Host-Bradyrhizobium relationships and nitrogen-fixation in the Bambarra groundnut *[Voandzeia subterranea (L.)* Thouars nom. cons.]

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23 strains of rhizobia, from 14 leguminous species, were evaluated on a Thai cultivar of Voandzeia* in Leonard jar trials. The symbiosis ranged from completely ineffective through moderate effectiveness to fully effective. *Bradyrhizobium sp.* TAL 169, isolated from *Vigna unguiculata*, ranked most-effective; the wide-spectrum *Bradyrhizobium* strain CB 756 was ineffective. In a cultivar x *Bradyrhizobium spp.* trial, the host-genotype significantly (P _ 0.01 or P 0.001) influenced shoot dry weight, shoot N, nodule dry weight and nitrogenase activity. The rhizobial strains were also significant sources of variation for shoot-dry weight (P = 0.05), shoot N (P < 0.001) and nodule dry weight (P < 0.01). There were no significant host x *Rhizobium* interactions, indicating that no specific combination was superior over the broad range of all treatments. Percentage shoot N and specific nitrogenase activity were poor indicators of N_2 fixation capacity in *Voandzeia. Voandzeia* cultivar No. 12 P.P. Rust in combination with *Bradyrhizobium sp.* TAL 169 showed high potential for growth, nodulation and N_2 fixation. This study indicated that *Voandzeia* is specific for its rhizobial requirement for effective symbiosis.

Keywords: Bambarra groundnut; Nitrogen fixation; *Bradyrhizobium*

Genetic factors of the host legume and *Rhizobium* influence nodulation and N_2 fixation. Therefore, in order to realize maximum benefits from N_2 fixation it is necessary to identify legume cultivars, strains of *Rhizobium* or combinations of these symbionts which have superior fixation capacity through the process of strain selection; this is the objective of the agricultural use of legumes.

The bambarra groundut *[Voandzeia subterranea* (L.) Thouars] is one of the most popular pulse legumes in Africa along with cowpeas, groundnuts, pigeon peas and common beans (NAS, 1979) and the third most important after groundnut and cowpea (Rachie and Silvestre, 1977). This crop features in the low-input farming systems of Africa as an intercrop of cereals and root crops, although it is also planted as a monocrop (Doku and Karikari, 1971). Most of the cultivars of the bambarra groundnut that exist today have arisen from casual selection in the course of cultivation and are therefore local in distribution (Hepper, 1970).

The bambarra groundnut has been reported to be nodulated in various geographical areas (Allen and Allen, 1981) but comparatively little attention has been given to its *Rhizobium* affinities or its N_2-fixing capacity in spite of its widespread occurrence and economic importance. The purpose of this study was to elucidate the cross-inoculation characteristics of *Voandzeia* and host-microsymbiont interactions to identify effective strains of rhizobia and superior host genotypes as a prelude to further experimentation with the legume's biological N_2 fixation potential.

Materials and methods

Rhizobia

All rhizobial strains used in this investigation are maintained in the NifTAL Project *Rhizobium* germplasm collection (Halliday and Somasegaran, 1984). The following rhizobial isolates, TAL 366, TAL 367, TAL 387, TAL 455, TAL 514, TAL 526, TAL 402, TAL 436, TAL 452, TAL 495, TAL 499, TAL 504 and TAL 508 were isolated from nodules of naturally nodulated *Voandzeia* plants grown at the Dryland Farming Research Station, Katumani, Kenya. Rhizobial isolates TAL 1552, TAL 1553, TAL 1554 and TAL 1555 were from *Voandzeia* plants grown in Hawaiian soils. Two *Bradyrhizobium spp.* (CB 756, and Nitragin 176A22) widely used in commercially prepared inoculants for `cowpea type' legumes were included in the tests as standard strains. Strains were cultured separately in yeast-mannitol broth (Vincent, 1970) prior to inoculation.

Seeds

Seed germplasm used in this study was obtained from Thailand, Kenya, Ghana and South Africa. The seed samples from Thailand were from an unknown cultivar grown in southern Thailand and were purchased at an open-air market at Bangkhen, Thailand. Cultivars No. 12 P.P. Rust, Swazi Homelands-1 and Swazi Homelands-2 were don-

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Voandzeia subterranea* (L.) Thours should now be regarded as belonging to the genus *Vigna Savi*, and has been renamed *Vigna subterranea* (L.) Verdc., comb. nov. (Kew Bull. **35(3)1980 474**).** The *Vigna* affinity may explain why the inoculation with TAL 169, derived from *V. unguiculata*, was highly effective - Editor
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ated by T.C. Nei, Pretoria, South Africa; TVS 747 (courtesy of E.V. Doku) and TVS 47 (courtesy of L. Ulsaker) were obtained from Ghana and Kenya, respectively.

Plant culture

In all experiments, undamaged seeds were selected for uniform size and surface sterilized in 3% (v/v) hydrogen peroxide for 5 min., rinsed in six changes of sterile water, and left to imbibe for 24 h in a refrigerator. Imbibed seeds were sown in moist, pre-sterilized (autoclaved) vermiculite. Pregerminated seeds with straight radicles (1.0-1.5 cm) were sown and inoculated (Somasegaran and Hoben, 1985) in modified Leonard jars (Vincent, *ibid.)* which used horticultural-grade vermiculite for the rooting medium and a nitrogen-free nutrient solution (Broughton and Dilworth, 1971). Plants were raised in a glasshouse under natural illumination.

Experiment 1

Cross-inoculation relationships were investigated in the unknown Thai cultivar of *Voandzeia* with 23 strains of diverse rhizobia (Table 1). The experiment was a randomized complete block design with noninoculated controls and non-inoculated controls plus 70 ppm N. Each treatment was set up in triplicate with one plant replication^{-1}. The experiment was terminated at 34 days. At harvest, nitrogenase activity was estimated by the acetylene reduction assay (Hardy *et al.*, 1971) as described previously (Somasegaran and Martin, 1986). Shoot and nodule dry weights were determined after oven drying plant material at 70°C for three days.

Experiment 2

Symbiotic variability among indigenous nodule isolates from bambarra groundnut grown in soils in Kenya and Hawaii was investigated in Leonard jars. 12 Kenyan and two Hawaiian rhizobial strains (Table 2) were investigated on cultivar No. 12 P.P. Rust. TAL 169 (Nitragin 176A22) was used as a standard strain based on its effectiveness in Experiment 1. The experiment was a randomized complete block design with four replications and an uninoculated control without nitrogen was included. The experiment was terminated at 42 days. Nitrogenase activity, shoot and nodule dry weights were determined as described in Experiment 1.

Experiment 3

Five cultivars (No. 12 P.P. Rust, TVS 47, TVS 747, Swazi Homelands 1 and Swazi Homelands 2) were evaluated against five strains of *Bradyrhizobium* spp. [TAL 169 (Nitragin 176A22), TAL 387, TAL 368, TAL 309 (CB 756) and TAL 1000] to study host-*Bradyrhizobium* interactions in *Voandzeia.* Plants were raised in Leonard jars. The experiment was a randomized block design with four replications and an uninoculated control without nitrogen was included. Two plants were raised replication^{-1} and at harvest (52 days) nitrogenase activity, shoot and nodule dry weights were determined by the method of Mitchell (1972).

Results

Experiment 1

The *Voandzeia* cultivar from Thailand nodulated

Table 1 Effectiveness of *Bradyrhizobium* species and *Rhizobium* species on the bambarra groundnut

Rhizobia	Shoot dry wt jar^{-1} (g)	Nodule dry wt jar^{-1} (g)	µmoles C_2H_4 plant^{-1} h^{-1}
TAL 22 – *Phaseolus lunatus*	2.07	0.15	9.6
TAL 102[a] (USDA 110)[b] – *Glycine max*[c]	1.28	0.15	6.7
TAL 133 – *Acacia mearnsii*	0.75	0.09	1.8
TAL 169 – (Nit. 176A22) – *Vigna unguiculata*	2.74	0.16	7.6
TAL 182 – *Phaseolus vulgaris*	0.51	0.04	2.5
TAL 309 (CB 756) – *Macrotyloma africanum*	0.64	0.01	1.1
TAL 344 – *Xylia dolabriformis*	0.69	0	0
TAL 362 – *Albizia lebbek*	0.57	0.02	0.2
TAL 366 – *Voandzeia subterranea*	1.28	0.13	2.8
TAL 367 – *V. subterranea*	1.8	0.16	11.4
TAL 455 – *V. subterranea*	1.0	0.09	5.5
TAL 514 – *V. subterranea*	1.32	0.1	3.4
TAL 526 – *V. subterranea*	1.36	0.1	11.1
TAL 569 – (MAR 472) – *Desmodium uncinatum*	2.02	0.16	8.9
TAL 620 – (ICRISAT 3889) – *Cicer arietinum*	0.79	0	0
TAL 651 – (UMKL 44) – *Calapogonium mucunoides*	1.67	0.16	10.1
TAL 766 (CIAT 255) – *Phaseolus acutifolius*	0.75	0.06	4.2
TAL 1000 – *Arachis hypogaea*	0.93	0.07	4.8
TAL 1145 – (CIAT 1967) – *Leucaena leucocephala*	0.64	0.02	1.5
TAL 1552 – *V. subterranea*	1.59	0.15	3.0
TAL 1553 – *V. subterranea*	2.33	0.23	8.7
TAL 1554 – *V. subterranea*	2.11	0.24	12.7
TAL 1555 – *V. subterranea*	2.05	0.15	12.1
Uninoculated	0.53	0	0
Uninoculated, plus 70 ppm N	3.53	0	0

[a,b] NifTAL and original strain designations, respectively; [c] host of isolation
LSD (P = 0.05): Shoot dry weight, 0.58; nodule dry weight, 0.05; µmoles C_2H_4 plant^{-1} h^{-1}, 8.4

Table 2 Symbiotic differences between rhizobia isolated from bambarra groundnut nodules

Bradyrhizobia	Shoot dry wt[a] plant^{-1} (g)	Nodule dry wt plant^{-1} (g)	µmoles C_2H_4 plant^{-1} h^{-1}
TAL 169[b]	3.62 a	0.24 a	9.3 ab
TAL 366	3.37 ab	0.20 ab	9.1 ab
TAL 367	2.53 abc	0.15 abc	8.7 abc
TAL 387	3.65 a	0.24 a	11.9 a
TAL 402	1.81 c	0.10 bc	4.1 bcd
TAL 436	1.80 c	0.09 c	3.9 bcd
TAL 452	2.02 bc	0.11 bc	4.3 bcd
TAL 455	1.92 c	0.10 bc	4.2 bcd
TAL 495	1.59 c	0.08 c	2.8 cd
TAL 499	1.39 c	0.08 c	2.1 d
TAL 504	2.09 bc	0.14 abc	7.0 abcd
TAL 508	2.07 bc	0.12 bc	6.3 bcd
TAL 514	1.82 c	0.10 bc	4.2 bcd
TAL 1552	1.75 c	0.08 c	3.6 bcd
TAL 1553	1.59 c	0.08 c	2.5 d
Uninoculated	1.08	0	0

[a] Means within a column followed by the same letter are not significantly different at $P = 0.05$
[b] *Bradyrhizobium* sp. isolated from cowpea

with almost all rhizobia derived from nodules of diverse legumes (Table 1) but the symbiosis ranged from completely ineffective to highly effective. The variability in the effectiveness of the *Rhizobium* treatments was reflected in highly significant effects in shoot dry weight ($P < 0.001$), nodule mass ($P < 0.001$) and nitrogenase (C_2H_2 reduction) activity ($P < 0.001$). Nodulation with *Rhizobium sp.* TAL 182 (*R. leguminosarum* biovar *phaseoli*) and TAL 1145 *(Rhizobium* sp. from *Leucaena leucocephala*) were ineffective. Chickpea rhizobia *(Rhizobium sp.* TAL 620) did not form nodules on Voandzeia.

Among the strains of *Bradyrhizobium spp.* that nodulated *Voandzeia, T*AL 169 (derived from *Vigna unguiculata)* was highly effective. Of equal effectiveness was a *Bradyrhizobium spp.* (TAL 1553) isolated from nodules of *Voandzeia* grown in a Hawaiian soil. The commercially available *Bradyrhizobium sp.* (CB 756 = TAL 309) was not significantly different from the uninoculated plants in shoot dry weight.

Experiment 2

Effectiveness tests on cultivar No. 12 P.P. Rust carried out with individual native strains derived from nodules of *Voandzeia* grown in a Kenyan soil showed marked differences in effectiveness as measured by shoot and nodule dry weights and nitrogenase activity (Table 2). Many of the strains compared poorly with standard strains TAL 169. However, the native strain TAL 387 was ranked as effective as TAL 169. The two Hawaiian isolates (TAL 1552 and TAL 1553) were not effective. Over the range of symbiotic variability observed among rhizobial isolates derived from *Voandzeia* nodules, nodule and shoot dry weights and nitrogenase activity parameters indicative of N_2 fixation capacity were highly correlated with one another. These linear correlations were as follows: shoot dry weight and C_2H_2 reduction, $r = 0.94$, $P < 0.001$; shoot dry weight and nodule dry weight, $r = 0.98$, $P < 0.001$; and nodule dry weight and C_2H_2 reduction, $r = 0.95$, $P < 0.001$.

Experiment 3

The effects of the host genotype and strain of *Bradyrhizobium* on the efficiency of the symbiosis are shown in the data on nitrogenase (C_2H_2 reduction) activities, nodule fresh and dry weights (Table 3), shoot dry weight, total and percentage shoot nitrogen (Table 4). No. 12 P.P. Rust in combination with TAL 169 was consistently superior in all the growth, nodulation and nitrogen fixation parameters determined. Other *Bradyrhizobium spp.* that were ranked of equal effectiveness or ranked close to TAL 169 for the nodulation and nitrogen fixation traits on No. 12 P.P. Rust were TAL 387, TAL 366. TAL 309 was of moderate to ineffective status. The peanut strain TAL 1000 was generally poor on all cultivars tested; Swazi Homelands-2 showed a low potential for N_2 fixation even when inoculated with TAL 169 as seen in its total shoot nitrogen. Inoculation of No. 12 P.P. Rust with TAL 169 resulted in a five-fold increase in the total shoot nitrogen compared with the uninoculated control plants. This cultivar was also superior to the rest in total shoot nitrogen.

The data for growth, nodulation and nitrogen fixation showed that these characteristics were significantly influenced independently by the *Bradyrhizobium* or the *Voandzeia* genotype as no genotype x microsymbiont interaction was observed. The host genotype showed highly significant ($P < 0.01$; $P \sim 0.001$) differences for all traits measured. However, for the strains of *Bradyrhizobium,* the shoot dry weight was not a highly significant source of variation, while TNA was not significant.

The linear correlations for the growth, nodulation and nitrogen-fixing parameters determined in the genotype x microsymbiont interaction investigation are presented in Table 5. Percentage shoot nitrogen was non-significantly correlated with all other parameters except with shoot nitrogen ($r = 0.62$, P
0.01). Also, SNA showed significant correlation only with TNA ($r = 0.51$, $P < 0.01$) and shoot dry weight ($r = 0.50$, P --< 0.01).

Discussion

This investigation has systematically examined the *Voandzeia-Bradyrhizobium symbiosis* with the aim of elucidating the cross inoculation group of *Voand-*

Table 3 Nodule weight and nitrogenase activities of bambarra groundnut cultivars inoculated with *Bradyrhizobium* species

	Bambarra groundnut cultivar									
	Nitrogenase activity					Nodule wt (g) plant^{-1}				
Bradyrhizobium	P.P. Rust	TVS 747	TVS 47	Swaz. #1	Swaz. #2	P.P. Rust	TVS 747	TVS 47	Swaz. #1	Swaz. #2
TAL 309	29.7[a] (23.5)[b]	9.8 (18.3)	11.9 (14.0)	12.8 (16.4)	3.6 (12.5)	0.17[c] (1.23)[d]	0.07 (0.5)	0.10 (0.77)	0.10 (0.72)	0.04 (0.27)
TAL 387	21.6 (13.6)	4.0 (6.1)	5.9 (8.3)	15.2 (16.2)	1.4 (3.0)	0.17 (1.6)	0.06 (0.72)	0.12 (0.89)	0.07 (0.80)	0.07 (0.60)
TAL 366	29.6 (18.9)	4.9 (9.3)	5.1 (5.7)	3.6 (9.0)	2.2 (9.0)	0.2 (1.53)	0.07 (0.51)	0.11 (0.99)	0.07 (0.48)	0.04 (0.26)
TAL 169	38.5 (31.8)	3.1 (45.5)	5.2 (11.7)	4.2 (13.8)	1.9 (6.8)	0.17 (1.16)	0.01 (0.55)	0.06 (0.48)	0.04 (0.22)	0.04 (0.31)
TAL 1000	20.6 (19.3)	1.4 (8.6)	11.1 (16.0)	10.4 (17.2)	1.5 (7.3)	0.12 (0.85)	0.03 (0.28)	0.07 (0.57)	0.07 (0.55)	0.03 (0.20)

[a] Total nitrogenase activity (TNA), μmoles C_2H_4 plant^{-1} h^{-1}; [b] Specific nitrogenase activity (SNA), μmoles C_2H_4 g fresh weight nodule^{-1} h^{-1}; [c] Nodule dry weight; [d] Nodule fresh weight
LSD ($P \leq 0.05$): TNA, 13.7; SNA, 16.8; nodule dry weight, 0.04; nodule fresh weight, 0.5

Table 4 Shoot dry weights and nitrogen content of bambarra groundnut cultivars inoculated with *Bradyrhizobium* species

	Bambarra groundnut cultivar									
	Shoot dry wt (g) plant^{-1}					Shoot nitrogen (mg) plant^{-1}				
Inoculation	P.P. Rust	TVS 747	TVS 47	Swaz. #1	Swaz. #2	P.P. Rust	TVS 747	TVS 47	Swaz. #1	Swaz. #2
TAL 169	2.52 –	1.20 –	1.63 –	1.86 –	0.92 –	51.63[a] (2.09)[b]	24.73 (2.07)	39.19 (2.40)	39.96 (2.12)	18.82 (2.06)
TAL 387	2.46 –	0.95 –	1.62 –	1.48 –	0.88 –	46.15 (1.88)	21.24 (2.29)	38.83 (2.40)	28.97 (1.91)	20.94 (2.46)
TAL 366	2.37 –	0.99 –	1.54 –	1.27 –	0.58 –	43.75 (1.84)	20.75 (2.00)	34.96 (2.21)	26.30 (1.35)	12.82 (2.26)
TAL 309	2.73 –	1.31 –	1.19 –	0.94 –	0.72 –	38.56 (1.40)	15.14 (1.15)	20.92 (1.70)	12.32 (1.34)	10.65 (1.40)
TAL 1000	1.93 –	0.95 –	1.17 –	1.61 –	0.67 –	22.09 (1.19)	13.09 (1.46)	15.54 (1.32)	12.71 (0.83)	7.23 (1.11)
Uninoculated	1.82 –	0.85 –	0.85 –	0.81 –	0.60 –	10.42 (0.58)	10.01 (1.18)	7.09 (0.84)	8.11 (0.99)	5.93 (0.98)

[a] Total shoot nitrogen (mg); [b] Shoot nitrogen (%)
LSD ($P \leq 0.05$): Total shoot nitrogen, 8.94; shoot nitrogen (%), 0.31; shoot dry weight, 0.64

Table 5 Linear correlation coefficients (*r*) for growth, nodulation and nitrogen fixation traits in the bambarra groundnut

Trait	Nodule dry weight	TNA	SNA	Shoot dry weight	Shoot nitrogen (%)	Total shoot nitrogen
Nodule fresh weight	0.93[c]	0.80[c]	0.28 NS	0.89[c]	0.24 NS	0.87[c]
Nodule dry weight		0.86[c]	0.14 NS	0.90[c]	0.25 NS	0.88[c]
TNA			0.51[b]	0.92[c]	0 NS	0.71[c]
SNA				0.50[b]	0.32 NS	0.20 NS
Shoot dry weight					0.14 NS	0.81[c]
Shoot nitrogen (%)						0.62[c]

[b,c] indicate significance at $P \leq 0.01$ and $P \leq 0.001$ levels of probability, respectively; NS, not significant

zeia and to select effective rhizobial strains for inoculating *Voandzeia* cultivars to maximize symbiotic nitrogen fixation.

The results of our study illustrate clearly that *Voandzeia is* a `cowpea-type' legume nodulated by slow-growing rhizobia *(Bradyrhizobium spp.)* which were derived from nodules of diverse leguminous species. Often the symbiosis was ineffective and non-beneficial even though the nodules were able to reduce acetylene, indicative of nitrogenase activity (Tables 1, 2 and 3).

It appears that *Voandzeia* will form a beneficial

and highly effective symbiosis with a few strains of *Bradyrhizobium spp.* from other `cowpea type' legumes, besides strains derived from its own nodules. Of interest in this investigation was strain TAL 169 which was derived from nodules of *Vigna unguiculata* or cowpea. This *Bradyrhizobium sp.* was consistently effective on all genotypes of *Voandzeia* tested. This observation is in contrast to that of Doku (1969) who showed that *Voandzeia* was not nodulated when inoculated with a crushed nodule suspension of effective nodules obtained from *Vigna unguiculata.* The same worker also showed that *Voandzeia* did not nodulate with nodule suspensions prepared from nodules of the lima bean *(Phaseolus lunatus*).

The effectiveness of native rhizobia on a particular legume species can vary considerably. For example, it was shown that in glasshouse-grown groundnut, of 21 rhizobial isolates from nodules collected in four groundnut-growing areas of Texas, only four strains were effective (Weaver, 1974). Virtually nothing is known about the efficiency of native bradyrhizobia that nodulate *Voandzeia. Though* we tested only twelve isolates (Table 2) derived from *Voandzeia* nodules from a single location in Kenya, our data demonstrated that most of the native strains were low to mediocre in effectiveness. This suggests that inoculation of selected strains would be beneficial at this location for increasing nitrogen fixation in *Voandzeia.*

A substantial part of an effective symbiosis depends on the compatibility factors between the host legume and the rhizobial strain. Careful selection of the host legume and microsymbiont characteristics can be utilized to achieve effective inoculation and nitrogen fixation (Gibson, 1980). In *Voandzeia,* marked differences in symbiotic variability exist in the host cultivar and the *Bradyrhizobium spp.* (Tables 3 and 4). The cultivar of *Voandzeia* influenced differences in all seven traits measured for growth, nodulation and nitrogen fixation. The absence of specific cultivar x microsymbiont interaction in this legume may simplify its inoculation, since a highly effective *Bradyrhizobium sp.* could be used with any *Voandzeia* cultivar which has a potential for high yield and N2 fixation. Our investigation has singled out *Bradyrhizobium sp.* TAL 169 and *Voandzeia* cultivar No. 12 P.P. Rust combination as the pair with a promising potential for symbiotic N_2 fixation in the bambarra groundnut.

Though no cultivar x microsymbiont interaction was shown in the symbiosis, the frequent ineffectiveness with *bradyrhizobia* from other legumes (Table 1) suggests that *Voandzeia* has a specific requirement of a particular strain of *Bradyrhizobium sp.* for effective symbiosis. This observation is supported by the fact that the cultivar from Thailand formed an ineffective symbiosis with *Bradyrhizobium sp.* CB 756 (TAL 309) (Table 1), a wide-spectrum strain effective on many new tropical legumes of agronomic importance (Date, 1969). Also, strain CB 756 was moderate to ineffective on the five cultivars tested (Tables 3 and 4).

When a new legume is being evaluated for its symbiotic performance with rhizobial strains of different effectiveness, reliable indicators to measure its nitrogen-fixing capacity need to be identified. Shoot and nodule weights, percentage and total nitrogen and nitrogenase (C_2H_2 reduction) activity are commonly used for this purpose (see Hardy *et al.,* 1971; van Berkum et *al.,* 1985; Wynne et *al.,* 1980; Somasegaran and Martin, 1986) and these indicators are significantly correlated with one another (Wynne et *al., ibid.;* van Berkum et *al., ibid.;* Somasegaran and Martin, *ibid.)* in glasshouse cultured plants grown under nitrogen-deficient conditions.

The linear correlations for indicators of nitrogen fixation capacity in *Voandzeia* are shown in Table 5. Percentage shoot nitrogen was a poor indicator and it was not significantly correlated with all established indicators except total nitrogen ($r = 0.62$, P 0.01). This is in contrast to work in *peanut-Bradyrhizobium* symbiosis (Wynne et al., *ibid.)* where percentage nitrogen was significantly correlated with nitrogenase activity, nodule mass and plant weight. Therefore, for *Voandzeia* percentage nitrogen does appear to be a reliable indicator of nitrogen fixation capacity.

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References

Allen, O.N. and Allen E.K. (1981) *The Leguminosae.* A Source *Book of* Characteristics, Uses and Nodulation, The University of Wisconsin Press, 812 pp

Broughton, W.J. and Dilworth, M.J. (1971) Control of leghemoglobin synthesis in snake beans, *Biochem. J.* 125 1075-1080

Date, R.A. (1969) A decade of legume inoculant quality control in Australia, J. Australian Inst. Agric. Sci. 35 27-37

Doku, E.V. (1969) Host specificity among five species in the cowpea cross-inoculation group, Plant Soil 30 126-128

Doku E.V. and Karikari S.K. (1971) Bambarra groundnut, Economic Bot. 25 255-262

Gibson, A.H. (1980) Host determinants in nodulation and nitrogen fixation, in: *Advances in Legume Science* (Summerfield, R.J. and Bunting, A.H., eds.), Royal Botanic Gardens, Kew, pp. 69-76

Halliday, J. and Somasegaran, P. (1984) *The Rhizobium* germplasm resource at *NifTAL* - catalogue of strains, NifTAL Project, University of Hawaii' 66 pp

Hardy, R.W.F., Bunns R.C., Herbert, R.R. Holsten, R.D. and Jackson, E.K. (1971) Biological nitrogen fixation: A key to world protein, Plant Soil **Special Volume** 561-590

Hepper, F.N. (1970) Bambarra groundnut *(Voandzeia subterranea), Field* Crops Abstracts 23 1-6

Mitchell, H.L. (1972) Microdetermination of nitrogen in plant tissues, J. Assoc. Offic. Analyt. Chem. 55 1-3

NAS (National Academy of Sciences) (1979) Tropical *Legumes, Washington,* D.C., National Academy of Sciences, 331 pp

Rachie K.O. and Silvestre, P. (1977) Grain Legumes, in: *Food* Crops of *the Lowland Tropics* (Leakey, C.L.A. and Wills, J.B. eds.), London, U.K., Oxford University Press, pp 41-44

Somasegaran, P. and Hoben, H.J. (1985) *Methods in Legume-Rhizobium* Technology, NitTAL Project, University of Hawaii, Paia, 367 pp

Somasegaran, P. and Martin, R.B. (1986) Symbiotic characteristics and *Rhizobium* requirements of Leucaena *leucocephala x Leucaena diversifolia* hybrid and its parental genotype, Appl. *Environ. Microbiol. 52* 1422-1424

van Berkum, P., Sloger C., Weber, D.F. Cregan, P.B. and Keyser, .H. (1985) elationship between ureide N and NZ fixation, aboveground N accumulation, acetylene reduction, and nodule mass in greenhouse and field studies with Glycine *max* L. (Merr.), Plant *Physiol. 77* 53-58

Vincent, J.M. (1970) A Manual *for* the Practical Study of *Root-Nodule Bacteria*, IBP Handbook *15*, Oxford, Blackwell, 164 pp

Weaver, R.W. (1974) Effectiveness of rhizobia forming nodules on Texas grown peanut, Peanut Sci. 1 23-25

Wynne, J.C. Elkan, G.H., Meisner, C.M., Schneeweis, T.J. and Ligon, J.M. (1980) Greenhouse evaluation of strains of *Rhizobium* for peanuts, Agron. J. *72* 645649