Biol Fertil Soils (2001) 33:3–9 

ORIGINAL PAPER

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# Effectiveness of foreign bradyrhizobia strains in enhancing nodulation, dry matter and seed yield of soybean (*Glycine max* L.) cultivars in Nigeria

Received: 25 May 1999

**Abstract** Field experiments were conducted to investigate the performance of three soybean cultivars with five foreign bradyrhizobia strains in different regions. The experiments at the two sites were designed with soybean (*Glycine max* L.) cultivars as the main factor and bradyrhizobia strains (USDA 136, TAL 122, USDA 6, TAL 377 and TAL 102) as the sub-factor. The experiments were arranged in randomised complete block design with four replications. Results show that nodule number, nodule dry weight and shoot dry weight, total N and seed yield were significantly increased when soybean cultivars were inoculated with foreign bradyrhizobia in two locations in the south east of Nigeria. At 63 days after planting the percentage increase in nodule number and dry weight after inoculation of soybean cultivars with bradyrhizobia strains ranged from 71 to 486% and from 0 to 200%, respectively. The percentage increase in shoot dry matter, %N and total N after bradyrhizobia inoculation ranged between 2–130%, 18–62% and 35–191%, respectively at Awka, and at the Igbariam site the percentage increase in shoot dry weight, %N and total N ranged between 3–76%, 0–43% and 19–125%, respectively. Seed yields after bradyrhizobia inoculation of soybean cultivar TGX 1485–1D at Igbariam ranged between 1.20 and 2.18 t $ha^{-1}$ against the uninoculated plants, which had seed yields of 1.05 t $ha^{-1}$. The poorest yield response after inoculation with bradyrhizobia strains was observed in soybean cultivar M-351, with a seed yield ranging from 0.60 to 0.98 t $ha^{-1}$. The fact that foreign bradyrhizobia strains were more effective than the indigenous strains for all the parameters studied suggests that there is a need to use bradyrhizobia inoculants for increased soybean production in Nigeria. The variations in the strain performance with the different soybean cultivars at the two sites, emphasises the need for careful *Bradyrhizobium* spp. strain selection. The fact that inoculation response was cultivar- and site-specific suggests that strategies for improving inoculation response in soybean cultivars should also consider the soil environment where the soybean is to be produced.

**Keywords** Bradyrhizobia · Soybean cultivar · Nodulation · Seed yield · Inoculation response

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## Introduction

Soybean (*Glycine max* (L.) Merrill) is gaining great prominence in the farming systems of Nigeria and other African countries because of its high protein content. Soybean inoculation with effective and efficient *Bradyrhizobium japonicum* strains may have a role in increasing biological N fixation and seed yield of legumes if properly exploited. Unfortunately, most African countries, including Nigeria, are not exploiting the benefits of bradyrhizobia inoculation technology due to lack of research on the inoculation response of foreign bradyrhizobia strains in different ecological zones and soils. Saint Macary et al. (1995) reported that farmers in African countries lagged behind in the use of inoculant technology. With the world-wide emphasis on sustainable agricultural systems, increase in soybean production will come mostly from the use of an alternative biotechnology rather than the use of fertiliser N in increasing seed yields.

The problem of inoculated soybean crops in most countries is the occurrence in their soils of highly competitive indigenous populations of bradyrhizobia strains which in many cases are less efficient $N_2$ fixers than the inoculated strains (Weaver and Frederick 1974; Minamisawa et al. 1992). In some instances there would be no response because of a failure of the inoculant rhizobia to establish due to competition from native rhizobial strains in the soil (Boonkerd et al. 1978). The expansion of soybean production in Nigeria into non-traditional soybean-growing areas would require inoculating soybeans with effective and competitive foreign bradyrhizobia strains in order to increase yield in a sustainable environment. Saginga at el. (1996) reported that only 33% of the time were increases in growth of soybean due to indigenous bradyrhizobia in 12 different farmers' fields in Nigeria. Rao et al. (1982) reported that tropical soils not previously used for the cultivation of soybeans contain few rhizobia capable of effectively nodulating soybeans.

Information on the effectiveness of foreign bradyrhizobia strains affecting soybean cultivars would be particularly beneficial in Nigeria, where bradyrhizobia inoculant production has been suggested as a means of increasing the production of soybeans in a sustainable environment. Research data in Nigeria on the performance characteristics of bradyrhizobia strains will make it possible to select adapted strains for commercial inoculant production. The objective of these multi-locational field experiments was to evaluate the potential for increasing nodulation, growth and seed yield of soybean cultivars by inoculation with known effective foreign bradyrhizobia strains.

## Materials and methods

### Location of the field experiments

The field experiments were located in a fields at Anambra State College of Agriculture, Igbariam, and at Nnamdi Azikiwe University, Awka, Nigeria. The soils had not been inoculated before with bradyrhizobia strains and soybean had not been grown on the sites. The sites have instead been used for maize (*Zea mays* L.) and cassava (*Manihot utilisima*) production in the previous years. There was no history of fertiliser application on the sites.

Some of the chemical and physical properties (0–20 cm) of the soils are shown in Table 1 Total N was determined by the semi-micro Kjeldahl digestion method of Bremner (1965). Extractable phosphorus was determined using Bray's method (Bray and Kurtz 1945). Organic carbon was determined by method of Walkley and Black (1934). The pH was measured at a 1:2.5 soil:water ratio. The soils belong to the order Ultisols and are classified as Typic Haplustults.

The initial indigenous bradyrhizobia population was determined by the plant infection technique, using dilution of soil for nodulation tests according to the method of Brockwell et al. (1963). Measured amounts of 30 ml sterilised N-free nutrient solution were placed in growth pouches. One ml of soil dilution of $10^{-1}$ to $10^{-10}$ was added to growth pouches. Each dilution was replicated four times. Sterilised soybean seeds were placed in each growth pouch. After 3 weeks, the presence or absence of nodules on the soybean roots was recorded for each dilution. The MPN was calculated from the most likely number, using the MPN tables of Vincent (1970).

### Source and preparation of bradyrhizobia inoculants

The foreign *Bradyrhizobium japonicum* strains USDA 136, TAL 122, USDA 6, TAL 377 and TAL 102 used for the study were obtained from Dr Peter van Berkum of USDA, Beltsville, USA. The strains which were originally lyophilised were reconstituted in yeast mannitol broth (YMB) containing mannitol, 10.0 g; $K_2HPO_4$, 0.5 g; $Mg_2S0_4$, $7H_20$, 0.2 g; NaCl, 0.1 g and yeast extract, 0.5 g (in 1 litre of distilled water). The strains were later maintained at 4 °C in yeast mannitol agar (YMA) slants in a test tube. To maintain viability, the strains were regularly subcultured. The bradyhizobia cultures mentioned above were prepared by growing bradyrhizobia strains in flasks containing 200 ml of sterilized YMB medium. Flasks were placed in darkness on a rotary shaker (100 rpm set at 28 °C for 5 days). After 5 days, the broth cultures were mixed with finely ground decomposed rice husk that had been dumped for 5 years on to a soil near a rice mill at Abakiliki, Ebonyi State, Nigeria. Bradyrhizobia inoculants were prepared by mixing 30 g of sterilised rice husk with 15 ml of broth cultures of the appropriate bradyrhizobium strain in polyethylene bags. The bags were properly kneaded to ensure proper mixing of the broth culture with the ground decomposed rice husks. The method of Miles and Misra (1938) was used to determine that the broth cultures of bradyrhizobia strains contained $1 \times 10^9$ cells $ml^{-1}$ at the time they were aseptically added to the sterilised rice husks. After incubating the inoculated rice husks for 2 weeks at 28 °C, the count of the bradyrhizobia was $1 \times 10^9$ $g^{-1}$ carrier material. To count the bradyrhizobia, 0.03 ml samples of serially diluted bradyhizobia inoculants were plated in Congo red yeast mannitol agar. Colonies that developed after incubation at 28 °C for 5–7 days were recorded.

**Table 1** Some characteristics of the soils at the Awka and Igbariam experimental sites

| Soil characteristics | Awka | Igbariam |
|---|---|---|
| Soil type texture | Sandy loam | Loamy sand |
| pH ($H_2O$) | 5.7 | 5.6 |
| Total N‰ (Kjeldahl) | 0.18 | 0.14 |
| Available-P ($\mu g\ g^{-1}$) | 3.1 | 3.2 |
| Total organic C (%) | 3.5 | 1.8 |
| Indigenous *Bradyrhizobium japonicum* (MPN) | $1 \times 10^2$ $g^{-1}$ soil | $1 \times 10^2 g$ $^{-1}$ soil |

### Source of soybean seeds

Soybean cultivars TGX 536-02D, TGX 1485-1D, and M-351 used for the study were supplied by Dr K. Dashiell of the Grain Legume Improvement Programme of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

### Experimental design

The experiments were designed as two-factor experiments in a randomised block design with four replications. The five bradyrhizobia strains (USDA 136, TAL 122, USDA 6, TAL 377 and TAL 102) were the main factor while soybean cultivars were the sub-factor. Identical field experiments were mounted in two locations; the only difference being that *Bradyrhizobium* strain TAL 122 was not included in the bradyrhizobia treatments for the experiment at Igbariam. The size of each experimental plot was 3 × 2 m. There were six rows per plot and the spacing was 50 × 8 cm.

Before planting, 20 g of the different bradyrhizobia inoculants was added to different polyethylene bags containing 200 g of soybean seeds. Gum arabic (6 ml) was added to each bag to enhance proper mixing and adhesion of the bradyrhizobium carrier material to the soybean seeds. Three inoculated seeds were planted by hand per hole and later thinned down to one per hole 1 week after germination. There was no liming and basal fertiliser application because the peasant farmer's method of cultivation without soil amendment was mimicked. Weeding was done manually when necessary. No pesticides were applied.

Harvest

At 42, 63 and 84 days after planting (DAP), six plants were harvested to record nodule number and nodule dry weight, while the shoot dry weight was estimated at 63 DAP. At pod maturity, 15 plants from the central rows, after leaving border rows, were harvested for grain yield estimation. Shoots of the plants were dried and later ground to pass a 0.5 cm sieve. Total N determinations were done by the semi-micro Kjeldahl method of Bremner (1965)

Statistical analysis

The data were analysed statistically by the method of Steel and Torrie (1960). Analyses of variance were performed and when a significant ($P<0.05$) treatment effect was found, the least significant difference (LSD) was calculated in order to compare treatment means.

## Results and discussion

The mean nodule numbers and dry weights at the Awka site are presented in Figs. 1 and 2. The few nodules that developed in the uninoculated soybean cultivars were small in size when compared with nodules from inoculated plants. At 42 and 63 DAP, soybean cultivar TGX 536–O2D recorded the highest nodule numbers of 30 and 64, respectively, after inoculation with TAL 102. Soybean cultivar M-351 inoculated with TAL 377 had the highest nodule numbers at 84 DAP. Nodule formation in soybean cultivar TGX 1485-1D was the lowest after inoculation with all the bradyrhizobia strains. The nodule dry weight for TGX 1485-1D ranged from 70 to 100 mg plant$^{-1}$, 50–299 mg plant$^{-1}$ for TGX 536-O2D and 80–50 mg plant$^{-1}$ for M-351 at 63 DAP (Fig. 2). Inoculation significantly increased nodule dry weight of the soybean cultivars after inoculation with bradyrhizobia strains TAL 102, TAL 377, but not USDA 136. The results indicate that soybean cultivar TGX 536-O2D had the best response to bradyrhizobia inoculation.

Bradyrhizobia strains varied in their ability to form nodules on soybean cultivars, probably due to the variation in the efficiency of each *Bradyrhizobium* strain in nodulating soybean cultivars. A report by Greder et al. (1986) shows that diversity exists within soybean genotypes for their ability to nodulate. Nodulation was higher in the inoculated soybean cultivars than in the uninoculated control, showing that for enhanced nodulation, soybean cultivars have to be inoculated by *Bradyrhizobium japonicum* before planting. Increased nodulation after bradyrhizobia inoculation may also be due to the fact that the experimental sites had not been planted with soybean before. This shows that a good inoculation response by soybean cultivars is more likely in fields that have not been previously inoculated with bradyrhizobia or planted with soybean. Vincent et al. (1979) reported that poor growth of legumes might be attributed to the absence of specific *Rhizobium* strains. Salema and Chowdhury (1980) observed that local varieties of soybean did not benefit from rhizobia inoculation, whereas indigenous soybean rhizobia in the soil were abundant. The fact that the indigenous bradyrhizobia did not appreciably increase the nodule number in uninoculated plants demonstrates that the indigenous bradyrhizobia were not efficient in nodulating the soybean cultivars. The MPN data of indigenous bradyrhizobia populations from the experimental sites suggest that the number of indigenous bradyrhizobia strains was inadequate for optimum nodulation of the uninoculated plants. Singleton and Tavares (1986) re-

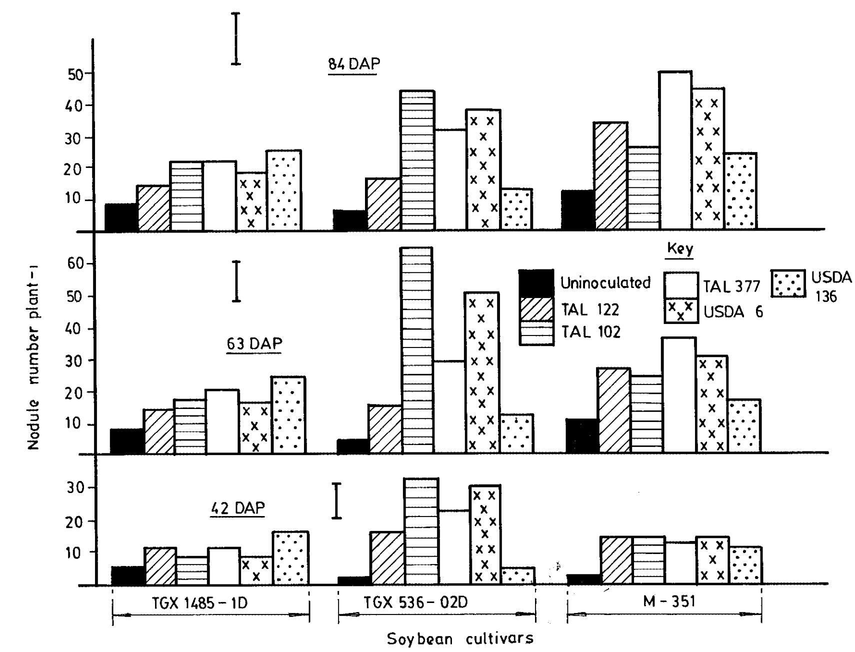


**Fig. 1** Nodule numbers after rhizobial inoculation of soybean cultivars at Awka

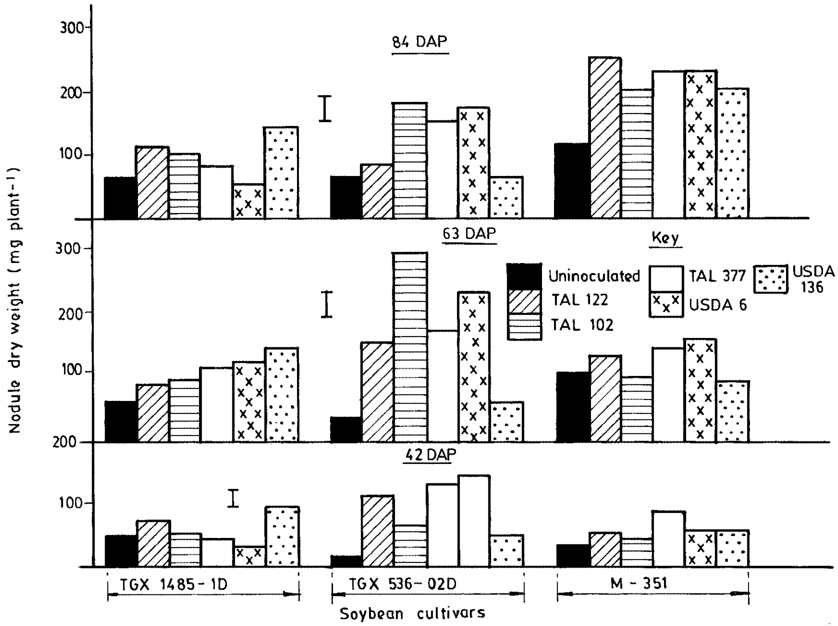


**Fig. 2** Nodule dry weight after inoculation of soybean cultivars at Awka

ported that even moderately effective strains could elicit large responses when native rhizobia populations are low in number or when the population is completely ineffective.

Okereke and Onochie (1996) demonstrated in a pot experiment, that foreign bradyrhizobia suited to local conditions can enhance nodulation. It was observed that the inner parts of the nodules formed on the uninoculated soybean cultivars were green, indicating the absence of leghaemoglobin. In an experiment in the Congo, Mandimba and Mondiboye (1996) reported that inoculation of soybeans enhanced nodule number per plant up to 355–380% and nodule dry mass per plant up to 336–382% for rhizobia strains TAL 377 and TAL 379, respectively. Simamungakalit et al. (1996) reported that in Bogo, Indonesia, nodule number and nodule dry weight responded to inoculation by up to 3- and 4-fold, respectively. They also reported the mean percentage of nodules formed by the introduced strains was 76, 74 and 41% for strains FCB 26, CB 809 and USDA 110, respectively.

The presence of nodules in the uninoculated controls signifies that the soybean cultivars were promiscuous, as they were nodulated by indigenous bradyrhizobia strains present in the soil. The range in the variability in nodulation between the three uninoculated soybean cultivars at 84 DAP suggests that the strains of bradyrhizobia that make up the native soil population may vary considerably in their effectiveness with soybean genotypes. Singleton and Tavares (1986) reported that nodules isolated from uninoculated plants in soils with indigenous bradyrhizobia ranged considerably in effectiveness with their hosts.

Increase in shoot dry weight varied according to the different bradyrhizobia strains and soybean cultivars at the Awka and Igbariam sites (Table 2) Inoculation also significantly increased percentage and total N of the different soybean cultivars. The percentage increase in shoot dry weight, %N and total N after bradyrhizobia inoculation ranged between 2–130%, 18–62% and 35–191%, respectively, at the Awka site (Table 3). At the Igbariam site the percentage increase in shoot dry weight, %N and total N ranged between 3–76%, 0–43% and 19–125%, respectively (Table 4). The highest total N accumulation was obtained at 84 DAP, when soybean cultivar TGX 536–02D was inoculated with bradyrhizobia strain TAL 102.

The increase in shoot dry matter, %N and total N were dependent upon the interaction between the soybean and the rhizobia strains, indicating that the responses to inoculation were strongly influenced by the host-microsymbiont interaction rather than the individual soybean cultivars and bradyrhizobial strains. The observed interaction between soybean cultivars and rhizobia strains have also been reported by Hungria and Neves (1987) for *Phaseolus vulgaris*. Chowdhury (1977) reported a strong interaction between soybean varieties and bradyrhizobia strains and thus recommended selection of appropriate bradyrhizobia strains for different soybean varieties. On the other hand, Pulver et al. (1982) showed that inoculation of the promiscuous soybean varieties Orba, TGM 686 and Malayan increased nodule mass but did not significantly increase shoot dry weight at 60 DAP. However, Hardarson et al (1984) and Chowdhury (1975) reported that total dry matter yield was not enough to measure rhizobia inoculation treatment effects in the field. Variations between plants in their ability to select nodulation competent strains have frequently been reported among legume species (Leung et al. 1994). Dowling and Broughton (1986) reported that legume hosts show preferential selection for rhizobia.

**Table 2** Shoot dry weight, percentage N and total N of soybean cultivars after bradyrhizobia inoculation at 63 days after planting at Awka and Igbariam. Values with the same letter in each column are not significantly different from each other at $P \leq 0.05$

| Soybean cultivar | Bradyrhizobia strain | Shoot dry matter (kg ha$^{-1}$) | Nitrogen (%) | Total N (kg ha$^{-1}$) |
|---|---|---|---|---|
| Awka site | | | | |
| TGX 1485-ID | Uninoculated | 2275[a] | 1.71[a] | 38.90[a] |
| | TAL 122 | 3200[b] | 2.65[b] | 84.16[b] |
| | TAL102 | 4250[c] | 2.64[b] | 112.20[c] |
| | TAL 377 | 3200[b] | 2.74[b] | 87.68[b] |
| | USDA 6 | 3125[b] | 2.77[b] | 68.56[b] |
| | USDA 136 | 5225[c] | 2.17[b] | 69.98[c] |
| TGX 536-02D | Uninoculated | 2150[a] | 1.91[a] | 41.07[a] |
| | TAL 122 | 3100[b] | 2.43[b] | 75.33[b] |
| | TAL102 | 4300[c] | 2.69[b] | 115.67[c] |
| | TAL 377 | 3225[b] | 2.26[b] | 72.89[b] |
| | USDA 6 | 3200[b] | 2.83[b] | 90.50[b] |
| | USDA 136 | 3275[b] | 2.73[b] | 89.41[b] |
| M-351 | Uninoculated | 3125[b] | 1.52[a] | 47.50[a] |
| | TAL 122 | 3225[b] | 2.15[b] | 69.34[c] |
| | TAL102 | 3175[b] | 2.02[b] | 64.14[c] |
| | TAL377 | 4200[c] | 2.21[b] | 92.82[b] |
| | USDA 6 | 4175[c] | 2.40[b] | 100.20[c] |
| | USDA136 | 4250[c] | 2.39[b] | 101.58[c] |
| Igbariam site | | | | |
| TGX 1485-ID | Uninoculated | 1975[a] | 2.14[c] | 40.27[a] |
| | TAL 102 | 2975[b] | 3.05[b] | 90.74[b] |
| | TAL377 | 2775[b] | 2.65[c] | 73.54[b] |
| | USDA 6 | 2100[a] | 2.73[c] | 57.33[a] |
| | USDA 136 | 2150[a] | 2.69[c] | 57.84[a] |
| TGX 536-02D | Uninoculated | 2775[b] | 2.31[a] | 64.10[a] |
| | TAL 102 | 4300[c] | 3.15[b] | 135.45[c] |
| | TAL 377 | 3350[d] | 2.63[c] | 88.11[b] |
| | USDA 6 | 4650[c] | 2.75[c] | 127.85[c] |
| | USDA136 | 3525[d] | 2.86 [c] | 100.82[b] |
| M-351 | Uninoculated | 3775[d] | 2.32[a] | 87.58[b] |
| | TAL 102 | 4625[c] | 2.69[c] | 124.41[c] |
| | TAL 377 | 6650[e] | 2.33[a] | 155.00[d] |
| | USDA 6 | 5400[c] | 2.43[a] | 125.82[c] |
| | USDA136 | 3900[d] | 2.67[c] | 104.13[b] |

**Table 3** Percentage increase (above the values for the inoculated plants) in nodule number, nodule dry weight, shoot and total N after bradyrhizobia inoculation at Awka

| Soybean cultivar | Bradyrhizobia strain | Nodule number | Nodule dry weight | Shoot dry weight | %N | Total N |
|---|---|---|---|---|---|---|
| TGX 1485-D | TAL 122 | 88 | 120 | 41 | 54 | 117 |
| | TAL 102 | 144 | 100 | 87 | 54 | 188 |
| | TAL 377 | 144 | 160 | 41 | 60 | 125 |
| | USDA 6 | 100 | 140 | 37 | 62 | 122 |
| | USDA 136 | 178 | 180 | 130 | 27 | 191 |
| TGX 536-02D | TAL 122 | 86 | 33 | 48 | 27 | 88 |
| | TAL 102 | 486 | 200 | 100 | 41 | 182 |
| | TAL 377 | 329 | 150 | 50 | 18 | 78 |
| | USDA 6 | 400 | 183 | 49 | 48 | 121 |
| | USDA 136 | 71 | 0 | 52 | 43 | 118 |
| M-351 | TAL 122 | 136 | 127 | 3 | 41 | 46 |
| | TAL 102 | 79 | 82 | 2 | 33 | 35 |
| | TAL 377 | 243 | 191 | 34 | 45 | 95 |
| | USDA 6 | 200 | 118 | 34 | 58 | 111 |
| | USDA 136 | 86 | 82 | 36 | 57 | 114 |

There was a significant increase in seed yield as a result of inoculation at the Awka site after bradyrhizobia inoculation of soybean cultivars (Fig. 3). Seed yield increase was only significant at Igbariam when bradyrhizobium strain TAL 102 was the inoculant (Fig. 4). At the Awka site the seed yield for soybean cultivar TGX 1485-1D, varied from 1.2 to 2.18 t ha$^{-1}$, 1.00 to 1.43 t ha$^{-1}$ in TGX 536-O2D and 0.60 to 0.98 t ha$^{-1}$ in

**Table 4** Percentage increase (above the values for the inoculated plants) in shoot dry weight, percentage N and total N after bradyrhizobia inoculation at Igbariam

| Soybean cultivar | Bradyrhizobia strain | Shoot dry weight | %N | Total N |
|---|---|---|---|---|
| TGX 1485-ID | TAL 102 | 51 | 43 | 125 |
| | TAL 377 | 41 | 24 | 83 |
| | USDA 6 | 6 | 28 | 42 |
| | USDA 136 | 9 | 26 | 44 |
| TGX 536-02D | TAL 102 | 55 | 36 | 111 |
| | TAL 377 | 21 | 14 | 38 |
| | USDA 6 | 68 | 19 | 100 |
| | USDA 136 | 27 | 24 | 57 |
| M-351 | TAL 102 | 23 | 16 | 42 |
| | TAL 377 | 76 | 45 | 63 |
| | USDA 6 | 43 | 58 | 33 |
| | USDA 136 | 3 | 15 | 19 |

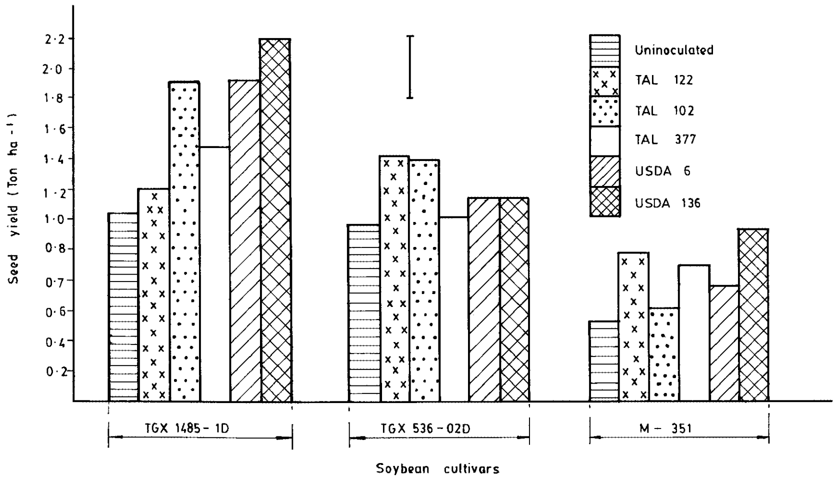


**Fig. 3** Seed yield of soybean at Awka

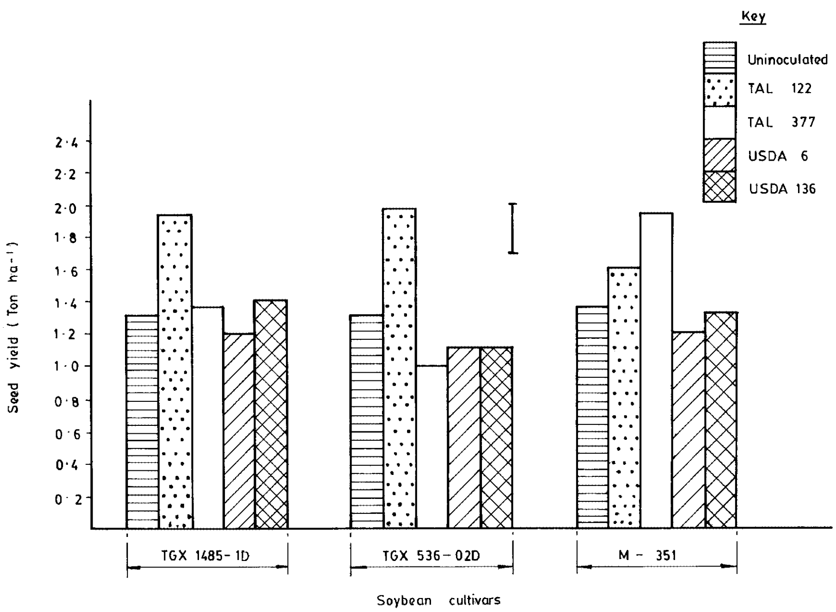


**Fig. 4** Seed yield of soybean at Igbariam

M-351 after inoculation with different bradyrhizobia strains. The uninoculated seed yields were 1.05, 0.98 and 0.45 t ha$^{-1}$ for soybean cultivars TGX 1485 1D, TGX 536-O2D and M-351, respectively.

Inoculation caused significant seed yield response, confirming a report by Thies et al. (1991), that initial soybean yield responses occur in field soils where indigenous populations of *Bradyrhizobium japonicum* are low or absent but contradicting that of Olufajo and Adu (1993), that foreign US strains failed to produce significant yield increases when compared with local isolates in Nigerian soils. Mandimba and Mondiboye

(1996) also reported a 35–55% increase in soybean seed yields by *Bradyrhizobium* inoculation in the Congo. At Bogor, Indonesia, Simamungakalit at el. (1996) reported that inoculation increased seed yield by 70–75%.

In conclusion, the field experiment demonstrates that nodulation, shoot dry weight, total N and seed yield of soybean cultivars can be appreciably increased by bradyrhizobial inoculation in Nigerian soils. The foreign bradyrhizobia strains were more effective than the indigenous strains for all the parameters studied. This suggests the need to use bradyrhizobia inoculants for increased soybean production in Nigeria. The apparently poor nodulation of the uninoculated soybean cultivars and the variations in strain performance, emphasises the need for careful *Bradyrhizobium* strain selection. The fact that inoculation response was cultivar- and site-specific suggests that strategies for improving inoculation response in soybean cultivars should also consider the soil environment where the soybean is to be produced.

**Acknowledgements** This study was supported by a research grant from the World Bank Assisted National Agricultural Research Project (NARP/RGS 017). The authors wish to thank Dr P.M. Nyadaitti and Mrs M.I. Udo-Aka for their logistic support and all the staff of National Agricultural Research Project, Abuja, Nigeria who in various capacities gave support to the successful execution of this study.

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