nitrogen fixing traits or pod yields, and did not equal the improvements obtained by adding 120 kg ha^{-1} N as ammonium sulfate. Control plots were heavily nodulated by the indigenous rhizobia. Serological analyses indicated that one of the identifiable strains, TAL 309, was capable of actively competing against indigenous rhizobia in occupying nodules.

The response to nitrogen fertilization suggests that not all of the nitrogen requirements were being met by nitrogen fixation. Perhaps in these soils of low organic carbon, low rates of nitrogen mineralization and the quantities of nitrogen contained in groundnut cotyledons are not sufficient to successfully establish the plant, without stress, before nitrogen fixation becomes fully established.

During trips to Sudan, Sudanese rhizobia were isolated from native plants growing in different soil types. Individual isolates were obtained from six legumes: groundnuts (*Arachis hypogaea*), green gram (*Phaseolus aureus*), lubia (*Dolichos lablab*), cowpea (*Vigna ungiculata*), pigeon pea (*Cajanus cajan*), and bambara groundnut (*Voandzeia subterranea*). The isolates were aseptically added to surface sterilized seeds of each legume and grown in sterile vermiculite. All the plant species were nodulated by each isolate, indicating that all isolates belonged to the cowpea miscellany. All isolates grew in

0.1% NaCl amended media, but growth was variable in 2.0% amended media. Most, but not all, isolates grew after exposure to moist heat for 15 minutes at 50 C. Aggultination reactions indicated that isolates from the native Sudanese legumes belong to several serological groupings. The isolates obtained from groundnut, in general, formed a larger number of nodules on the groundnut cultivar than did isolates from other legume species.

One particular strain from Kadugli (Western Sudan) was found to be more efficient in nitrogen fixation than other Sudanese strains evaluated. The Kadugli strain was tested in studies on three Sudanese groundnut cultivars (MH383, Barberton, and Ashford) for competitiveness in forming nodules against an isolate from Wad Medani and the commercial strain TAL 309. The host genotype little influenced the nitrogen fixation efficiency of the strains tested or their relative competitive indexes. Whenever TAL 309 was included in inoculum mixtures, it consistently occupied the majority of nodules. A calculated competitive index revealed that TAL 309 was seven and eleven times more competitive than the Sudanese strains, Wad Medani and Kadugli, respectively. Between Sudanese strains, the Wad Medani strain was four times more competitive than the Kadugli strain. Further studies indicated that a high inoculation rate of the Kadugli strain could completely replace the other two strains from occupying nodules.

The Kadugli strain isolated from Western Sudan presumably is adapted to Sudanese conditions and holds promise as a strain for future field testing. The inoculation level required for this less competitive strain to occupy nodules in the field should be evaluated. Of the work conducted during this project, the isolation and characterization of the Kadugli strain probably holds the most promise for improving nitrogen fixation of groundnuts in Sudan. The fact that peanut plants responded to nitrogen fertilizers is

encouraging. This indicates that native strains are not supplying all the nitrogen needed for maximum growth, and that there is potential for improved yields through inoculation with an adaptive, competitive, and efficient *Rhizobium* strain.

PART I

INOCULATION TRIALS ON GROUNDNUTS (*Arachis hypogaea*) IN SUDAN (1st year studies)

INOCULATION TRIALS ON GROUNDNUTS (*ARACHIS HYPOGAEA*) IN SUDAN

M.A. Hadad,[1] T.E. Loynachan [1] and M.M. Musa[2]

Summary

Field plots established on heavy cracking clays near Wad Medani, Sudan, examined the effect of inoculation method, rate, and depth of placement of inoculant on the nodulation and yield of two adapted groundnut cultivars, Ashford and Barberton.

Most probable number (MPN) counts with siratro as host indicated approximately 2 x 10^4 cowpea-group rhizobia/g of soil at Wad Medani. These were highly infective on groundnut, but did not supply the crop with all the nitrogen (N) it required. Thus, addition of 40 kg N/ha increased the yield of 'Barberton' by 38%, while 120 kg N/ha increased yields of 'Barberton' by 52% and of 'Ashford' by 23%. The added inoculant had little effect on any trait associated with N_2 fixation. This might suggest the need for inoculant strains competitive with the naturally occurring rhizobia that are infective for groundnut.

A survey of 30 sites throughout Sudan indicated only one location — in the sandy soils of western Sudan — with less than 10^2 cowpea-type rhizobia/g of soil.

INTRODUCTION

Groundnuts (*Arachis hypogaea* L.) are both a source of protein for the local inhabitants and a major cash crop for the Sudan. Currently more than 385,000 ha are planted, but the area sown is increasing, and the crop could occupy several million ha in the near future if current rates of expansion are maintained.

Research in the Sudan on groundnut as a nitrogen (N_2) fixing legume has been limited (Musa, 1972; Muktar & Yonsif, 1979). Inoculation of groundnut with effective compatible rhizobia may result in yield increases, especially if

[1] Agronomy Dept., Iowa State University, Ames, Iowa, USA.
[2] Agricultural Research Corporation, Wad Medani, Sudan.

the indigenous strains are not effective (Ham, 1980). Deeper placement of the inoculant may be necessary to protect the rhizobia from surface temperatures as high as 60°C (Musa, 1972).

As Sudanese soils are known to be deficient in N (Said, 1973) a series of experiments was undertaken to test the response of groundnuts to inoculation and N fertilization at Wad Medani, and to survey outlying areas of Sudan to determine the incidence, and efficiency in symbiotic N_2 fixation, of native cowpea-type rhizobia in soil.

MATERIALS AND METHODS

Field study at Wad Medani

Initial experiments were undertaken at the Research Center, Agricultural Research Corporation, Wad Medani, Sudan. The soils were heavy cracking clays classified as Vertisols, containing free $CaCO_3$ and with a pH close to 8.5. The site was previously sown to cotton.

Two cultivars, Ashford, which is late maturing, semispreading, and alternately branched, and Barberton, an early maturing, sequentially branched cultivar, were used in a randomized complete block design having eight treatments (see Table 1). The inoculant used contained four groundnut *Rhizobium* strains (176A22 and 25B7 from the Nitragin Company, Milwaukee, WI; 3G4b4, from the USDA collection at Beltsville, MD; and TAL 309, obtained from North American Plant Breeders, Princeton, IL). The strains were selected on the basis of greenhouse results at Iowa State University (ISU) with the two Sudanese cultivars. Two rates of inoculum (10^4 and 10^8 rhizobia/two seeds) were applied to plots fertilized with P, K, S, and all the micronutrients except Mo, while the 10^8 treatment was applied both with the seed and 8 cm below the seed. The two rates of N (40 and 120 kg N/ha) were applied as ammonium sulfate.

Five plots each 3 x 9.6 m with five ridges 60 cm apart, were sown for each treatment, and irrigated within 24 h after application of the inoculant. Two samplings were made to monitor growth during the season; the first at the flowering stage (six weeks after sowing) and the second during pod filling (10 weeks after sowing). Both samples were taken from 50 cm of the outer two rows. Nodule number, nodule dry weight, shoot dry weight, and N content were determined. Harvest of the plots for yield was at 90 days after sowing of 'Barberton' and 120 days after sowing for 'Ashford'.

Most probable number (MPN) counts for the Wad Medani location used siratro (*Macroptilium atropurpureum*) as the test plant, and the method detailed by Vincent (1970).

TABLE 1: Summary of treatments imposed in the field study.

Treatment	*Rhizobium*	N	P	K	S and micro nutrients[1]
1	–		–		–
2	–	1	–	–	–
3	–	3	1	1	1
4	10^4/2 seeds	–	1	1	1
5	10^8/2 seeds	–	1	1	1
6	10^8/2 seeds	–	–	–	–
7	10^8/2 seeds	–	1	1	–
8[2]	10^8/2 seeds[2]	–	–	–	–

[1]Fertilizer rates are as follows:

N_1: 40 kg N per ha as $(NH_4)_2SO_4$; N_3 : 120 kg N per ha as $(NH_4)_2SO_4$

P_1: 40 kg P_2O_5 per ha as triple superphosphate

K_1: 40 kg K_2O per ha as mutriate of potash

S and micronutrients

S : 45 kg S per ha as elemental S

B : 0.5 kg B per ha as borax

Fe: 0.5 kg Fe per ha as chelate (6 % Fe)

Cu: 2 kg Cu per ha as $CuSO_4.5H_2O$

Mn: 15 kg Mn per ha as $MnSO_4.H_2O$

Zn: 0.6 kg Zn per ha as chelate

[2]Deep placement.

Survey of outlying regions

To gain a better understanding of groundnut rhizobia throughout Sudan, samples were collected from the surface 15 cm of soil from outlying regions of the Sudan and taken to Wad Medani for a MPN determination using siratro as the test plant. The history of the field at each site was obtained. Also at Wad Medani and at several of the outlying areas, quantities of the inoculum previously used in the Wad Medani study were supplied to cooperators who established observation trials with a varying number of cultivars with and without inoculation. The inoculated cultivars were grouped together to minimize contamination while the same cultivars, without inoculation, were grown in an adjacent area. Fifty cm of the planted rows were dug for evaluation of nodulation and to determine an average shoot dry weight of the inoculated and control plants. This study was designed mainly to gain survey information and the treatments were not replicated.

RESULTS AND DISCUSSION

Field study at Wad Medani

Native rhizobia and the need for inoculation

MPN counts with siratro showed that the Wad Medani site contained approximately 2 x 10^4 rhizobia/g in the top 15 cm at planting. Many of these rhizobia were apparently infective with both groundnut varieties tested, as there was heavy nodulation of all control plots (see Table 2). Despite this, plants in the control plots of both cultivars were decidedly chlorotic during the early flowering stage when compared with N-fertilized treatments. This indicated both the inefficiency of the native rhizobia in supplying the plants with sufficient N, and the need for N fertilization or inoculation with effective and competitive strains of *Rhizobium*. Pod yields at harvest were 4638, 5628, and 5725 kg/ha respectively for the control, 40, and 120 kg/ha treatments with 'Ashford'; and 2335, 3229, and 3559 kg/ha, respectively, for the same treatments with 'Barberton'. By evaluating orthogonal comparisons (treatment 1 vs. treatment 2, etc.), 40 kg N/ha with 'Barberton' produced greater yield than the control, and at 120 kg N/ha (treatment 3 vs. treatment 4), yields of both 'Ashford' and 'Barberton' were statistically increased over the control.

Response to inoculation

Little benefit from inoculation was observed in this study (see Table 2), and yields following inoculation were not significantly different from control plots. Since identification of nodule rhizobia was not attempted in this study, the ability of the introduced rhizobia to compete for nodule sites under the high-temperature conditions of this trial cannot be evaluated. Further studies with marked or identifiable strains are required.

Survey of outlying regions

MPN counts with siratro as host showed that cowpea-group rhizobia abounded in most of the soils tested, irrespective of the time since peanuts were last planted (see Table 3). The one exception was a sandy soil from El Obeid which contained only 3 x 10^2 rhizobia/g of soil. High numbers were even found in virgin soils not previously planted to groundnut. Apparently the native leguminous plants in the Sudan can serve as an inoculant source.

Trial plantings were made at Wad Medani, Abu Naama, Rahad, El Obeid, and Sennar. Fourteen experimental cultivars were included in the Medani study, and six at Rahad. Since the study was not replicated, only general observations can be made. All of the cultivars at the five locations were nodulated without the addition of an inoculant (see Table 4). It appears, however, that there was a slight advantage in overall nodule numbers to added inoculum. The variety MH383, at Wad Medani and Rahad, had

considerably more nodules with inoculation. This is a variety that is being considered for replacement of 'Ashford' as an irrigated variety within the country. Overall growth of shoots at all sites appears to have benefited from inoculation.

TABLE 2: The effect of added inoculant on nitrogen-fixing traits.

Trait		6 weeks after sowing		10 weeks after sowing	
		'Ashford'	'Barberton'	'Ashford'	'Barberton'
Lateral-root nodules (no/plant)					
Control	(trt 1)	39.2 a[2]	20.4 abc	55.7 a	16.2 a
1N	(trt 2)	36.7 a	11.0 c	49.6 a	24.4 a
3N	(trt 3)	22.6 a	7.5 c	56.1 a	17.6 a
10^8	(trt 6)	41.9 a	16.6 abc	26.2 a	23.7 a
10^8	(trt 8)[1]	42.0 a	33.0 ab	53.5 a	24.1 a
Main-root nodules (no/plant)					
Control	(trt 1)	33.0 ab	24.1 a	45.4 a	17.0 a
1N	(trt 2)	30.8 ab	14.2 a	43.2 a	13.2 a
3N	(trt 3)	17.2 b	12.2 a	67.6 a	15.6 a
10^8	(trt 6)	25.4 ab	14.7 a	36.5 a	17.6 a
10^8	(trt 8)[1]	45.0 a	25.2 a	52.1 a	24.9 a
Total nodule dry wt (mg/plant)					
Control	(trt 1)	19 b	11 a	395 a	227 a
1N	(trt 2)	15 b	9 a	441 a	225 a
3N	(trt 3)	10 b	6 a	376 a	184 a
10^8	(trt 6)	17 b	10 a	264 a	320 a
10^8	(trt 8)[1]	117 a	10 a	471 a	323 a
Shoot dry wt (g/plant)					
Control	(trt 1)	3.6 a	5.3 a	15.1 b	15.1 a
1N	(trt 2)	4.9 ab	4.3 a	26.9 a	17.6 a
3 N	(trt 3)	6.9 a	7.2 a	23.8 ab	21.6 a
10^8	(trt 6)	4.4 b	3.6 a	16.0 ab	13.8 a
10^8	(trt 8)[1]	4.8 ab	4.6 a	23.5 ab	23.7 a

[1]Inoculant placed at 8 cm below the seed.

[2]Means, within the same column having common letters are not significantly diferent at the 0.05 level by DMRT.

TABLE 3: The number of viable cowpea-group rhizobia found in Sudanese soils.

Number of soils	Years since last planted to groundnut	Ave. MPN count with siratro (Viable cells/g soil)
11	1	2.3×10^5
6	2	3.6×10^4
4	3	2.9×10^4
3	4	3.7×10^4
6	–[2]	4.9×10^5

[1] One of the six samples had 2.4×10^6 rhizobia/g soil; one sandy sample from western Sudan had fewer than 3×10^2 rhizobia/g (lowest detection limit of the test), and was not included in the average.

[2] Never planted to groundnut.

FUTURE RESEARCH

Many areas for future research are evident from this initial study with some additional studies already underway at ISU. Thus:

Five isolates collected during the survey of outlying regions are being evaluated for efficiency in comparison with other known groundnut strains (25B7, 26Z6, 176A22, TAL309, 8A11, 3Gb10, 3Gb20). Tests of efficiency and infectivity with both 'Ashford' and 'Barberton' will be made;

Antibiotic-resistant markers are being developed in the above strains to determine their competitive ability in the field. Antiserum will also be developed for the best selected strains in order to evaluate their characteristics against those of the Sudanese strains;

The Sudanese and other strains are being evaluated for resistance to high temperatures and desiccation in a growth chamber study at ISU. Inoculant strains must be able to compete with the native strains in the hot, dry soils of Sudan in order to form nodules.

If facilities permit, the field work in Sudan will be extended to important production areas other than Wad Medani. With the low number of native rhizobia and the potential for groundnut expansion in the El Obeid region, this is a logical choice for future work.

TABLE 4: Summary of observation plots in groundnut-producing regions of Sudan.

Location	Cultivar	Nodules/plant[1]		Top dry wt[1]	
		Inoculated	Uninoculated	Inoculated	Uninoculated
Wad Medani:	Ashford	150	(missing)	70.0	13.3
	MH383	244	55	32.5	15.0
	Virginia	59	135	50.0	4.0
	C/5/B-2	30	32	30.0	6.8
	C/5/B-1	77	94	60.0	5.0
	MH372	76	125	53.3	11.7
	C/5-1	132	71	57.5	7.5
	Wadie No. 2	223	44	16.7	25.0
	NA/2/40	108	86	22.0	20.0
	AM-22	196	157	40.0	24.0
	Nigerian	141	176	23.3	33.0
	430AD9G	80	164	60.0	11.7
	NC-2	81	194	15.0	20.0
	C/5/6	98	99	38.0	14.3
	Average	121	110	40.6	15.1
Rahad:	Ashford	95	71	10.0	28.0
	MH383	136	78	21.3	16.7
	MH372	119	95	15.0	20.0
	Wadie No. 2	99	82	20.8	17.0
	Nigerian	111	83	16.0	28.0
	430AD9B	99	106	18.0	16.0
	Average	110	86	16.9	16.0
Abu Naama:	Ashford	85	93	10.3	7.6
	Barberton	75	75	6.2	7.6
	Average	80	84	8.3	7.6
El Obeid:	Barberton	75	71	5.0	4.4
Sennar:	Ashford	35	21	2.0	1.5
	Barberton	34	15	2.1	1.4
	Average	35	18	2.1	1.5

[1]Values are averages of 1/2 m of row, and depending upon plant density, ranged from four to eight plants.

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PART II

INOCULATION TRIALS ON GROUNDNUTS (*Arachis hypogaea*) IN SUDAN (2nd and 3rd year studies)

INTRODUCTION

Little is known concerning the factors affecting nitrogen fixation of groundnut (peanuts) (Arachis hypogaea L.) in Sudan, one of the largest groundnut-producing countries in the world. Initial studies indicated that (a) commonly used herbicides and insecticides did not decrease yields, (b) the soil contained high populations of rhizobia capable of nodulating groundnut, and (c) yields were not improved by adding P, K, S, Ca, Mg or micronutrients, but were improved by adding nitrogen fertilizer (Hadad et al., 1982). Mukhtar and Yousif (1979) also have reported that groundnuts in Sudan respond to nitrogen fertilization.

Since yield responses were found due to nitrogen additions, the native rhizobia apparently were not able to satisfy the complete nitrogen needs of the growing plants. The groundnut-nodulating rhizobia in Sudanese soils have been little studied (Musa, 1972), and no previous inoculation trials have been conducted. If the native strains are less than completely efficient in fixing nitrogen, they must be replaced in nodules by better strains if improved yields are to be realized. Hot, dry conditions in this tropical country during planting (60° soil temperatures have been reported by Musa (1972)), or other environmental factors may hinder establishment of inoculant strains. Also, the inoculant strains may be less competitive than the established native strains in forming nodules.

Several methods of improving survival and persistence of inoculant strains in soils have been reported. These include deep placement of

inoculants (Schiffman and Alper, 1968; Marcarian, 1982; Wilson, 1975), mixing inoculant strains (Nambiar and Dart, 1982), and selection of inoculant carriers (Kremer and Peterson, 1982). The deep placement of inoculants may be beneficial in Sudan to reduce exposure to high temperatures that occur in the surface soil. Even though some advocate mixed inoculant strains, others cite the dangers in mixed cultures from (a) differential multiplication rates of the strains resulting in large differences in the final prepared inoculant, and (b) competition between the strains resulting in the least effective strain occupying the nodules (Date and Brockwell, 1976). Studies involving competition between inoculant strains and the existing native rhizobia require identification of the competing organisms. Serological differences using agglutination reactions is one technique that has been used to verify strain recovery (Roughly et al., 1976).

This paper reports on investigations in Sudan to improve the nitrogen-fixing ability of groundnut. Specifically, strains that were found to be infective and efficient in nitrogen fixation in greenhouse tests on Sudanese cultivars were added to field-grown groundnuts at Wad Medani, Sudan. Additions were made as single or a mixture of strains, formulated in peat or an oil-base carrier, and placed with the seed or at 5 and 10 cm below the seed.

MATERIALS AND METHODS

The field work was conducted in 1981 and 1982 during the rainy season (mid-June through October) on the heavy clay soils located at Wad Medani, Sudan. Total rainfall during the rainy season at this location is approximately 200 mm and supplemental irrigation is required. Wad Medani is located between the Blue and the White Nile rivers, 120 km southeast of Khartoum. The soils at this location are classified as Entic Pellusterts, fine, smectic, isohyperthermic, and the dominant clay is montmorillonite. The research site (Table 1) was previously planted to cotton. To ensure that nutrients other than nitrogen were not limiting, a uniform application of macro- and micronutrients was hand applied to each plot (Table 2). Nitrogen fertilization at 120 kg ha^{-1} N as ammonium sulfate was applied as a treatment variable. The treatments were laid out in a randomized, complete block design with four replications in 1981 and with five replications in 1982.

Groundnuts were hand planted on ridges 60 cm apart with an in-row spacing of 20 cm. Two seeds were placed in each hole. The plot size was 2.4 x 12 m, with four ridges per plot. The two cultivars used were 'Ashford', a late-maturing, spreading variety (Virginia type) and 'Barberton', an early-maturing, semibunch variety (Spanish type). Planting dates were the 18th and 19th of June in 1981, and the 16th and 17th of June in 1982 for 'Ashford' and 'Barberton', respectively. Plots were irrigated approximately every fortnight, depending upon rainfall. All plots were hand weeded during the season to eliminate competition from weeds.

Table 1. Soil characteristics[a] (0 to 20 cm) of the experimental site at Wad Medani, Sudan

pH	O.C.	Total N	CEC	EC	ESP	Sand	Clay
	%	$\mu g\ g^{-1}$	$cmol(NH_4^+)kg^{-1}$	— mmho/cm —		— % —	
8.4	0.47	473	50	0.52	3.6	21	55

[a]Characterization of this soil reported earlier by Glenn et al. (1969).

Table 2. Treatments[a] applied to the Sudanese cultivars 'Ashford' and 'Barberton' during the 1981 and the 1982 growing seasons at Wad Medani, Sudan

Treatment No.	Growing season	
	1981	1982
1	Uninoculated control	Uninoculated control
2	Nitrogen control[b]	Nitrogen control[b]
3	8A11 (peat)	8A11 (peat)
4	TAL 309 (peat)	TAL 309 (peat)
5	Peat mixture (s)[c]	Peat mixture (8A11 & TAL 309)
6	Peat mixture (5)[c]	Oil mixture (8A11 & TAL 309)
7	Peat mixture (10)[c]	8A11 (oil)
8	--	TAL 309 (oil)

[a]Treatments were replicated four times in 1981 and five times in 1982. All treatments received a uniform application of macro- and micronutrients as shown below:

Phosphorus: 40 kg P_2O_5 ha^{-1} (triple superphosphate)
Potassium: 40 kg K_2O ha^{-1} (muriate of potash)
Sulfur: 45 kg S ha^{-1} (elemental sulfur)
Boron: 0.5 kg B ha^{-1} (borax)
Iron: 0.5 kg Fe (6% Fe in chelate) ha^{-1}
Copper: 2 kg Cu ($CuSO_4 \cdot 5H_2O$) ha^{-1}
Manganese: 15 kg Mn ($MnSO_4 \cdot H_2O$) ha^{-1}
Zinc: 0.6 kg Zn (14.2% Zn in chelate) ha^{-1}.

[b]Nitrogen: 120 kg N ha^{-1} (ammonium sulfate).

[c]Mixture of strains 8A11, TAL 309, 25B7, and 26Z6 applied at the sowing depth(s), and at 5 and 10 cm below the seeding depth for treatments 5, 6, and 7, respectively, in 1981.

Rhizobium Strains

Two strains were used in the field studies both years (Table 2): 8A11 (obtained from the Nitragin Company of Milwaukee, Wisconsin) and TAL 309 (obtained from North American Plant Breeders, Princeton, Illinois). Strains 26Z6 and 25B7, supplied by the Nitragin Company, were additionally used in a peat carrier in the 1981 field studies, but data are not presented.

Strain selection for field study was based on greenhouse efficiency testing. Seeds of two popular Sudanese cultivars, 'Ashford' and 'Barberton', were planted in 4-L containers of a sand-perlite mixture, inoculated with $1x10^8$ cells of a specific *Rhizobium* strain, and grown in the greenhouse for two months. Color, top dry weights, and nodule numbers were determined at harvest.

Methods of Inoculation

The peat carrier (1981 and 1982 seasons) was prepared as a granular inoculant by the Nitragin Company. The oil carrier (1982 season) consisted of freeze-dried *Rhizobium* cells prepared by Drs. Peterson and Kremer at Mississippi State University, Starkville, Mississippi. Both inocula were added at rates to give approximately 10^7 viable *Rhizobium* cells per two groundnut seeds. Viable populations were determined in growth pouches (Weaver and Frederick, 1972) by the most probable number (MPN) technique (Vincent, 1970) with sirratro (*Macroptilium atropurpureum*) as the test plant.

In all but the deep placement studies, the inoculant was furrow applied by hand at the sowing depth (5 cm below the soil surface). A

scooping device was prepared that allowed the workers to add a consistent volume of the granular inoculant to each pair of groundnut seeds. The oil-base inoculant was seed applied after the Rhizobium cells were suspended in groundnut oil (Kremer and Peterson, 1982). Mixtures of the strains to give a total of 10^7 viable Rhizobium per two groundnut seeds in peat and oil-base carriers were applied at the seeding depth in the 1982 season. Seeds and applied inoculant were immediately hand covered with soil after sowing and were irrigated within 24 h. For the deep placement treatments (1981), a stick was used to open a furrow at 10- and 15-cm depths on top of each ridge, where the inoculant was placed (the inoculant contained a mixture of strains in peat to give approximately 10^7 cells per two groundnut seeds). The furrows were immediately filled with soil to within 5 cm of the soil surface and the groundnut seeds were planted. The ridges were then completely reformed by hand to their original contour.

Sampling and Harvest

For assessment of the nitrogen-fixing traits, a section of row containing one dozen plants beginning 1/2 m from the end of the plots from the outer two rows was sampled during the early flowering stage one month after sowing. Nodule counts, shoot dry weights, and Kjeldahl nitrogen of the shoots were determined. Fifty representative nodules, 25 main-root and 25 lateral-root, were randomly selected for each treatment. The nodules were transferred to test tubes containing silica gel for desiccation and transported to the laboratory at Iowa State

University for Rhizobium isolation and the determination of nodule occupancy.

Plot harvest was preceded by light irrigation to minimize pod losses. The plants in 10 m of the center two rows in each plot were dug for pod yield determinations. The harvests were approximately 90 and 120 days after sowing for 'Barberton' and 'Ashford', respectively.

Determination of Nodule Occupancy

Agglutination reactions were used in identifying strains recovered from nodules. Antisera were prepared for strains 8A11, TAL 309, and for a native strain from the research site (Wad Medani). For antigen preparation, the Rhizobium suspensions were heated in a waterbath at 100°C for 30 min. to destroy the heat-labile, nonspecific flagellar antigens (Vincent, 1970). Titers of at least 1280 were obtained for each after rabbit injection. Results were reported as positive or negative agglutination.

RESULTS AND DISCUSSION

The *Rhizobium* strains used as inoculants in the field were selected based on greenhouse efficiency testing with the Sudanese cultivars 'Ashford' and 'Barberton' (Table 3). Statistically significant differences were found among strains in color, top dry weights, and nodule numbers. Strains 8A11, TAL 309, 25B7, and 26Z6 appeared to be four of the better strains on both cultivars and were selected for field application in 1981. Only the two best strains, 8A11 and TAL 309, were used in 1982 because the addition of the oil-base carrier dictated a reduction in the total number of strains tested.

Strains and Cultivars

Data for common treatments in both 1981 and 1982 are presented in Table 4. The 'Ashford' cultivar (Virginia type) was generally better nodulated (significant at the 0.01 level of probability) and the pod yields were higher than those of the 'Barberton' cultivar (Spanish type).

Added nitrogen fertilizer tended to decrease nodule number and nodule mass but increased top dry weight, tissue nitrogen, and pod yield. The top dry weights of the 'Ashford' cultivar with nitrogen fertilizer were excelled only by inoculation with 8A11 in 1981 (although not significant) and the top dry weights of both 'Ashford' and 'Barberton' with nitrogen fertilizer exceeded all other treatments in 1982 ($P<0.05$). The effect of nitrogen also was evident in pod yield. 'Barberton' yields with added nitrogen were statistically ($P<0.05$) better than the control and the two inoculated treatments in 1982. While the yields of 'Ashford'

Table 3. Greenhouse screening for nitrogen-fixing traits with the cultivars 'Ashford' and 'Barberton'

Treatment	'Ashford'			'Barberton'		
	Color[a]	Top dry weight	Nodule number	Color[a]	Top dry weight	Nodule number
		g plant^{-1}	No. plant^{-1}		g plant^{-1}	No. plant^{-1}
Uninoculated control	2.00d[b]	0.60ab	0.0e	2.40e	0.42cd	0.0c
Nitrogen[c] control	5.00a	1.15a	0.0e	5.00a	1.24a	0.0c
Wad Medani[d]	3.75abc	0.65ab	23.0cde	2.75e	0.45bcd	10.8c
Sennar[d]	4.00abc	0.79ab	66.8abc	3.60cd	0.49bcd	31.2bc
26Z6[e]	5.00a	0.67ab	43.2bcde	4.40abc	0.61bcd	72.6a
8A11[e]	5.00a	0.98ab	83.0ab	4.40abc	0.67bcd	63.8a
USDA 3184[f]	3.50bc	0.43b	26.5cde	3.00de	0.37d	17.8a
USDA 3187[f]	3.00cd	0.55b	4.5de	2.60e	0.43cd	1.3c
25B7[e]	5.00a	0.81ab	56.0bcd	4.40abc	0.77b	64.6a
USDA 3179[f]	3.33bc	0.68ab	112.3a	3.75cd	0.44cd	75.0a
176A22[f]	4.50abc	0.69ab	54.3bcde	4.00bc	0.50bcd	29.2bc
TAL 309[g]	5.00a	0.94ab	64.8abc	4.80ab	0.73bc	47.6ab

[a]Visual color rating two months after planting: 5 for dark green, 4 and 3 for intermediate colors, and 2 for yellow.

[b]Values are averages of five replications; means within the same column followed by a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

[c]Ten ml of 8 μg N ml^{-1} NH_4NO_3 solution applied three times during the 8-week growing period.

[d]Isolates collected from Sudan in the summer of 1981.

[e]Supplied by the Nitragin Company, Milwaukee, WI.

[f]Supplied by Nitrogen Fixation Laboratory, Beltsville, MD.

[g]Supplied by North American Plant Breeders, Princeton, IL.

Table 4. Effect of the common treatments in 1981 and 1982 on the nitrogen-fixing traits of the cultivars 'Ashford' and 'Barberton', Wad Medani, Sudan

Treatment	Nodulation				Yield					
	Number		Mass		Top dry weight		Tissue nitrogen		Pod	
	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
	No. plant^{-1}		mg plant^{-1}		g plant^{-1}		%		kg ha^{-1}	
Ashford										
Uninoculated control	73.0a[a]	95.0ab	19.00b	69.20a	3.33a	6.28b	3.48a	2.72ab	1743.1a	1628.5a
N-control[b]	38.0a	81.0b	26.25ab	66.40a	3.65a	10.82a	3.49a	3.07a	1899.3a	1711.8a
8A11	63.0a	128.0a	40.50a	84.00a	4.58a	6.25b	3.26a	2.83ab	1697.9a	1597.2a
TAL 309	69.0a	96.0ab	29.25ab	78.00a	3.55a	5.22b	3.36a	2.51b	1625.0a	1743.1a
Barberton										
Uninoculated control	52.0a	70.0a	17.25a	50.00ab	2.91a	4.58b	3.82ab	2.67a	1350.7a	579.9b
N-control[b]	36.0a	64.0a	17.00a	33.60b	3.58a	9.48a	3.93a	2.72a	1437.5a	909.7a
8A11	47.0a	61.0a	18.50a	55.60a	3.18a	5.12b	3.63bc	2.68a	1333.3a	680.6b
TAL 309	41.0a	78.0a	25.75a	67.20a	2.95a	5.08b	3.40c	2.58a	1454.9a	684.0b

[a]Values are averages of four observations in 1981 and five observations in 1982; means for each cultivar within the same column followed by a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

[b]Added at sowing as ammonium sulfate [$(NH_4)_2SO_4$] at 120 kg ha^{-1} N.

were not statistically enhanced at the 0.05 level of probability, a trend favoring the nitrogen treatment was present, especially in 1981. Pod yields in 1982 were consistently lower, especially with 'Barberton', than yields in 1981. In the author's experience, fluctuation in groundnut yields under Sudanese conditions is not an uncommon phenomenon.

Little differences were found between the plots inoculated with 8A11 or TAL 309 and the uninoculated controls. There was a trend favoring tissue dry weights and tissue nitrogen with the application of strain 8A11 over TAL 309, but this was not reflected in improved pod yields. An analysis of variance showed a significant cultivar by strain interaction for nodule mass, but only at the 0.10 level of probability (Table B10). Strain 8A11 produced significantly ($P<0.05$) greater nodule mass than the uninoculated control with the 'Ashford' cultivar in 1981 and strain TAL 309 tended to produce greater nodule mass than the uninoculated control both years with the 'Barberton' cultivar.

Mixing Inoculant Strains

Commercial inoculants usually contain two or more strains to safeguard against the failure of a single strain addition. The data for comparing single Rhizobium strains in peat and oil-base carriers versus mixed strain additions are presented in Table 5. No differences were found, in general, between the single and mixed additions in either carrier. Since this soil contained a large population of native rhizobia that was successful in nodulating groundnuts (as evident by the high number of nodules in the uninoculated controls), the responses of single versus mixed cultures may have been obscured by the native

Table 5. Effect of peat and oil-base carriers on groundnut nitrogen-fixing traits in the 1982 season, Wad Medani, Sudan

Treatment[a]	Nodulation		Yield		
	Number	Mass	Top dry weight	Tissue nitrogen	Pod
	No. plant^{-1}	mg plant^{-1}	g plant^{-1}	%	kg ha^{-1}
		Uninoculated			
Uninoculated control	82.0a[b]	59.16bc	5.43b	2.70ab	1104.2abc
N-control[c]	73.0a	50.00c	10.15a	2.89a	1309.0a
		Single Inoculant			
8A11					
Peat	95.0a	69.80ab	5.69b	2.76ab	1138.9abc
Oil	84.0a	58.80bc	5.01b	2.59b	1000.0bc
TAL 309					
Peat	88.0a	74.30a	5.14b	2.55b	1211.8ab
Oil	84.0a	62.40abc	4.87b	2.60b	899.3c
		Mixed Inoculant[d]			
Peat	87.0a	72.60a	5.15b	2.55b	1211.8ab
Oil	97.0a	65.80ab	5.79b	2.61b	1062.5abc

[a]Except for the uninoculated treatments, viable rhizobia were added at 10^7 per two groundnut seeds.

[b]Means of 10 observations, since there was no significant cultivar by treatment interaction ($P<0.05$) (Appendix B), the means for both cultivars 'Ashford' and 'Barberton' were pooled; means within the same column followed by a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

[c]Added at sowing as ammonium sulfate [$(NH_4)_2SO_4$] at 120 kg ha^{-1} N.

[d]Mixture of the *Rhizobium* strains 8A11 and TAL 309.

rhizobia.

Inoculant Carriers

Inocula used in MPN determinations were carried to the field during planting, handled in similar fashion as the applied inoculant, and then returned to the laboratory for determination of viable counts. When calculated for the number of viable Rhizobium cells per two groundnut seeds, both peat and oil-base inocula gave counts exceeding 10^7. The population of the native rhizobia was 2.1×10^4 rhizobia g^{-1} of soil.

The peat carrier appeared to be superior to the oil carrier (Table 5). Pod yields with strain TAL 309 were statistically greater with the peat carrier. Furthermore, although differences were not significant at the 0.05 level, most values for nodule number, nodule mass, top dry weights, and pod yields were consistently greater with the peat carrier compared with the oil carrier. When exposed to 16 d of temperature-moisture stress, Kremer and Peterson (1982) earlier reported higher rhizobia populations from seeds inoculated with an oil-base carrier than seed inoculated with a peat-base inoculant. In this study, plots were irrigated within 24 h after sowing.

Shelled groundnuts are very fragile for direct inoculation. The decrease in the nitrogen-fixing traits and pod yields with the oil-base inoculant (Table 5) may suggest (a) poor adherence of the bacteria to the seed following inoculation, (b) poor survival on the seed after inoculation, or (c) the oil application to the seed may have interfered with plant growth. The latter suggestion is supported by the low top

dry weights, tissue nitrogen, and pod yields obtained with the oil treatments compared with the uninoculated controls. Both the single inoculants and the mixed inoculants in oil had lower values for most of these traits.

Nodule occupancy data (Table 6) indicated little differences in the percentage of TAL 309 occupying nodules due to oil or peat carriers.

Table 6. Groundnut nodule occupancy in the treatments receiving strain TAL 309, Wad Medani, Sudan, 1982

Inoculant carrier	Occupancy[a]	
	Main root nodules	Lateral root nodules
	%	
Peat	38.0±4.7	36.0±7.1
Oil	30.0±2.0	44.0±4.3

[a]Means ± S.E. of 50 nodules identified per treatment. The indigenous rhizobia in Wad Medani soils (Sudan) were serologically distinct from TAL 309; the native population was estimated by the MPN to be 2.1×10^4 groundnut-nodulating rhizobia g^{-1} in the top 15 cm of soil. The inoculant was added at 10^7 rhizobia per two groundnut seeds.

The Wad Medani isolates, obtained from the control plots, were serologically identical to 8A11 and antisera reactions could not be used to determine 8A11 strain recovery. The peat carrier resulted in equal percentages of main and lateral-root nodules but the oil carrier gave a higher percentage of lateral-root nodules occupied by TAL 309. The increase in strain TAL 309 recovery from the oil carrier in the lateral-root nodules may indicate late infection that was not reflected in the

measured nitrogen-fixing traits (Table 5). The oil carrier might have protected the rhizobia longer than the peat carrier.

Depth of Inoculant Placement

As the depth of inoculant placement increased, top dry weights increased and pod yields tended to improve (Table 7). The number of

Table 7. Effect of the placement depth of inoculant on nitrogen-fixing traits of groundnuts, Wad Medani, Sudan, 1981

Treatment	Nodulation		Yield		
	Number	Mass	Top dry weight	Tissue nitrogen	Pod
	No. plant^{-1}	mg plant^{-1}	g plant^{-1}	%	kg ha^{-1}
Uninoculated control	62.0a	18.13a	3.11b	3.65ab	1545.1a
N-control[a]	36.0a	34.13a	3.11b	3.71a	1666.7a
Inoculum mixture(s)[b]	43.0a[c]	27.13a	3.13b	3.43bc	1638.9a
Inoculum mixture (5)[b]	58.0a	22.00a	3.31ab	3.39c	1666.7a
Inoculum mixture (10)[b]	46.0a	26.13a	3.98a	3.48bc	1753.5a

[a]Added at sowing as ammonium sulfate [$(NH_4)_2SO_4$] at 120 kg ha^{-1} N.

[b]Mixture of the Rhizobium strains 25B7, 8A11, TAL 309, and 26Z6 in peat-base inoculant placed at the seeding depth(s), and 5 and 10 cm below the seeding depth, respectively.

[c]Values represent an average of 8 observations; since there was no cultivar by treatment interaction ($P<0.05$) (Tables B14-B18), the observations for both cultivars 'Ashford' and 'Barberton' were pooled; means within the same column followed by a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

nodules per plant and the weight of nodules per plant were unaffected by the placement depths. This disagrees with the weight-compensation phenomenon suggested by Schiffman and Alper (1968). Also, unlike the

results in soil with no (Wilson, 1975) or few (Marcarian, 1982) rhizobia, the distribution of nodules along the root in this soil with a high indigenous population was not related to the depth of inoculant placement. Visual observations of the nodules indicated a rather uniform distribution throughout the rooting system.

Results of these studies failed to demonstrate decided yield advantages with inoculation, regardless of method or depth of placement. The soils contained an active, native population of groundnut-nodulating rhizobia. An added strain (TAL 309), however, could successfully compete for nodule sites with the indigenous rhizobia. Adding 120 kg N ha^{-1} resulted in improved growth parameters, suggesting not all of the nitrogen requirements could be met by nitrogen fixation. Perhaps in these soils of low organic carbon, low rates of nitrogen mineralization and the quantities of nitrogen in groundnut cotyledons are not adequate to successfully establish the plant, without stress, before nitrogen fixation becomes fully active. Further, carbohydrate partitioning between nodules and plant tissue may reduce growth and yields when plants are required to depend on fixation for their nitrogen supply. The results of this study suggest that additional work is needed to identify aggressive, temperature adaptive, efficient strains for use in Sudan and to more fully understand factors affecting symbiotic nitrogen fixation in tropical soils.

SUMMARY AND CONCLUSIONS

Greenhouse, field, and laboratory studies were conducted to evaluate nitrogen fixation by groundnuts (*Arachis hypogaea* L.) in Sudan. *Rhizobium* isolates were screened in the greenhouse for infectivity and efficiency on the two Sudanese groundnut cultivars 'Barberton' and 'Ashford'. Significant differences in both traits were found among the eight strains screened. Strains 8A11 obtained from the Nitragin Company and TAL 309 obtained from North American Plant Breeders were two of the more effective strains. In studies in Sudan on soil (Entic Pellusterts) between the Blue and White Nile Rivers, both 'Ashford' and 'Barberton' were inoculated with selected strains (a) added singly or in mixture, (b) formulated in peat or oil-base carriers, and (c) placed with the seed or at 5 and 10 cm below the seed. Inoculation, in general, resulted in little improvement in nitrogen-fixing traits or pod yields, and did not equal the improvements obtained by adding 120 kg ha^{-1} N as ammonium sulfate. Control plots were heavily nodulated by the indigenous rhizobia. Trends did favor the peat carrier over the oil carrier and placing the inoculant at the 10-cm depth below the seed. Antisera were prepared for strain identification. Of the strains used in the field studies, only TAL 309 was serologically distinct from the native population. This distinct strain occupied 38 and 36% of the main and lateral-root nodules, respectively, when applied in the peat carrier and 30 and 44% of the main and lateral-root nodules when applied in the oil-base carrier. Both treatments were applied at $>10^7$ viable

Rhizobium cells per two groundnut seeds. With a native population of 2.1×10^4 rhizobia g^{-1} of soil, the inoculant strain seemed to be fairly competitive with the indigenous rhizobia in spite of the hot, dry conditions present at the time of groundnut planting.

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PART III

CHARACTERIZATION OF SUDANESE GROUNDNUT-NODULATING RHIZOBIA

INTRODUCTION

Rhizobium strains and legume species with little symbiotic specificity are much more common in the tropics than in temperate regions of the world. The large heterogeneous cowpea group is characterized by a high degree of symbiotic promiscuity. Its classification, however, is supported mainly by data from a rather narrow range of legumes characteristic of temperate zones (Norris, 1956). Further investigations of tropical isolates have been emphasized (Williams, 1967).

Groundnut (Arachis hypogaea L.) is nodulated by members of the cowpea miscellany (Fred et al., 1932; Buchanan and Gibson, 1974). Members of this group are mainly thought to be slow growing and alkaline producing. Some inconsistencies were reported in Sudan by Habish and Kheiri (1968) who claimed that their groundnut Rhizobium isolates were fast growing and acid producing. Furthermore, their isolates did not nodulate cowpea, which made them separate from the cowpea group. Elsewhere, several strains of fast-growing rhizobia capable of nodulating cowpea and soybean have been reported (Zablotowicz and Focht, 1981).

In Sudan, differences are often noted in soil characteristics regarding pH, organic matter, and total nitrogen. Environmental conditions that prevail plus different crop rotations may affect the abundance of indigenous groundnut rhizobia. The distribution of Rhizobium strains has been shown to vary with soil pH (Damirgi et al., 1967; Ham et al., 1971), temperature (Ahmed et al., 1981), available and organic nitrogen (Bezdicek, 1972), and with the frequency of occurrence of

soybeans in crop rotations (Weaver et al., 1972). Only limited research, however, has been conducted on the effect of such soil factors on groundnut rhizobia, especially in the tropics.

Variations between groundnut rhizobia in efficiency in fixing atmospheric nitrogen are well-documented. Tropical rhizobia are apparently highly infective but are not able to supply crops with all the required nitrogen needed to obtain maximum yields (Nambiar and Dart, 1982). Gathering basic information concerning groundnut rhizobia may help in identifying the factors that limit the legume-*Rhizobium* symbiosis. Accordingly, this work was conducted to:

(1) Assess groundnut rhizobia abundance in different production regions of Sudan and correlate the data to soil properties and the duration, presence, or absence of groundnuts in crop rotations.

(2) Obtain *Rhizobium* isolates from fresh nodules of the commonly grown legumes in Sudan and study their growth and serological characteristics. Test under greenhouse conditions the infectivity and efficiency of the isolates with a cultivar of groundnut from Sudan.

MATERIALS AND METHODS

Soils and *Rhizobium* Abundance

Thirty-two sites in Sudan of varying soil types and management practices were sampled at the beginning of the rainy season (June) for the first part of this study (Table 1). The sites were chosen in geographical regions with existing groundnut production (Central and Southcentral Sudan), and with potential groundnut expansion in Western Sudan, Western Sudan is considered west of the Nile River (Fig. 1). Three subsamples from each site were randomly taken from a 0 to 15-cm depth. Moisture and rhizobia number determinations were made within 24 h after sampling and the remainder of the soil samples were dried, crushed, passed through a 3-mm screen, and thoroughly mixed before chemical analyses were made.

Rhizobium numbers were determined in growth pouches (Weaver and Frederick, 1972) using the MPN-technique (Vincent, 1970) with sirratro (*Macroptilium atropupureum*) as the test plant. The data were compiled and analyzed by constructing a correlation matrix.

Characterization of Sudanese Rhizobia

In the second part of the study, *Rhizobium* isolates were made from nodules collected in August from the commonly grown legumes in Sudan (Table 2). Difficulties with transportation via Landrover (Fig. 1) did not allow the Kazgail and Kadugli sites to be sampled for rhizobia number determinations in June but did allow nodule collections in August. The procedures described by Vincent (1970) for isolation and isolate authentication of rhizobia were followed. Colony characteristics were

Table 1. Soil characteristics[a] of the survey locations in Sudan

Soil No.	Location	Moist.	pH Suspension	pH Paste	Organic C	Organic N	Rhizobium No.	Years[b]
		%			%	$\mu g\ g^{-1}$	$No.g^{-1}$ soil	
1	Abunaama	9.89	8.2	7.0	0.431	599	$1.1x10^4$	1
2	Abunaama	10.13	8.9	7.9	0.463	588	$2.4x10^6$	∞[c]
3	Abunaama	10.13	8.6	7.7	0.414	613	$2.9x10^4$	1
4	Abunaama	9.65	8.8	7.8	0.406	588	$4.4x10^4$	1
5	Abunaama	10.13	8.6	7.7	0.414	641	$1.1x10^5$	1
6	Abunaama	10.01	8.6	7.7	0.406	616	$5.3x10^4$	2
7	Abunaama	10.13	8.8	8.0	0.431	613	$1.5x10^4$	1
8	Abunaama	10.13	8.7	7.7	0.378	578	$4.2x10^4$	2
9	Abunaama	10.13	8.8	7.7	0.395	588	$7.5x10^4$	4
10	Abunaama	10.01	8.8	7.7	0.378	574	$4.4x10^4$	3
11	Abunaama	9.76	8.8	7.7	0.446	574	$1.6x10^5$	1
12	Abunaama	ND[d]	ND	ND	ND	ND	$1.1x10^4$	∞
13	Wad Medani	9.65	8.8	7.8	0.492	616	$1.1x10^4$	∞
14	Wad Medani	15.20	8.6	7.9	0.481	602	$1.5x10^4$	2
15	Wad Medani	10.25	8.9	8.0	0.492	630	$2.0x10^4$	1
16	Wad Medani	6.26	9.0	8.0	0.424	574	$3.5x10^4$	3
17	Wad Medani	6.38	9.0	8.5	0.460	564	$3.5x10^4$	2
18	Wad Medani	7.52	8.7	7.9	0.427	588	$2.1x10^4$	4
19	Wad Medani	6.04	8.6	8.0	0.416	602	$7.3x10^4$	1
20	Sennar	6.49	8.4	7.8	0.378	560	$1.5x10^4$	1
21	Sennar	9.52	8.5	7.9	0.359	613	$1.1x10^4$	3
22	Sennar	5.93	8.9	8.2	0.424	620	$2.4x10^4$	1
23	Sennar	6.38	8.5	8.0	0.323	560	$1.5x10^4$	2
24	Sennar	6.83	8.7	7.9	0.402	557	$2.0x10^4$	4
25	Sennar	6.26	8.7	7.9	0.323	585	$2.0x10^4$	3
26	Rahad	6.95	8.8	7.8	0.543	700	$1.5x10^4$	1
27	Rahad	10.37	8.5	7.7	0.477	641	$1.5x10^3$	∞
28	Rahad	10.37	8.7	8.0	0.158	406	$2.7x10^3$	∞
29	Rahad	8.93	8.0	7.4	0.101	333	ND	1
30	El Obeid	8.93	6.6	5.9	0.082	322	$<3.0x10^2$	1
31	El Obeid	8.93	6.9	6.5	0.101	333	$1.1x10^3$	1
32	El Obeid	10.13	6.8	6.1	0.946	1246	$1.5x10^4$	∞

[a]Values are averages of three replications. The moisture percentage is the moisture content at time of sampling, suspension pH was measured with a glass electrode at a soil:H_2O ratio of 1:5, paste pH was measured in a saturated soil, organic carbon was measured by the Walkley-Black method, and organic nitrogen was measured by the Kjeldahl procedure.

[b]Years since last planted to groundnut.

[c]∞ Refers to never planted to groundnut.

[d]ND not determined.

Fig. 1. Locations in Sudan where samples were collected for abundance determinations and characterization of groundnut-nodulating rhizobia

Table 2. Legume of isolation and origin of the *Rhizobium* isolates used in the characterization study

Isolate No.	Legume of isolation		Origin
	Common name	Scientific name	
1	Green gram	*Phaseolus aureus*	Central Sudan (Wad Medani)[a]
2	Pigeon pea	*Cajanus cajan*	Central Sudan (Wad Medani)[a]
3	Cowpea	*Vigna ungiculata*	Central Sudan (Wad Medani)[a]
4	Lubia	*Dolichos lablab*	Central Sudan (Wad Medani)[a]
5	Bambara groundnut	*Voandzeia subterranea*	Western Sudan (El Obeid)[b]
6	Groundnut	*Arachis hypogaea*	Central Sudan (Wad Medani)[a]
7	Groundnut	*Arachis hypogaea*	Southcentral Sudan (Sennar)[a]
8	Groundnut	*Arachis hypogaea*	Southcentral Sudan (Sennar)[a]
9	Groundnut	*Arachis hypogaea*	Western Sudan (Kazgail)[b]
10	Groundnut	*Arachis hypogaea*	Western Sudan (Kazgail)[b]
11	Groundnut	*Arachis hypogaea*	Western Sudan (Kadugli)[a]
12	8A11	--	Supplied by the Nitragin Company, Milwaukee, WI

[a]Textural soil type is clay.

[b]Textural soil type is sand.

studied on a Yeast Extract Mannitol (YEM) agar medium, containing bromthymol blue as a pH indicator. Growth in medium with different pHs, different sodium chloride levels, and following moist-heat exposure followed the methods of Graham and Parker (1964). Mannitol and glucose utilization was tested at 28°C in basal mineral medium (YEM minus carbon source) in Wheaton-Nephelo flasks placed on a waterbath shaker. Daily changes in percentage transmittance were monitored using a spectrophotometer at 525 nm.

Agglutination reactions were used to study the serological identity of the isolates. For antigen preparation, the Rhizobium suspensions were heated in a waterbath at 100°C for 30 minutes to destroy the heat-labile, nonspecific flagellar antigens. Antisera production followed the method of Vincent (1970). Readings were reported as strong agglutination (+4), partial agglutination (+3, +2, +1), or negative agglutination (-).

Infectivity and the relative efficiency of the isolates in fixing atmospheric nitrogen were determined under greenhouse conditions using the Sudanese groundnut cultivar 'Barberton'. A nitrogen control (10 ml of 8 $\mu g\ ml^{-1}$ NH_4NO_3 solution added three times during the growth period), a standard Rhizobium strain known to be efficient on groundnut (8A11), and an uninoculated control were included. All treatments were replicated four times in a completely randomized design. After two months of growth, nodulation and tissue dry weights were evaluated as indicators of nitrogen fixation.

RESULTS AND DISCUSSION

Rhizobium Abundance as Influenced by Soil Characteristics

Since the soils at Kazgail and Kadugli could not be sampled in June before planting and at the beginning of the rainy season when all other soils were sampled (Table 1), data for these sites are missing. We were concerned that the presence of a crop and the different time of year would affect the number of groundnut rhizobia in the soil.

The soils that were sampled in June varied considerably in the parameters measured (Table 1). Soil pHs (paste) ranged from 5.9 to 8.5. In general, the soils from Central and Southcentral Sudan had neutral to alkaline pHs and the soils from Western Sudan had acid pHs. Also, the organic carbon content ranged from 0.08% for a sandy soil collected in Western Sudan to a characteristic 0.3 to 0.4% C for soils from Central and Southcentral Sudan. One exception was a soil from Western Sudan that was collected under Hashab (*Acacia senegal*) seedlings, and had 0.9% organic carbon, and 1,246 $\mu g\ g^{-1}$ organic nitrogen.

The correlation coefficients obtained between the different soil parameters and *Rhizobium* abundance are presented in Table 3. Only pH paste and pH suspension, and organic carbon and organic nitrogen were found to be significantly correlated. There was no apparent relationship between MPN counts and soil parameters, or between MPN counts and the length of time since groundnuts were last in the rotation. Another soil parameter that possibly should be included in future studies is nitrate levels. Gibson et al. (1982) recently reported that the level

Table 3. Correlation coefficients (r) between soil characteristics and numbers of groundnut-nodulating rhizobia

Parameter	Moisture	pH (suspension)	pH (Paste)	Organic carbon	Total nitrogen	MPN	Years[a]
Moisture		-0.10	-0.20	-0.12	0.08	0.11	-0.02
pH (suspension)			0.94**	0.11	0.12	0.29	0.14
pH (paste)				0.04	0.05	0.16	0.13
Organic carbon					0.96**	0.11	0.18
Total nitrogen						0.08	0.14
MPN							-0.19

[a]Years since last planted to groundnut.

**Significant at the 1% level of probability.

of nitrates in the soils of Senegal during the dry months preceding groundnut planting could be responsible for depressed nodulation.

Counts of the native rhizobial population, with sirratro as the test plant, showed that most of the soils in Sudan harbor a large population of rhizobia that potentially can nodulate groundnuts. Rhizobia were found in sites never planted to groundnuts (Table 1). With the exception of a sandy soil from Western Sudan that contained fewer than $3x10^2$ rhizobia g^{-1} (lowest detection level of the test), five soils never before planted to groundnuts contained an average of $4.9x10^5$ rhizobia g^{-1}; one of the samples had $2.4x10^6$ rhizobia g^{-1}. This compares with averages of $2.3x10^5$, $3.6x10^4$, $2.9x10^4$, and $3.7x10^4$ rhizobia

g^{-1} obtained from soils last planted to groundnuts 1, 2, 3, and 4 years ago, respectively. Either rhizobia are naturally part of the indigenous soil population or native legumes are serving as an inoculant source in Sudan for groundnut-nodulating rhizobia.

Cultural and Physiological Characteristics

All the isolates tested were short, gram-negative rods. The colonies were circular with entire margins and with raised to convex elevations. The colony sizes ranged from less than 1 mm to 3 mm in 12 days when grown on YEM-agar. Most of the colonies absorbed bromthymol blue, but colonies of cowpea, lubia, and pigeon pea isolates did not absorb the dye.

All isolates grew in YEM-agar amended with 0.1% NaCl (Table 4), but tolerances to 2% NaCl differed. The isolates from Sennar (groundnut), cowpea, and bambara groundnut did not grow at the 2% NaCl concentration. None of the isolates tolerated 3% NaCl (this is in agreement with data for the cowpea miscellany from Graham and Parker (1964)).

Most of the strains survived the moist-heat treatment for 15 minutes at 50°C, except the isolates from green gram, bambara groundnut, and a groundnut strain from Kazgail (Table 4). These data agree, in general, with the findings of Habish and Kheiri (1968) but disagree with the findings of Graham and Parker (1964).

Utilization of glucose and mannitol was similar (Table 4), except that the isolates from lubia and pigeon pea utilized mannitol somewhat less efficiently than glucose (Fig. 2).

Table 4. Carbohydrate utilization and pH, sodium chloride, and moist-heat tolerance of Sudanese *Rhizobium* isolates

Treat-ment	Isolate	Growth[a]									
		pH[b]				Carbo-hydrate[b]		Sodium chloride%[b]			Moist-heat[b]
		4	6	8	10	Glu-cose	Man-nitol	0.1	2	3	Expo-sure
1	Green gram	-	+	+	-	+	+	+	+	-	-
2	Pigeon pea	-	+	+	-	+	±	+	+	-	+
3	Cowpea	-	+	+	-	+	+	+	-	-	+
4	Lubia	-	+	+	-	+	±	+	+	-	+
5	Bambara groundnut	-	+	+	-	+	+	+	-	-	-
6	Groundnut (Wad Medani)	-	+	+	-	+	+	+	+	-	+
7	Groundnut (Sennar)	-	+	+	-	+	+	+	-	-	+
8	Groundnut (Sennar)	-	+	+	-	+	+	+	-	-	+
9	Groundnut (Kazgail)	-	+	+	-	+	+	+	-	-	-
10	Groundnut (Kazgail)	-	+	+	-	+	+	+	+	-	+
11	Groundnut (Kadugli)	-	+	+	-	+	+	+	+	-	+

[a] +, ±, - refers to positive, partial, and negative growth, respectively.

[b] pH tolerance was determined in YEM-broth adjusted with NaOH or HCl; carbohydrate utilization was determined in basal salts medium with 10g L^{-1} glucose or mannitol; sodium chloride tolerance was determined in YEM-broth adjusted to 0.1, 2, or 3% NaCl; moist heat evaluated growth after 15 min. exposure to 50°C in YEM-broth.

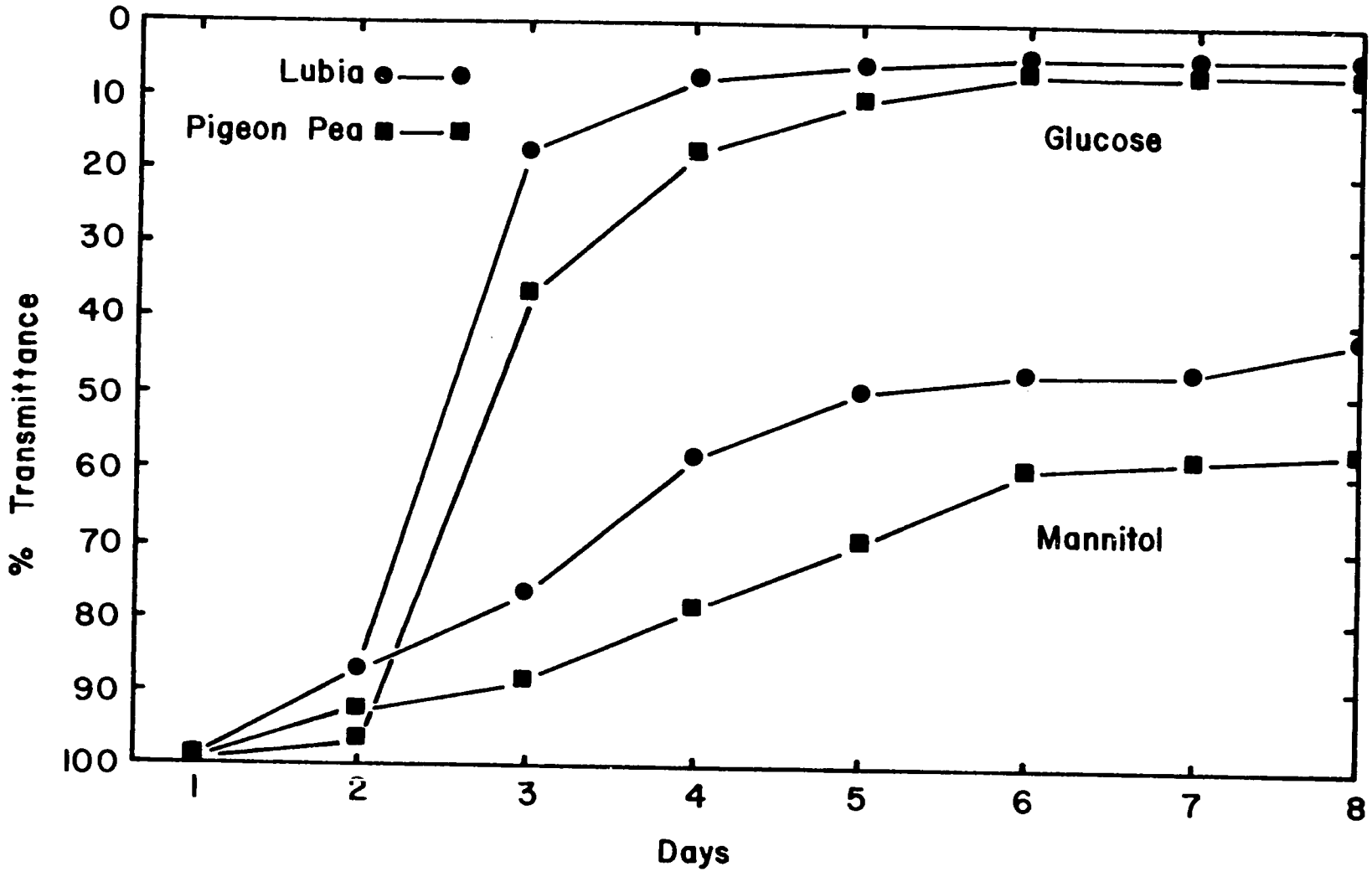

Fig. 2. Mannitol and glucose utilization of lubia and pigeon pea rhizobia

None of the strains grew at pHs 4 and 10, but did grow at pHs 6 and 8 (Table 4). Isolates could not be differentiated based on their rates of growth in media adjusted to the wide range of pHs tested.

Infectiveness of the Isolates

Each *Rhizobium* strain was identified by original host and tested for its ability to nodulate both sirratro and the groundnut cultivar 'Barberton'. The presence or absence of nodules was assessed after two months of growth in the greenhouse (Table 5).

All the isolates tested nodulated both sirratro and groundnut; this disagreed with the results of Habish and Kheiri (1968) who reported that groundnuts were not nodulated by isolates from cowpea, pigeon pea, and lubia (*Dolichos lablab*). The groundnut cultivar tended to produce more nodules when inoculated with isolates obtained from groundnuts than when inoculated with isolates obtained from other plant species. For example, the groundnut isolate from Wad Medani yielded a total of 161 nodules per plant, while the isolate from pigeon pea gave only five nodules per plant. The two groundnut isolates from Sennar (trts 7 and 8) and one isolate from Kazgail (trt 9) gave relatively lower numbers of nodules, suggesting that these strains were not as infective as other groundnut isolates.

The infectiveness of the isolates in forming nodules varied within as well as between locations. While one isolate from Kazgail (Western Sudan) gave 107 nodules per plant, the other isolate from the same location produced significantly ($P<0.05$) fewer nodules (48 nodules per plant).

Table 5. Nitrogen fixation traits of groundnut after inoculation with Sudanese *Rhizobium* isolates

Treat-ment	Isolate	Viable cells added	Nodules	Top dry weight	Root fresh weight
		No. ml^{-1}	No. $plant^{-1}$	g $plant^{-1}$	
1	Green gram	$1.7x10^6$	54.0def[a]	0.62ab	1.58ab
2	Pigeon pea	$9.8x10^6$	5.0b	0.53ab	2.25ab
3	Cowpea	$1.9x10^7$	62.3cde	0.40b	1.40ab
4	Lubia	$1.5x10^7$	31.3efg	0.57ab	1.60ab
5	Bambara groundnut	$8.9x10^6$	93.3bcd	0.68ab	1.28ab
6	Groundnut (Wad Medani)	$1.0x10^7$	161.3a	0.61ab	1.77ab
7	Groundnut (Sennar)	$3.36x10^7$	36.0efg	0.42b	1.50ab
8	Groundnut (Sennar)	$1.82x10^7$	19.0fg	0.41b	1.28ab
9	Groundnut (Kazgail)	$1.0x10^7$	48.3ef	0.46ab	1.17ab
10	Groundnut (Kazgail)	$1.22x10^7$	107.0b	0.67ab	1.28ab
11	Groundnut (Kadugli)	$3.8x10^6$	109.7b	0.82a	1.43ab
12	8A11	$1.0x10^6$	95.0bc	0.60ab	1.80ab
13	Uninoculated control	--	0.0g	0.58ab	2.50a
14	Nitrogen control	--	1.0g	0.81a	1.88ab

[a]Values represent an average of four replications; means within the same column having a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

Based on these nodulation results, it is justifiable to classify all the tested rhizobia as belonging to the cowpea group (Fred et al., 1932).

Nitrogen Fixation

Nodulation and top dry weights of groundnuts have been shown to be positively correlated to Rhizobium efficiency in nitrogen fixation (Wynne et al., 1980). The isolated rhizobia in this study varied significantly in the number of nodules and top dry weights produced (Table 5). The groundnut isolate from Wad Medani (trt 6) produced significantly higher nodule numbers than the other isolates, including the standard strain 8A11, but most of the nodules in this treatment had white interiors and approximately 90% were small and located on lateral roots. These traits suggest inefficiency in nitrogen fixation and agreed with the observations made by Hadad et al. (1982). Other groundnut isolates that resulted in high nodulation included Kazgail (trt 10) and Kadugli (trt 11). The nodules of these isolates were mostly located on the main root, had red interiors, and gave good top dry weights. Differences due to isolates in top dry weights and root fresh weights generally were small in this relatively short study. The large groundnut cotyledons were not removed and they presumably supplied some of the needed nitrogen.

Isolates from legumes other than groundnuts generally performed poorly with the groundnut cultivar. The only exception was the isolate from bambara groundnut, which enhanced both nodulation and top dry

weights. Bambara groundnut (Voandzeia subterranea) is a legume native to the rain-fed areas in Western Sudan where 'Barberton' is commonly grown. This isolate could be characterized as an efficient strain with the 'Barberton' cultivar.

Serological Identity of the Isolates

Several of the groundnut rhizobia reacted with antisera produced from isolates from Wad Medani, Kazgail, and the cowpea isolate (Table 6). An isolate from Kadugli and an isolate from Kazgail failed to react with any of the tested antisera. One isolate from Sennar failed to react with antisera from the Kazgail isolate and cowpea isolate, but agglutinated well with the antiserum from Wad Medani. Antigenically different strains therefore existed at different sites. This is similar to the findings of Damirgi et al. (1967), who studied soybean rhizobia from different locations in Iowa.

Agglutination reactions with isolates from other legumes indicated that only the isolates from bambara groundnut and lubia were serologically distinct, since they failed to react with antisera from groundnut (Wad Medani and Kazgail) or from cowpea (Table 6). The isolate from green gram failed to react with the antiserum from the Wad Medani isolate, and the pigeon pea isolate did not react with the antiserum from the Kazgail isolate.

This is an initial attempt in characterizing Sudanese isolates. Groundnut-nodulating rhizobia obviously vary at different locations and alternate hosts are capable of providing rhizobia that can nodulate.

Table 6. Serological cross reactivity of rhizobia isolated from different legumes and different locations in Sudan

Antiserum	Antigen										
	Groundnut (Wad Medani)	Groundnut (Kazgail)	Groundnut (Kazgail)	Groundnut (Sennar)	Groundnut (Sennar)	Groundnut (Kadugli)	Cowpea	Green gram	Pigeon pea	Lubia	Bambara Groundnut
Groundnut (Wad Medani)	+4[a]	+1	-	+4	+2	-	+4	-	+1	-	-
Groundnut (Kazgail)	+4	+4	-	+2	-	-	+2	+4	-	-	-
Cowpea	+4	+2	-	+2	-	-	+4	+4	+4	-	-

[a]Reactivity was evaluated by agglutination with +4 = strong agglutination, +3, +2, +1 = partial agglutination, and - = negative agglutination.

groundnut. Alternate-host rhizobia do not appear to be as infective as groundnut rhizobia. Work is needed to further classify the Sudanese rhizobia into various serogroups and to characterize serogroup distributions.

SUMMARY AND CONCLUSIONS

Sudan is the fourth largest exporter of groundnuts in the world, yet little is known concerning the plant-rhizobia symbiosis. This study reports on the abundance of groundnut-nodulating rhizobia in the soils of Sudan as related to soil properties and the duration since groundnuts were last planted, and the physiological, serological, and nitrogen-fixing characteristics of Sudanese rhizobia. Thirty-two sites were sampled; all but one contained greater than 3.0×10^2 rhizobia g^{-1} soil capable of forming nodules on sirratro (*Macroptilium atropupureum*). Several of these soils had never been planted to groundnut. A correlation matrix indicated no relationship was present between soil rhizobial populations and the time since groundnuts were last planted in the rotation. Individual isolates of *Rhizobium* from six legumes: groundnuts (*Arachis hypogaea*), green gram (*Phaseolus aureus*), lubia (*Dolichos lablab*), cowpea (*Vigna ungiculata*), pigeon pea (*Cajanus cajan*), and bambara groundnut (*Voandzeia subterranea*) were obtained from four locations in Sudan. The isolates were aseptically added to surface-sterilized seeds of each legume grown in sterile vermiculite and the ability to form nodules was determined. All the plant species were nodulated by each of the isolates. All isolates grew in 0.1% NaCl amended media, but growth was variable in 2.0% amended media. Most, but not all, isolates grew after exposure to moist heat; exposure was for 15 min. at 50°C. Optimum pH for growth was, in general, between pH 6 and 8. Agglutination reactions indicated that isolates from

groundnuts, as well as isolates from other legumes, belonged to several serological groupings. Some of the isolates were found to form a large number of nodules on a Sudanese groundnut cultivar while other isolates formed only few nodules.

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PART IV

NITROGEN FIXING EFFICIENCY AND COMPETITIVENESS OF THREE SEROLOGICALLY DISTINCT GROUNDNUT (PEANUT)-NODULATING RHIZOBIA

INTRODUCTION

In many developing countries, especially in Africa, groundnut (*Arachis hypogaea* L.) is an important protein source in the diet of the local inhabitants. The groundnut plant relies upon its symbiotic association with root-nodule bacteria for nitrogen fixation. Several workers have demonstrated differences in nitrogen fixation efficiency of groundnut rhizobia (Graham and Donawa, 1981; Weaver, 1974; Wynne et al., 1980), but little is known concerning differences in the competitive ability among strains. Further, very little is known about the groundnut-nodulating rhizobia of the tropics. In one study, isolates of *Rhizobium phaseoli* from tropical soils were found to be more beneficial for use as inoculants than exotic strains and resulted in improved yields of common beans (Elnadi et al., 1971). This suggests that local isolates should be screened for infectivity and efficiency before introduction of alien strains.

The role of the host plant in the symbiosis is well-documented (Vincent and Walters, 1953; Caldwell and Vest, 1968). Wynne et al. (1980) also reported a significant host genotype by strain interaction for several nitrogen-fixing traits with groundnut. The effect of the environment is less well-documented and little is known concerning environmental influences on the competition among strains to occupy nodule sites. Graham and Donawa (1981) showed with groundnut that increasing the pH of an acid soil from 4.6 to 6.5 increased the percentage of inoculant recovery in nodules, but further increasing the pH to 7.1

reduced the percentage recovery below that found at pH 4.6.

Competition among Rhizobium strains to occupy nodule sites may be the determining factor in the success of inoculation practices, especially if the soil contains indigenous rhizobia. Host genotype (May and Bohlool, 1983), Rhizobium strains (Trinick, 1982), and pH (Graham and Donawa, 1981; Weaver et al., 1972) all may influence the outcome of competition studies. The use of ultra high inoculum rates, suggested by Kapusta and Rouwenhorst (1973), also may influence nodule occupancy by the competing strains.

For identification of competing strains, Nicol and Thorton (1941) utilized differences in colony morphology of rhizobia growing on yeast extract mannitol (YEM) agar to determine nodule occupancy in inoculated peas. If the competing strains are serologically distinct, however, one of the most commonly used techniques for strain identification is agglutination reactions (Vincent, 1970). Agglutination of Rhizobium japonicum with specific antisera can be made by direct reaction with nodule juices (Means et al., 1964). Unfortunately with groundnut nodules, we found that the isolates must first be streaked on YEMA before serological identity can be determined (unpublished observations). If colony morphology can be used for identification of groundnut rhizobia, considerable time may be saved in determining nodule occupancy. Also, based on previous work in our laboratory, there is considerable cross reactivity between isolates of groundnut rhizobia, making identification via agglutination reactions tedious.

The effects of cultivar, Rhizobium strain, pH, and the rate of

inoculation on nitrogen-fixing traits and competitive nodulation were investigated under greenhouse conditions. The study used native Sudanese groundnut cultivars, native and commercial *Rhizobium* strains, and two pH levels that are characteristic of Sudanese soils. Determination of nodule occupancy was based on colony morphology of rhizobial isolates, which was verified by serological reactions.

MATERIALS AND METHODS

Strains

Three serologically distinct groundnut-nodulating Rhizobium strains were evaluated. The Wad Medani strain was isolated from a clay soil in Central Sudan and the Kadugli strain from a clay soil in Western Sudan. The commercial strain, TAL 309, was supplied by Dr. Wacek, North American Plant Breeders, Princeton, Illinois.

Cells were grown in yeast extract mannitol (YEM) broth for six days at 28°C on a waterbath shaker. Cell counts were made by using a counting chamber and a phase contrast microscope.

Cultivars

Three Sudanese groundnut cultivars were used in this study: 'Ashford' and 'MH383' (both Virginia type), and 'Barberton' (a Spanish type). The seeds were obtained from the Agronomy Section, Agricultural Research Corporation, Wad Medani, Sudan.

Strain-Cultivar Interaction

The competitive ability and nitrogen-fixing efficiency of the strains were evaluated in combination with three Sudanese groundnut cultivars (Study I). The strains were added singly and in mixtures (Table 1). The mixtures were prepared by adding an equal volume (0.5 ml) of two broth cultures. Because the cell counts were not identical, the resulting ratios of added inocula varied from 1.6:1 to 10:1 (Table 1). Both the uninoculated and the nitrogen controls received no

Table 1. Inoculants used in the evaluation of efficiency and competitive ability of groundnut *Rhizobium* in the greenhouse

Study I[a]			Study II[b]		
Trt. No.	Strain	Inoculant size	Trt. No.	Strain	Inoculant size
		No. ml^{-1}			No. ml^{-1}
1	Wad Medani (W.M.)	2.0×10^8	1	Wad Medani (W.M.)	2.0×10^4
2	Kadugli (K.)	1.25×10^8	2	Kadugli (K.)	1.25×10^4
3	TAL 309 (T)	2.0×10^7	3	Kadugli (K.)	1.25×10^8
4	W.M./K. mixture	$1.0 \times 10^8/6.3 \times 10^7$ (1.6:1)	4	W.M./K. mixture	$1.0 \times 10^4/6.3 \times 10^7$ ($1:6.3 \times 10^3$)
5	W.M./T. mixture	$1.0 \times 10^8/1.0 \times 10^7$ (10:1)	5	Uninoc. control	None
6	K./T. mixture	$6.3 \times 10^7/1.0 \times 10^7$ (6.3:1)			
7	Uninoc. control	None			
8	Nitrogen control[c]	None			

[a]Inoculants applied to Sudanese cultivars 'Ashford', 'Barberton', and 'MH383' in a greenhouse study. Plants were grown in a sand-vermiculite mixture (pH 6.5) for seven weeks. Numbers in parentheses are the ratios of applied cells in mixed inoculants.

[b]Inoculants applied to the Sudanese cultivar 'Ashford' in a greenhouse study. Plants were grown in a sand-vermiculite mixture adjusted to pH 6.5 or 8.0 for seven weeks. Number in parentheses is the ratio of applied cells in the mixed inoculant.

[c]Nitrogen added as 10 ml of 8 μg ml^{-1} NH_4NO_3 solution three times during the growth period.

rhizobial additions, but the nitrogen controls did receive 10 ml of 8 $\mu g\ ml^{-1}$ NH_4NO_3 solution three times during the seven-week growth period.

Seeds were surface sterilized by immersion in 30% H_2O_2 for 15 min. followed by five rinses in sterile water (Vincent, 1970), pre-germinated, and planted into pots containing a sterile sand:vermiculite mixture (1:1 by volume). The mixture initially received 100 ml of a complete nutrient solution lacking nitrogen. Each treatment was replicated four times, and the pots were positioned in the greenhouse in a completely randomized design.

At harvest, seven weeks from sowing, plant color was rated using 4 for dark green, 3 and 2 for intermediate colors, and 1 for yellow. The plant shoots were then dried at 72°C for two days and dry weights determined. Nitrogenase activity was measured by the acetylene reduction assay (Hardy et al., 1968). Nodulation was assessed and five nodules were randomly sampled from each plant for determination of the nodulation percentage by the competing strains. Colony morphology and agglutination reactions were used for occupant identification.

pH Effect

The soils of Western Sudan have a neutral to slightly acid pH, while the soils in Central Sudan characteristically contain free $CaCO_3$ and pHs are between 8.0 and 8.5 (Hadad and Loynachan, Agronomy Department, I.S.U., unpublished data). In a separate study (Study II), sand:vermiculite mixtures (1:1 by volume) were adjusted with $CaCO_3$ additions

RESULTS AND DISCUSSION

Wynne et al. (1980) reported that nodulation, plant color, tissue weight, and nitrogenase activity were statistically correlated with each other as nitrogen-fixing traits in greenhouse-grown groundnuts. Data in Table 2 summarize the significant ($P<0.05$) correlation coefficients found in these studies. In general, the results are in agreement with those of Wynne et al. (1980). In the second study, we further subdivided the nodules into lateral and main-root nodules. Main-root nodulation and nodule weights were significantly correlated ($P<0.05$) with nodule activity (C_2H_2 reduced). Several parameters, therefore, apparently can serve as reliable indicators in the screening of rhizobia for efficiency under greenhouse conditions.

Cultivar-Strain Interaction

No statistical differences were found in any nitrogen-fixing traits other than nodulation among the single-strain additions in Study I (Table 3). The isolate from Wad Medani (W.M.) significantly ($P<0.05$) improved nodulation of these cultivars without obvious benefit to the plant, which may imply inefficient nodulation. Strain TAL 309 (T) gave the lowest nodulation but the majority of the nodules with TAL 309 were large and located on the main root. Tissue weights and nodule activity also tended to improve following inoculation with strain TAL 309. The host genotype apparently had little influence on strain efficiency, since consistent results were obtained with the three tested cultivars.

to pH levels of 6.5 and 8.0 (Table 1). The $CaCO_3$ was thoroughly mixed with the sand and vermiculite before placement in pots. Only the two Sudanese rhizobial isolates were evaluated on the Sudanese cultivar 'Ashford'. The first two treatments represented levels of rhizobia found g^{-1} soil in the field (Hadad and Loynachan, Agronomy Department, I.S.U., unpublished data), the third treatment was a high level of inoculation comparable to levels of rhizobia g^{-1} of inoculant, and the fourth treatment was a mixture of strains representing a more competitive strain (Wad Medani strain) at levels found g^{-1} soil and a less competitive strain (Kadugli strain) at levels found g^{-1} inoculant.

Statistical Analysis

A pooled correlation matrix was constructed to evaluate the relationship between the measured nitrogen-fixing traits in both studies. Treatment differences within studies were evaluated using Duncan's Multiple Range Tests.

Table 2. Significant correlation coefficients (r) between nitrogen-fixing traits for combined studies I and II

	Top dry weight	C_2H_2 reduced	Total nodulation	Main-root nodulation	Lateral-root nodulation	Main-root nodule weight
Color	0.40*** (0.61)[c]***	NS[a] (0.24)	NS (NS)	ND[b] (NS)	ND (NS)	ND (NS)
Top dry weight		0.63*** (0.53)***	0.35*** (0.42)**	ND (NS)	ND (0.41)**	ND (0.48)**
C_2H_2 reduced			0.29** (0.39)**	ND (0.58)***	ND (0.28)*	ND (0.74)***
Total nodulation				ND (0.36)**	ND (0.98)***	ND (0.39)**
Main-root nodulation					ND (NS)	ND (0.84)***
Lateral-root nodulation						ND (NS)

[a]NS - Not significant at the 0.05 level of probability.

[b]ND - Not determined; the subdivision of nodules into main and lateral-root nodules was only conducted for the second study.

[c]Bottom values in parentheses represent the correlation coefficients obtained with the second study.

*,**,***Significant at the 0.05, 0.01, and 0.001 levels of probability, respectively.

Table 3. Nitrogen-fixing traits as influenced by single-strain *Rhizobium* additions to three Sudanese groundnut cultivars (Study I)

Trt. No.[a]	Strain[a]	Plant growth: Fresh top wt.	Plant growth: Dry top wt.	Plant growth: Color rating	Nitrogenase activity	Total nodulation
		g plant^{-1}			μmol plant^{-1} h^{-1}	No. plant^{-1}
			Ashford			
1	W.M.	3.58a[b]	0.63ab	2.50bc	0.352a	103.0b
2	K.	3.59a	0.60ab	3.13ab	0.362a	69.0c
3	T.	3.93a	0.65ab	2.63bc	0.424a	56.0c
7	Uninoc. control	3.41a	0.57ab	2.00c	0.190b	0.0d
8	Nitrogen control	4.19a	0.73a	3.75a	0.123b	0.0d
			MH383			
1	W.M.	3.10a	0.53a	3.13a	0.286a	71.0ab
2	K.	3.04a	0.51a	3.25a	0.321a	62.0bc
3	T.	3.14a	0.55a	2.67a	0.331a	28.0cd
7	Uninoc. control	2.57a	0.44a	2.83a	0.0b	0.0d
8	Nitrogen control	3.69a	0.70a	3.67a	0.0b	0.0d
			Barberton			
1	W.M.	2.36b	0.40b	1.63c	0.237ab	70.0b
2	K.	2.71b	0.45b	2.00bc	0.254ab	35.0c
3	T.	2.60b	0.44b	2.25bc	0.286ab	34.0c
7	Uninoc. control	2.13b	0.39b	2.13bc	0.0c	0.0d
8	Nitrogen control	3.62a	0.66a	3.38a	0.0c	0.0d

[a]Treatments discussed in Table 1.

[b]Values are averages of four replications; means within the same column for each cultivar followed by a common letter (both Tables 3 and 5) are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

Similarly, changing the pH from 6.5 to 8.0 had little influence on the nitrogen-fixing efficiency of the two Sudanese strains in Study II (Table 4). The main differences with pH change involved lateral-root nodules. Fewer lateral-root nodules were consistently found at the higher pH. This may reflect more rapid die off of rhizobia at pH 8.0; thus, fewer organisms were present during the formation of lateral-root nodules. The pH range used in this study, although reflective of pHs in Sudanese soils, may have been too narrow to cause major differences in nitrogen-fixing traits.

Increasing the inoculant size of the Kadugli strain 10^4 (treatments 2 vs. 3) did affect nodulation (Table 4). Numbers of lateral-root nodules of the 'Ashford' cultivar were significantly ($P<0.05$) reduced, while the main-root nodules and nodule weights increased. The higher inoculation rate and the early establishment of main-root nodules may have interfered with lateral-root nodule initiation. There was no apparent interaction between pH and inoculation rate.

Competition for Nodule Sites

The percentage of nodules occupied by each strain in Study I and the ratio of rhizobia applied and found are detailed in Table 5. When the Wad Medani (W.M.) or TAL 309 (T) strain was added singly, 100% of the nodules were occupied by the applied strain. Since antiserum was not prepared for the strain from Kadugli (K), negative reactions obtained with the treatments receiving either TAL 309 or the Wad Medani strain in combination with the Kadugli strain were considered as occupancy by the

Table 4. Influence of pH and inoculum size on nitrogen-fixing traits of the Sudanese cultivar 'Ashford' (Study II)

Trt. No.[a]	Strain[a]	pH	Plant growth			Nodulation				Nitrogenase activity
			Tissue dry wt.	Root dry wt.	Color rating	Total	Main root	Lateral root	MR wt.[b]	
				g plant^{-1}			No. plant^{-1}	No. plant^{-1}	g plant^{-1}	μmol plant^{-1} hr^{-1}
1	W.M.	6.5	0.44a[c]	0.20a	3.17a	98.0c	9.3cd	88.7d	0.0218cd	0.028cd
	W.M.	8.0	0.39a	0.19a	2.86a	76.4e	5.7de	70.7e	0.0100d	0.003d
2	K.	6.5	0.45a	0.19a	3.00a	111.0b	8.5de	102.5b	0.0190cd	0.080bcd
	K.	8.0	0.49a	0.23a	3.24a	65.0f	11.7bcd	53.3g	0.0284cd	0.210abc
3	K.	6.5	0.53a	0.19a	3.86a	111.0b	19.7ab	91.3c	0.0640ab	0.276ab
	K.	8.0	0.54a	0.24a	3.93a	56.4g	17.7abc	38.7i	0.0678ab	0.313a
4	W.M./K.	6.5	0.35a	0.20a	2.67a	90.0b	24.0a	66.0f	0.0955a	0.207bc
	W.M./K.	8.0	0.50a	0.19a	3.33a	72.0e	23.0a	49.0h	0.0896a	0.212abc
5	Uninoc. control	6.5	0.39a	0.19a	2.67a	0.0	0.0	0.0	0.0	0.0
	Uninoc. control	8.0	0.43a	0.24a	3.00a	0.0	0.0	0.0	0.0	0.0

[a]Treatments discussed in Table 1.

[b]Main-root nodule dry weight.

[c]Values are averages of three replications; means within the same column followed by a common letter are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

Table 5. Influence of host genotype on the competitive nodulation of three Sudanese groundnut cultivars (Study I)

Trt.[a] No.	Strain[a]	Total nodulation	Nitrogenase activity	Percentage occupancy			Occupancy ratio	
				W.M.	T.	K.	Applied	Found
		No. plant^{-1}	µmol plant^{-1} hr^{-1}	%				
				Ashford				
4	W.M./K.	63.0c[b]	0.404a	86.7±6.6[c]	0.0	13.3±6.6	1.6:1	6.7:1
5	W.M./T.	132.0a	0.349a	56.3±6.3	43.75±6.3	0.0	10.0:1	1.3:1
6	K./T.	58.0c	0.383a	0.0	73.3±14.7	26.7±14.7	6.3:1	0.4:1
				MH383				
4	W.M./K.	78.0ab	0.333a	92.3±6.6	0.0	7.7±6.6	1.6:1	12.0:1
5	W.M./T.	114.0a	0.306a	55.6±7.4	44.4±7.4	0.0	10.0:1	1.3:1
6	K./T.	83.0ab	0.373a	0.0	90.9±22.5	9.5±22.5	6.3:1	0.1:1
				Barberton				
4	W.M./K.[d]	49.0bc	0.310a	66.7±9.3	0.0	26.7±9.3	1.6:1	2.5:1
5	W.M./T.	98.0a	0.245ab	61.5±6.9	38.5±6.9	0.0	10.0:1	1.6:1
6	K./T.	40.0c	0.217b	0.0	45.5±6.3	54.5±6.3	6.3:1	1.2:1

[a]Treatments discussed in Table 1.

[b]Values are averages of four replications; means within the same column for each cultivar followed by a common letter (both Tables 3 and 5) are not significantly different at the 0.05 level of probability by the Duncan's Multiple Range Test.

[c]Means ± S.E.

[d]Mixed infections accounted for 6.6%.

Kadugli strain.

Strain TAL 309 appeared to be quite competitive when compared with the Sudanese strains on all three cultivars. The ratio of applied cells with Wad Medani/TAL 309 was 10:1 but the ratios of nodule occupants was 1.3 to 1.6:1 (Table 5). If one calculates the competitive index as the ratio of cells applied to the ratio of strains found in nodules, TAL 309 was seven times more competitive than the Wad Medani strain. In comparison with the Kadugli strain, TAL 309 averaged eleven times more competitive. Of the two Sudanese strains, the Wad Medani strain was more competitive than the Kadugli strain. When applied at a ratio of 1.6:1 (W.M./K.), the Wad Medani strain occupied 87, 92, and 67% of the nodules on cultivars 'Ashford', 'Barberton', and 'MH383', respectively. This gave the Wad Medani strain an average competitive index of 4.4 compared with the Kadugli strain. The competitive ratings were therefore TAL 309 > Wad Medani > Kadugli. Relatively consistent occupancy data were obtained with all three cultivars. Under field conditions when strain TAL 309 was applied to groundnuts in a Sudanese soil populated with the Wad Medani strain, the strain was fairly competitive and occupied 30 and 40% of the main and lateral-root nodules, respectively (Hadad and Loynachan, Agronomy Department, I.S.U., unpublished data).

Since data from Study I suggested that the Wad Medani strain was more competitive than the Kadugli strain, a treatment was considered in Study II (Table 1) where the more competitive strain (Wad Medani) was added at levels found g^{-1} soil and the less competitive strain (Kadugli) was added at levels found g^{-1} inoculant. Further, the Wad Medani

strain was originally isolated from an alkaline soil and the Kadugli strain from a slightly acid soil. Regardless of pH of the medium, the Kadugli strain formed all nodules that were typed. The use of ultra high inoculum rates, as suggested by Kapusta and Rouwenhorst (1975), appears promising as an effective means of replacing inefficient strains and improving the competitive ability of less-competitive strains of groundnut rhizobia. More work is needed to quantify the inoculum size needed with the Kadugli strain to compete successfully with the Wad Medani strain under actual field conditions.

Strain Identification

Morphological identification of nodule occupants by observing growth characteristics would greatly aid in reducing the tedious task of serological identification. Furthermore, as often observed with groundnut rhizobia, if the competing strains cross react, serological reactions cannot be successfully used for strain identification.

The Rhizobium strains tested in this study varied considerably in their growth characteristics when isolated from nodules and grown on YEMA containing 25 μg ml^{-1} bromthymol blue as a pH indicator. Time of appearance, colony shape, size, elevation, and absorption of the bromthymol blue varied among the strains (Figure 1, Table 6). Absorption of the bromthymol blue, most evident by the Kadugli strain, resulted in a decided yellowishness of the colony. In fact, the Kadugli and Wad Medani strains could easily be separated based solely on color.

Identification of the isolates based on colony morphology was

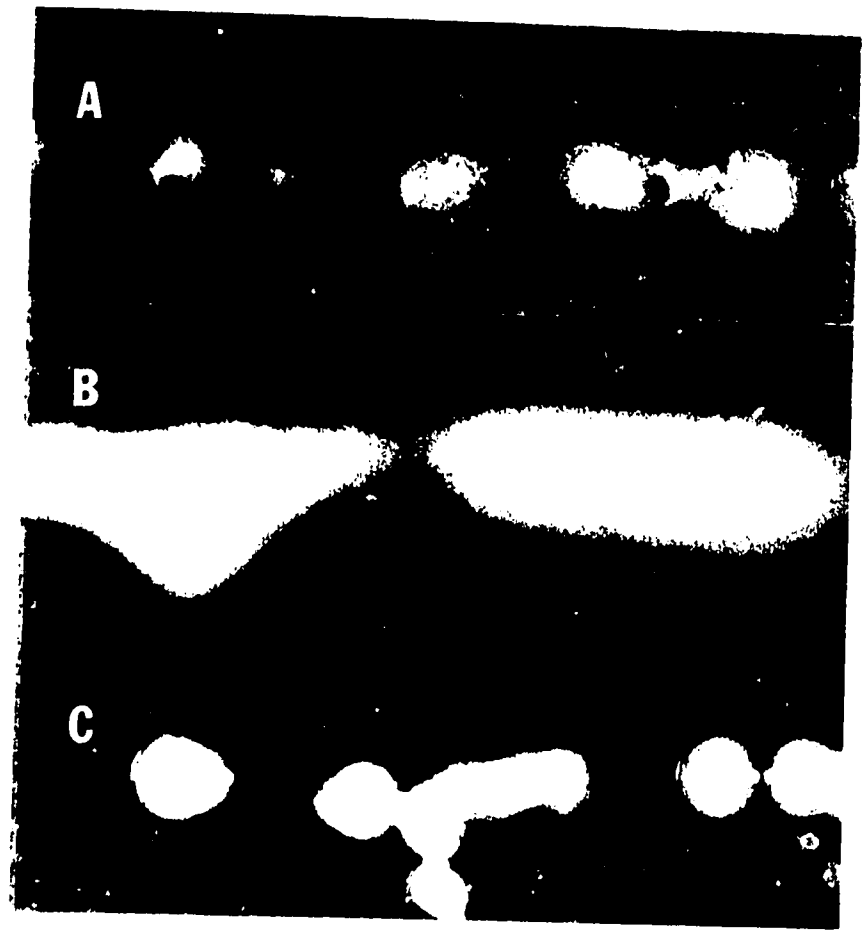

Fig. . Colonial morphology of Kadugli (A), TAL 309 (B), and Wad Medani (C) Rhizobium strains (magnification 6X)

Table 5. Morphological characteristics of the *Rhizobium* isolates used in the competition study

Rhizobium strain	Time for appearance on YEMA	Colony size at day[a]			Colony margin	Colony elevation	BTB[b] absorption	Polysaccharide secretion
		8	12	16				
	days	mm						
Kadugli	12	--	<1.0	1.5	Entire	Raised	+4	Slight
TAL309	8	<1.0	1.0	>3.0	Irregular	Flat	+1	Abundant
Wad Medani	10	--	<1.0	2.0	Entire	Convex	+2	Slight

[a]Growth was at 28°C on yeast extract mannitol agar amended with 25 μg ml^{-1} bromthymol blue (BTB) as a pH indicator.

[b]All isolates were alkaline producers; BTB rating was +4 for maximum absorption, +2 for moderate absorption, and +1 for slight absorption.

verified by using serological techniques (agglutination reaction). In all instances, positive agreement was obtained. This suggests that colony morphology, at least with selected strains, could be used as a means of rapidly screening nodule occupants with groundnut rhizobia.

SUMMARY AND CONCLUSIONS

In order to improve symbiotic nitrogen fixation, strains of rhizobia must be found that are both efficient and competitive. This study evaluated three serologically distinct cultures of *Rhizobium*, one commercial strain (TAL 309) and two Sudanese isolates, in greenhouse studies for efficiency and competitiveness on three groundnut (*Arachis hypogaea* L.) cultivars. The commercial strain, TAL 309, was more efficient in nitrogen fixation than either of the Sudanese strains, which were identified as the Wad Medani strain and Kadugli strain. The host genotype little influenced the nitrogen-fixing efficiency of the strains tested. A pooled correlation matrix indicated that plant color at harvest, acetylene reduction, and the total number of nodules were positively correlated with shoot dry weights. In addition to being serologically distinct, the strains varied in colony morphology when grown on yeast extract mannitol (YEM) agar. Both agglutination reactions and colony appearance were used for the identification of nodule occupants.

Whenever the TAL 309 strain was included in inoculum mixtures, it consistently occupied the majority of nodules with all three groundnut cultivars. A calculated competitive index revealed that TAL 309 was seven and eleven times more competitive than the Sudanese strains, Wad Medani and Kadugli, respectively. Between Sudanese strains, Wad Medani was four times more competitive than the Kadugli strain. No cultivar x strain interaction was present. In a separate study, the effect of pH and inoculum size was evaluated by using the Sudanese strains and the

groundnut cultivar 'Ashford'. Varying the pH from 6.5 to 8.0 had no influence,on the relative competitive ability of strains. Even though the Wad Medani strain was more competitive, increasing the inoculum size of the Kadugli strain to 10^4 times that of the Wad Medani strain completely eliminated the Wad Medani strain from occupying main-root nodules. This suggests that high inoculation rates of groundnut rhizobia could be used to replace inefficient strains.

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PERSONNEL INVOLVED

A. Agricultural Research Corporation, Wad Medani, Sudan.

Many individuals from the Agricultural Research Corporation were actively involved in the project. Dr. Musa M. Musa, a trained soil microbiologist, and the Deputy Director General of the Agricultural Research Corporation was tremendously helpful in setting up the cooperative agreement and in conducting the first year of study. He also was of tremendous assistance in organizing the collection of soils from remote sites of Sudan during the initial survey.

Dr. Nuri Osman Mukhtar took over Dr. Musa's responsibility in the soil microbiology laboratory in 1981 after Dr. Musa moved to the Arab Agricultural Organization in Khartoum. Dr. Nuri was very helpful in supervising preparation of the field plots, maintenance of the field plots, isolation of rhizobia, and coordination of the Sudanese efforts with the project.

Mr. Abdalla Hamdown, a pesticide specialist, helped in the first year of the study in establishing the herbicide and fungicide plots. He also was available for counsultation concerning the research.

Dr. Hassan M. Ishag, the Chief Agronomist in the Agricultural Research Corporation, helped in plot sampling and sample analyses.

Dr. Ali Taha Ayob, the Head of the Soil Science Section, helped by directing the station inspector and technicians involved in routine laboratory analyses to give priority to the project's work.

Dr. Hassan Hag Abdalla, the Director of the Soil Survey Department, also helped by conducting some of the analyses of the soil samples brought

in from the survey locations.

Dr. Fathi Ahmed Kahlifa was our regional coordinator in southcentral Sudan at the Abu Naama Research Station. He helped by establishing survey plots with inoculation under irrigation and rain-fed conditions.

Mr. Faroug Elhadi was our cooperator in Western Sudan at the El-Obeid Research Station. With his help, two farmers in the area evaluated the inoculants under rain-fed conditions.

Dr. Mohamed Bekheit, Agricultural Research Corporation Director, was of tremendous aid in making logistical arrangements for visits to various locations of the country. Also, we very much appreciated his interests in following the progress of the project.

Dr. D. A. Dafalla, Western Sudan Agricultural Research Corporation Director, and Dr. Jim Riley, Western Sudan Agricultural Research Project Advisor to the Director General, both from Khartoum, were of great assistance in arranging accommodations and transportation to Western Sudan for collection of samples and for plot establishment.

Finally, we would like to thank the AID Office at Khartoum and especially the Agricultural Officer, most recently Mr. Witt, who was of assistance in coordinating our research efforts within the country.

B. Iowa State University, Ames, Iowa.

The major contributor to the project from Iowa State University was Dr. Mohamad Hadad, a Sudanese graduate student, who completed both his M.S. and Ph.D. degrees while working on this project. Dr. Hadad completed his studies under the direction of Dr. Thomas Loynachan, Principal Investigator of the project.

During the first year of the study, help was received from Dr. David Staniforth in arranging and establishing the pesticide studies. He

was available for consultation at other times as well.

C. Others.

Several others should be mentioned as having an important part in the successful completion of the project. Drs. Miller and Elkan were USAID Program Managers, Dr. Frederick was USAID techincal advisor, Drs. Peterson, Kremmer, Burton, Smith, and Wacek assisted in technical advise and/or providing strains and inoculants. Finally, the field evaluation would not have been possible without the gracious assistance of the Nitragin Company, who provided the granular inoculant for each of the three years of field evaluation.

FINANCIAL SUMMARY

January 1980 - January 1984

Expense	Source of Funds USAID	ISU	ARC	Total
Principal and co-investigators	-	14,500	10,280	24,780
Employee benefits (19.3%)	-	2,799	-	2,799
Research Assistantship	-	-	39,600	39,600
Hourly Labor				
ISU	9,500	-	-	9,500
ARC	1,800	-	-	1,800
Field Support (ARC)	19,500	-	-	19,500
Travel and Subsistence*	27,500	-	-	27,500
Expendable supplies				
ISU	7,650			7,650
ARC	1,850			1,850
Computer Time (ISU)	1,200	-	-	1,200
Indirect costs (37% of salaries and wages)	-	20,017**	-	20,017
TOTAL	69,000	37,316	49,880	156,196

*Includes travel expenses between countries and within each country for samplings, meetings, etc.

**Includes indirect costs of salaried individuals at ISU (37% of $14,500 + $39,600).

IMPACT AND CONCLUSIONS

These studies represent the first reports on the practice of inoculation of groundnut in Sudan. Perhaps, the most direct benefit coming from the project is the education of a young Sudanese scientist, Dr. Hadad, in the very practical problems involving nitrogen fixation within his country. Certainly many questions remain unanswered, but a groundwork and basis for future investigation have been established.

The presence of high numbers of *Rhizobium* in virgin soils, or soils never planted to groundnut, indicates that other plants native to the surveyed locations are presumably serving as hosts for groundnut-nodulating rhizobia. The survival of such rhizobia in the soils in Western Sudan, however, especially with the large potential for expansion of groundnut production in Western Sudan, merits further investigation since one location contained fewer than 3.0×10^2 rhizobia g^{-1} of soil. The dominant vegetation of the sample area is Western Sudan was gum arabic trees (*Acacia senegal*) and, according to previous reports, gum arabic trees do not host groundnut rhizobia.

The rhizobia of Sudan apparently have survival mechanisms for living as free-living organisms in the hot, dry months that precede the rainy season. Possibly attachment to clay or sand particles, or another unknown mechanism of survival is important and future work is needed along these lines. The importance of montmorillinitic clay should be investigated.

Many of the strains that cross reacted serologically with the cowpea isolates were found to share at least one antigenic site. Serological variability within the strains was found among different sites of the same soil type. Characterization of Sudanese rhizobia into serogroups and their distribution with soil types need further study.

The rhizobial population needed for maximum nodulation depends, among other factors, on the method of inoculation. The soil conditions in Sudan

during groundnut planting are usually adverse because rhizobia are exposed to desiccation and high soil temperatures. Further work on methods to protect the sensitive rhizobia, either by inoculation methods or carriers, is warranted.

Very little is known concerning the general biology of Sudanese soils. The presence of inhibiting or stimulating organisms in the rhizosphere and their effect on rhizobial strains should be examined.

The finding that the Kadugli strain from Western Sudan is more efficient in nitrogen fixation than strains isolated from Central Sudan is important. Certainly, further work with this strain deserves consideration.

The finding that peanut rhizobia, at least a few, can be quickly identified morphologically will greatly assist in evaluation of competition studies. This avoids the tedious work involved with serology and antibiotic markers, especially important with the serological cross reactivity of the cowpea group. These strains may have merit for use in competition studies in other developing countries where sophisticated equipment for isolate identification is not readily available.

In summary, several important questions concerning the nitrogen fixation efficacy of Sudanese rhizobia on groundnut have been answered in these studies, but important questions remain unanswered. The studies were successful in meeting many of the initial objectives outlined when the project began. As the project developed, other questions and other areas of direction equally as important as the original objectives became apparent. The study of *Rhizobium* ecology in soil is obviously a very complicated undertaking. Even in developed countries on major crops, such as with soybean production in Iowa, much remains unknown concerning why one strain is more competitive and tends to dominate nodule occupancy. Considerable work remains ahead.

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