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J. A. Okogun · N. Sanginga

Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria?

Abstract Promiscuous soybean lines have been bred on the basis that they would nodulate freely without artificial inoculation. However, our recent studies have demonstrated that the indigenous rhizobia are not able to meet their full nitrogen (N) requirement. Rhizobia inoculation might be necessary. We examined the competition for nodule formation among native *Rhizobia* spp. and two inoculated *Bradyrhizobia* strains (R25B indigenous strain and a mixture of R25B+IRj 2180A indigenous strain from soybean lines in the savanna of northern Nigeria), their effect on N fixation, and their contribution to the yield of four soybean cultivars, grown in the field in three different agroecological zones in the moist savanna of Nigeria. About 34% of nodules were formed by the mixture of introduced R25B+IRj 2180A, while R25B formed only about 24% of the nodules but did not influence biomass and grain yield production. The indigenous rhizobia strains that nodulated the soybean varieties fixed up to 70% of their accumulated total N, confirming the promiscuous nature of these soybean varieties. Even though these varieties fixed about 75 kg N ha^{-1}; this was not enough to sustain their optimum grain yield, as earlier reported. However, the grain yield from inoculated soybean was not significantly higher than that from the uninoculated soybean, showing a degree of competitiveness among the introduced rhizobial strains and the native rhizobia population.

Keywords Agroecological zone · Altitude · Enzyme-linked immunosorbent assay · Moist savanna · Nitrogen fixation · Promiscuous soybean · Rhizobia

J. A. Okogun (✉) · N. Sanginga
International Institute of Tropical Agriculture (IITA),
Oyo Road, PMB 5320, Ibadan, Nigeria
e-mail: n.aokogun@iita.exch.cgiar.org
Tel.: +234-22-412626, Fax: +234-22-412221

Present address:
J. A. Okogun, IITA, c/o L.W. Lambourn & Co,
Carolyn House, Dingwall Road, Croydon, CR9 3EE, UK

J. A. Okogun
Agronomy Department, University of Ibadan, Ibadan, Nigeria

Introduction

Between the late 1970s and the early 1980s, IITA soybean scientists had two different approaches to improving biological nitrogen fixation (BNF) to meet the demand for nitrogen (N) in soybean. The first was to breed promiscuous soybean varieties that would nodulate with indigenous soil rhizobia to make inoculation unnecessary; the second was to encourage the production and uses of inoculant in soybean production (Sanginga et al. 1997). Even though there were reports of a yield response by non-promiscuous soybean to inoculation, this was not significant in the promiscuous soybean lines (Pulver et al. 1982; Rao et al. 1981). The pertinent question currently asked is whether the indigenous rhizobial strains are able to meet the optimum N requirement for good establishment of soybean even though there had been no significant differences in the responses of promiscuous soybean to rhizobial inoculation. This question required some answers, as breeding work on promiscuous soybean in IITA had continued for over a decade without microbiological input to quantify the effectiveness of the indigenous rhizobial strains on the soybean lines developed between 1993 and the present (Sanginga et al. 1997). Because soybean is not indigenous to Africa, compatible *Bradyrhizobium japonicum* populations are not widely distributed there. Soybean crops generally require large supplies of N to achieve their maximum yield potential (George and Singleton 1992). The deficiency could be supplemented with N fertilizer or BNF in poor soils (Abaidoo 1997). However, in a soybean inoculation trial (Sanginga et al. 1996), no consistent advantages were obtained. Results showed a positive but inconsistent response, depending on the plant hosts, farmers' fields, and the previous history of the farms. The response of soybean to inoculation occurred in situations where indigenous bradyrhizobial cells were fewer than 10 cells g^{-1} soil, or where the rhizobial populations were not effective. Such situations had been reported (RENEASA 1996) where inoculation increased yields by as much as 179%.

Thus, it might be difficult for all soybean lines to have effective nodulation with the indigenous rhizobia to sustain the N requirement of the cultivars in all environments, especially since *B. japonicum* is host-specific. This means that it might be necessary to inoculate soybean cultivars with elite rhizobial strains that could have advantages over the native rhizobial strains.

Thus, the objectives of this study were (1) to study the competition of introduced elite *Bradyrhizobia* strains with indigenous rhizobia in the soil, (2) to assess the response of soybean cultivars to inoculation, and (3) to quantify the contribution of the introduced rhizobial strains to crop yield in three agroecological regions (derived, southern Guinea, and northern Guinea savannas) of the moist savanna zone (MSZ) of Nigeria.

Materials and methods

Site selection, characterization and land preparation

Fields that had no history of soybean growth were selected in the three sites and the topsoil (0 – 15 cm) was sampled before the fields were prepared for planting. Thirty cores of soil in each of four blocks (which represented four replications for the trial) were collected using a soil auger 6.0 cm in diameter. Cores were bulked and subsamples from each block were collected for rhizobial population estimation. The most probable number (MPN) analysis of the sites was done using soybean lines TGx 1456-2E, TGx 1660-19F, and cowpea in growth pouches (Weaver and Frederick 1972). The test species were surface-sterilized as described by Vincent (1970) and germinated in Petri dishes that contained moist sterile cotton wool at ambient temperature. Two seedlings of the test crops were placed on the grooves of the pouches that contained Jensen's N-free nutrient solution. Each seedling was inoculated with 1.0 ml of soil solution 2 weeks afterwards. Nodulation was examined 30 days after inoculation while the plants were grown under daylight fluorescent tubes at 28°C. The total N content of soil was low (0.06%).

The MPN count and some characteristics of the three sites selected for the trials are shown in Table 1.

Fields were cleared of grasses and ridged at 75 cm intervals to a depth of 30 cm. Each treatment plot was 10×5 m. Before seed sowing, 15 kg P ha^{-1} as single superphosphate (SSP) and 15 kg K ha^{-1} as muriate of potash were basally applied to all plots by broadcasting.

Soybean source

Four soybean breeding lines were obtained from the Grain Legume Improvement Program Unit of the Crop Improvement Division of IITA for the study. They were early-maturing varieties TGx 1485-1D and TGx 1456-2E, and late-maturing lines TGx 1448-2E and TGx 1660-19F. The degree of promiscuity of these lines has been reported (Sanginga et al. 2000). Seeds were surface-sterilized with alcohol and hydrogen peroxide (Vincent 1970).

Inoculation and planting

Two *Bradyrhizobia* strains isolated from promiscuous soybean in 1994 (R25B) and a combination of R25B with *Bradyrhizobium japonicum* IRj 2180A isolated from soybean in 1979 were inoculated on soybean seed (10^7 cells seed g^{-1}) following the method described by Somasegaran and Hoben (1994).

At planting, the soybean seeds were drilled in shallow open grooves in the ridges, then covered with light soil. Uninoculated plots were planted before inoculated plots to avoid contamination. At 2 weeks after planting (WAP), the seedlings were thinned to 1 plant per 10 cm. Each plot received 15 kg P as SSP and there were four rows of ridges per plot. Periodically, plots were hand-weeded to avoid cross-contamination.

Experimental design

The trials were laid out in a split-plot design with the soybean as the main treatment and inoculation as the subtreatment. The control plot received no inoculation. Each treatment was replicated four times.

Sampling

In both years, at 50% podding, five representative plants were cut at about 5 cm above soil level. Shoot biomass of the plants was determined after oven-drying for 72 h at 70°C. Immediately after cutting, bleeding sap was collected from the stumps into vials for the determination of N fixation by the ureide analysis method (Peoples et al. 1989).

The roots of the plants were carefully dug up, packed in polythene bags, together with the nodules that had become detached during digging, and kept in coolers while being transported to the laboratory. Roots were washed and nodules were counted. The nodules were stored at 4°C until enzyme-linked immunosorbent assay (ELISA) analysis was done. Nodules formed by the introduced rhizobia were identified by ELISA (Nambiar and Anjaiah 1985) using 100 nodules randomly selected for each treatment.

Grain yield was measured at physiological maturity by randomly uprooting 60 dry soybean plants per plot. The pods were removed from the plants and air-dried in a glasshouse for 72 h until ready for threshing. The threshed seeds were oven-dried to a moisture content of about 8% and the weights were recorded.

Statistical analysis

The Statistical Analysis System (SAS 1989) was used for statistical analyses of data. Analysis of variance was done using 'PROC GLM' and correlations using 'PROC CORR'. Comparisons of treatment levels were done by using Duncan's test in specific pairwise comparisons at P=0.05.

Table 1 Some characteristic features of the three sites

Site	Coordinates	Elevation (masl)[a]	Vegetation	Rhizobial MPN[b] count before planting	Rhizobial MPN count at the end of season
Fasola	7°54′N, 3°46′E	228[b]	Derived savanna	4×10^2	6.9×10^9
Mokwa	6°5′N, 9°48′E	308	Southern Guinea savanna	1.7×10^4	8.5×10^8
Zaria	11°11′N, 7°38′E	685	Northern Guinea savanna	2.7×10^4	23×10^5

[a] Metres above sea level
[b] Most probable number

Results

Nodulation

In 1996, 30% of the nodules in the roots of the soybean cultivars were formed by the mixture of R25B + IRj 2180A and 17% by R25B. In 1997, 30% of the nodules were formed by R25B and 38% by the mixture of IRj 2180A and R25B (see Table 2). Inoculation did not have any significant effect on soybean nodule fresh weight in 1996 and 1997, and there was no significant effect of inoculation on nodule number in 1997.

Nodulation among the soybean cultivars differed significantly in 1996 and 1997. Soybean variety TGx 1456-2E had the highest mean nodule number and fresh weight and TGx 1448-2E had the lowest in both years (Table 3). There were no significant differences in nodulation across the locations in both years (Table 4).

Shoot biomass and grain yield

Shoot biomass and grain yield production of soybean were not affected by inoculation, and the interaction between bradyrhizobial inoculation and soybean breeding lines was not significant (Table 5). The grain yields were not significantly different within years but significantly different between years. All the cultivars had a higher grain yield in 1996 than in 1997 (Table 6). When the grain yields in the two years were compared, the differences ranged from 23% in TGX-1448-2E (the lowest) to 41% in 1485-1D and 1456-2E. Cultivar TGx 1448-2E was the most consistent in grain yield in both years (Table 6). There were strong relationships between shoot dry matter yield and the soybean grain yield ($P<1\%$, $r=0.557$, $n=252$). Grain yield in 1996 was 3 t ha^{-1} in Zaria, 2.7 t ha^{-1} in Mokwa, and 1.6 t ha^{-1} in Fasola (Table 7).

Table 2 Influence of *Bradyrhizobia* inoculation on the nodulation of soybean. Means that have same letters are not significantly different at $P=0.05$

Treatment	1996			1997		
	Nodule number (no. plant^{-1})	Nodule fresh weight (mg plant^{-1})	Inoculated rhizobial strain occupancy (%)	Nodule number (no. plant^{-1})	Nodule fresh weight (mg plant^{-1})	Inoculated rhizobial strain occupancy (%)
Control	21[b]	1,030	3[c]	13	490	11[c]
R25B	24[ab]	1,070	17[b]	15	600	29[b]
R25B+IRj2180A	29[a]	1,240 NS	30[a]	15 NS	630 NS	36[a]

NS, not significantly different

Table 3 Effect of inoculation on nodulation of different soybean cultivars. Means that have same letters are not significantly different at $P=0.05$

Cultivar	1996			1997		
	Nodule number plant^{-1}	Nodule fresh weight mg plant^{-1}	Inoculated rhizobial strain occupancy (%)	Nodule number plant^{-1}	Nodule fresh weight mg plant^{-1}	Inoculated rhizobial strain occupancy (%)
TGx 1485-1D	21[b]	1000[b]	15	17[a]	569[b]	21[b]
TGx 1456-2E	36[a]	1860[a]	14	19[a]	907[a]	17[b]
TGx 1448-2E	16[c]	630[b]	16	8[b]	405[c]	24[a]
TGx 1660-19F	24[b]	870[b]	23 NS	15[a]	465[c]	28[a]

NS, not significantly different

Table 4 Effect of location on soybean nodule number, nodule fresh weight and % inoculated rhizobia strain occupancy. Means that have same letters are not significantly different at $P=0.05$

Location	1996			1997		
	Nodule number (no. plant^{-1})	Nodule fresh weight (mg plant^{-1})	Inoculated rhizobia occupancy (%)	Nodule number (no. plant^{-1})	Nodule fresh weight (mg plant^{-1})	Inoculated rhizobia occupancy (%)
Fasola	30	1,040	14	13	500	11[c]
Mokwa	24	1,150	20	15	600	29[b]
Zaria	23 NS	1,120 NS	16 NS	15 NS	600 NS	37[a]

NS, Not significantly different

Table 5 Influence of *Bradyrhizobia* inoculation on shoot dry weight, shoot N uptake, % Ndfa, amount Ndfa, and grain yield. Means that have same letters are not significantly different at *P*=0.05

Treatment	1996					1997				
	Shoot dry weight (g plant^{-1})	Shoot N (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})	Shoot dry weight (g plant^{-1})	Shoot N (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})
Control	29	77	72	55	2,409	19	96[b]	58	56	1,335
R25B	29	85	74	63	2,480	19	100[b]	58	58	1,300
R25B+ IRj2180A	30 NS	86 NS	76 NS	65	2,482 NS	20 NS	115[a]	57 NS	66	1,418 NS

NS, not significantly different

Table 6 Effect of inoculation on soybean shoot dry weight, shoot N uptake %Ndfa, amount Ndfa, and grain yield. Means that have same letters are not significantly different at *P*=0.05

Cultivar	1996					1997				
	Shoot dry weight (g plant^{-1})	Shoot N (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})	Shoot dry weight (g plant^{-1})	Shoot N (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})
TGx 1485-1D	27	107[a]	73[ab]	78	1,977[b]	12[b]	82[b]	55	45	1,161[c]
TGx 1456-2E	30	80[bc]	69[b]	55	2,764[a]	25[a]	136[a]	61	83	1,638[b]
TGx 1448-2E	31	68[c]	76[a]	52	2,590[a]	25[a]	135[a]	60	81	1,992[a]
TGx 1660-19F	29 NS	86[b]	78[a]	67	2,558[a]	24[a]	133[a]	53 NS	71	1,678[b]

NS, not significantly different

Table 7 Effect of location on soybean shoot dry weight, shoot N uptake %Ndfa, amount Ndfa, and grain yield. Means that have same letters are not significantly different at *P*=0.05

Location	1996					1997				
	Shoot dry weight (g plant^{-1})	Shoot N uptake (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})	Shoot dry weight (g plant^{-1})	Shoot N uptake (kg ha^{-1})	N$_2$ fixed (%)	Amount N$_2$ fixed (kg ha^{-1})	Grain yield (kg ha^{-1})
Fasola	18c	40[b]	57[a]	23	1,555[b]	17[b]	61[b]	58	35	1,382[b]
Mokwa	37[a]	106[a]	57[a]	60	2,729[a]	20[a]	52[b]	58	30	957[c]
Zaria	28[b]	108[a]	36[b]	37	3,046[a]	21[a]	98[a]	57 NS	56	1,716[a]

NS, not significantly different

Nitrogen fixation and N accumulation

Inoculation did not enhance BNF within the treatments and within years (Table 5) but BNF was higher in all the treatments in 1996 than in 1997. The proportion of BNF varied between 53 and 78%, depending on the soybean variety and year (Table 6). Within the year, Ndfa was not significantly different. However, all the cultivars fixed more biological N in 1996 (30%), than in 1997.

The effect of treatments on shoot N uptake was significantly higher in the mixture of R25B + IRj 2180A than in R25B and in the control in 1997 but was not significantly different in 1996 (Table 5). Shoot N uptake was higher in 1997 in all cultivars except TGx 1485-1D, but this was not translated into grain yield (Table 6).

There were significant relationships between shoot biomass and N uptake (*r*=0.84, *n*=123) and between shoot biomass and grain N uptake (*r*=0.51, *n*=123) in 1996. Correlations were significant between grain yield and N uptake (*P*=0.05, *r*=0.66, *n*=75) and between N fixed and shoot N uptake (*r*=0.55, *n*=75).

There were no significant interactions between treatments and locations as observed in shoot biomass yield, nodule number, nodule fresh weight, grain yield, and rhizobial strain infection. There were no significant differences in infection by the elite strains across the locations.

Discussion

Our study showed that the introduced rhizobial strains were not effective in fixing enough biological N to sustain soybean growth, indicating the need for a starter dose of N or a search for more effective rhizobial strains to enhance the growth of soybean and the grain yield (Sanginga et al. 2000). Although the introduced rhizobial strains produced increases in percentage nodule formation in both years, they could not compete favourably with the indigenous rhizobia as they neither enhanced N_2 fixation nor increased the grain yield compared with the control. The introduced strains were not effective enough to influence the desired growth, thus confirming the earlier reports of the promiscuity of IITA soybean lines (Abaidoo 1997; Sanginga et al. 2000).

Soybean lines performed better in all sites in 1996 than in 1997 in terms of nodulation, N_2 fixation, shoot biomass and grain yield. There were varietal differences to the inoculation of rhizobial strains. Cultivar TGx 1456-2E showed more promiscuity than the others. However, the increased promiscuity exhibited by TGx 1456-2E did not have any significant advantage in N fixed or grain yield over the two late-maturing soybean varieties. Late-maturing soybean varieties were more tolerant to the introduced rhizobial strains in 1997 as against 1996, as more roots were infected by introduced rhizobia than in 1996. This confirmed that the performances of rhizobial strains are influenced by prevailing conditions such as moisture (Nantakorn and Weaver 1982) and competition with the local strains for nodule sites (Harold and Fudi 1992), among other factors, even though these were not measured across the zones. However, the weather data at Zaria, for instance, for the years 1996 and 1997, showed similarities in relative humidity and soil temperature at 5 cm and at 30 cm depth (data not shown). The only substantial difference in the weather report was in the precipitation. Excess moisture from rainfall in 1997 (1,058.2 mm) was probably too much for a proper symbiotic association between soybean and introduced strains. In 1996, rainfall was 232.3 mm less than that in 1997. The Zaria weather data showed that much of the rainfall was concentrated between the months of July and September (data not shown), which incidentally was within the period of sampling. This also affected nodulation and BNF of the strains in 1996 and 1997 (Tables 2 and 5). The optimal soil moisture content for rhizobial activities is 10%, because a high soil moisture content has adverse effects on the population of *Rhizobium japonicum* and *R. trifolii* (Osa-Afiana and Alexander 1979). Thus, the performance of rhizobia could vary among periods, depending on the prevailing environmental conditions.

The trials showed that strain R25B was more aggressive in 1997 in forming nodules in soybean (Table 2). In 1996, it was a different picture as there was almost a ratio of 1:2 for R25B and the mixture of R25B and IRj 2180A. It is difficult to deduce what conferred this advantage on R25B in 1997. It was possible that interstrain competition between R25B and IRj 2180A was more serious than

natural antagonism (Vidor 1982), and strain R25B appeared to be more tolerant to high moisture content but failed to influence any of the parameters measured.

Although low soil nutrients play a role in the reduction of legume/rhizobia symbiotic activities, soybean is able to source N through BNF. In the MSZ, cereals are grown extensively and thus export a lot of the nutrients, especially N and P, from the soil and this might result in a negative net N-balance soil condition (Sanginga et al. 1997). Inoculation of effective rhizobial strains into areas where *Bradyrhizobia* number is low, such as experienced in this study (Table 1), is necessary to sustain soybean production.

Earlier reports (Sanginga et al. 1997) showed that promiscuous soybean varieties derived about 85 kg N^{-1} from BNF and this amount could not sustain these soybean lines. Results from the current trials showed also a range of fixed N from the atmosphere of 69–81 kg N ha^{-1}. Thus, to achieve the development of sustainable cropping systems through the improvement of N fixation in promiscuous soybean, as proposed by Sanginga et al. (1997) and Mpepereki et al. (2000), a search for more aggressive rhizobia colonizers with higher N fixation potentials than the present strains is clearly needed. This study also showed that the inoculated strains were aggressive in colonizing the soil because positive rhizobial reactions to the control treatment during ELISA analysis were observed. There were differences in the symbiotic compatibility of the soybean cultivars and the introduced strains. The soybean variety TGx 1456-2E was more compatible with the local rhizobia and this confirmed the earlier report of Sanginga et al. (2000). This was evidenced in the high nodule number and biomass but less occupancy of the nodules by the introduced strains. Despite the high rate of nodulation by the indigenous rhizobia, this did not enhance the performance of the soybean cultivar over the two late-maturing varieties, but it significantly increased its grain yield over the early-maturing cultivar TGx 1485-1D.

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