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Nitrogen fixation and N contribution by promiscuous nodulating soybeans in the southern Guinea savanna of Nigeria

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Abstract

Attention is being paid to improving the N_2 fixation of promiscuous nodulating soybeans in an attempt to develop sustainable cropping systems in the moist savanna. There is however a dearth of reliable estimates of N_2 fixation by these promiscuous soybeans and hardly any quantitative information is available on their residual N benefits to subsequent cereal crops grown in the southern Guinea savanna zone. The ^{15}N isotope dilution method was used to assess symbiotic N_2 fixation and response to inoculation and N contribution of three IITA promiscuous and two Brazilian soybean lines grown in the field at Mokwa (southern Guinea savanna) for two years. Rhizobial inoculation increased total N and grain yield of early maturing cultivars IAC 100 and TGX 1456–2E but did not affect the late maturing cultivar TGX 1660–19F. Both fixed N (Ndfa) and N derived from the soil were the major sources of N accounting for 84 and 75 kg N ha^{-1} or 46 and 43%, respectively, of the plant total N. A line effect was, however, apparent with the late maturing line TGX 1660–19F deriving on the average 126 kg N ha^{-1} or 52% of plant total N from N_2 fixation compared to the early maturing line IAC 100 with 37 kg N ha^{-1} or 38%. Total N accumulated and amounts of N_2 fixed were low during early growth (V_2/V_3 and R_1/R_2 stages), but increased rapidly after this period to reach the maximum at R_3/R_4 and then dropped after R_3/R_4. The proportion of Ndfa, however, increased with the growing period. At the physiological maturity (R_8), N_2 fixed accounted for an average of 70% of total N accumulated in the seeds. Roots accumulated about 13% while leaves and stems had 53 and 32% of the entire plant N at R_3/R_4, respectively. It was estimated after grain removal, that soybean growth led to a net contribution of an average of 18 kg N ha^{-1} to soil N. However, the N contribution ranged from - 8 to 43 kg N ha^{-1} depending on the soybean cultivars and inoculation treatment..

Introduction

Soybean is a relatively new crop to most African countries but in recent times its cultivation in countries such as Nigeria, Ghana, Cote d'Ivoire, Benin, Zambia, Zimbabwe, Rwanda and Tanzania is gaining popularity as a consequence of the increasing needs for protein from food and fodder. It is also being considered for soil fertility improvement in cereal-based cropping systems in the Guinea savanna (Carsky et al., 1996).

While rhizobial inoculants for soybean have affected agricultural economics in USA, Brazil, and Australia (Eaglesham, 1985; Peoples and Crasswell, 1992) adoption of rhizobial inoculants by small-holder, subsistence farmers has lagged behind in African countries (Saint Macary et al., 1993). Since 1977 IITA scientists target the improvement of biological nitrogen fixation for soybean through two approaches: (i) a breeding program to develop promiscuous soybean varieties that nodulate with indigenous soil rhizobia eliminating the need for inoculation (Kueneman et al., 1984) and (ii) a program to increase the availability of seed inoculants (Rao et al., 1981).

While the breeding programme for promiscuous soybean at IITA has continued, the studies on the associated rhizobia have been spasmodic. Reports of studies on the effectiveness of rhizobia indicate signifi-

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cant yield responses to inoculant and fertilizer N for two non-promiscuous lines, but responses were small and generally not significant for two promiscuous lines (Pulver et al., 1984; Rao et al., 1981). This raised the question of the effectiveness of the rhizobia used as inoculants by these authors. It was important to find out whether inoculants, if available, can be used on promiscuous soybean varieties to promote rapid nodulation or whether indigenous rhizobia will outcompete the inoculant strains. Active field studies stopped at IITA in 1983. As far as is known, none of the rhizobium strains used before 1983 has been tested for compatibility with recent selections from the breeding programme under controlled or field conditions. This is because the breeding programme has proceeded with promiscuity as a major selection criterion for over 10 years without microbiological inputs.

In the only published studies so far available on field testing of selections from IITA breeding programme with selected IITA *Bradyrhizobium japonicum* (IRj) strains, IRj 2133 and 2144, Olufajo and Adu (1993) demonstrated significant yield responses, while a US strain failed to do so. However, Okereke and Eaglesham (1993) showed that commercial inoculants increased nodulation and % N_2 fixed of IITA promiscuous soybean and concluded that none of the genotypes used could be considered to be compatible with the indigenous rhizobia. Sanginga et al. (1996) in a study conducted in 13 different farmers' fields in Nigeria showed that increases in growth of two promiscuous soybean cultivars TGX 1660–19F and TGX 1456–2E were explained by rhizobial inoculation in 30% of the cases and 33% by the indigenous population of rhizobia. Mpepereki and Makonese (1996) recently showed that nodulation of 12 promiscuous soybean lines was detected in 41% of the 71 arable soils in communal areas in Zimbabwe.

The somewhat confusing results obtained in recent experiments reflect the need for more information both on the rhizobia and new cultivars being used and their effect on the actual proportion and amount of N_2 fixed by promiscuous soybean. Even though many early reports have indicated that cultivation of legumes results in enrichment of soil N, this has been dependent on the proportion of the legume's N that is fixed, N harvest index and its distribution in various plant organs. Earlier field studies performed in Nigeria have shown that soybean derived less than 60% of its N from fixation with a negative contribution to the N balance in the cropping system (Eaglesham et al., 1982). In the present study we hypothesize that genotypic variation in soil N uptake potential exists between promiscuous soybean lines and those with a low N harvest index will have a positive N balance. This information could be useful in developing management practices, including the application of fertilizer, to increase soybean yields by optimizing N_2 fixation and N nutrition. The objectives of this work were to: (i) assess the growth response and variability of promiscuous soybeans to rhizobial inoculation; (ii) quantify N_2 fixation and the contribution of other sources of N (soil and fertilizer N) to plant total N and grain yield and (iii) assess the influence of the soybean cultivars on the soil N balance.

Materials and methods

The field experiments were conducted in 1994 and 1995 at Mokwa. Mokwa is located in the southern Guinea savanna in Nigeria (Longitude 6°5′; Latitude 9°48′) and has a mean rainfall of 1100 mm per annum. The soil is characterized as an Alfisol.

Soil samples were collected before clearing at 0 to 20 cm depth (composite of 20 cores for each of the four blocks). They had low total N (0.06%) and about 32 rhizobial cells per gram of soil. The population of infective soybean rhizobia was estimated by the plant infection technique (Weaver and Frederic, 1972). A basal application of 15 kg P as single superphosphate and 15 kg K ha^{-1} as muriate of potash was applied to the soil by broadcasting before planting.

Seed sources and preparation

Five soybean lines were selected from a screening trial for promiscuity done by the Grain Legume Improvement Programme (GLIP) at IITA: (1) Early maturing lines (i) IAC 100, (Brazilian line) (ii) TGX 1456–2E (IITA) and (iii) TGX 1519–1D (IITA) and the two late maturing lines were (i) TGX 1660–19F (IITA) and (ii) Br 17060 (Brazilian line). Three maize cultivars Oba Sup 2, Tze Comp and 8644 maturing early, medium and late were used as reference plants to estimate nitrogen fixation in soybeans. All seeds were surface sterilized with H_2O_2 (3minutes and alcohol for 10 seconds to avoid rhizobia contamination).

Rhizobial inoculation

No rhizobial inoculation was performed in 1994. In 1995 soybean lines were inoculated with a mixture of rhizobia isolates, IRJ 2180 (*Bradyrhizobium* *japonicum* isolated from soybean in 1979) and R25 (*Bradyrhizobium* sp. isolated from promiscuous soybean in 1994) were inoculated following the practice described by Vincent (1970). These two strains were selected from an earlier study conducted to reassess promiscuity and effectiveness of rhizobia nodulating IITA breeding lines (Sanginga et al., 1997). Seeds were mixed with peat containing rhizobial inoculant at a population density of approximately 10^7 cells of rhizobia per seed.

Labelled nitrogen fertilizer

Nitrogen fertilizer (urea) enriched with 5 atom % excess was applied at 20 kg N ha^{-1} to all soybean lines and maize cultivars growing in ^{15}N microplots. The remaining plot received unlabelled urea at the same rate.

Experimental layout

The experimental design was a split plot in 1994 and a split-split-plot in 1995 both arranged in a randomized complete block design with 4 replications. Main treatments in 1995 were uninoculated and inoculated plants; sub treatments were soybean varieties and maize reference plants and sub-sub treatments were the harvesting periods: V_2/V_3 (2 or 3 trifoliate leaves) 25 days for all varieties; R_1/R_2 (flowering) 36 days for early and 53 days for late maturing varieties; R_3/R_4 (podding) 65 days for early and 75 days for late maturing varieties; and R_8 final (harvesting at maturity).

Plants were not inoculated in 1994 and therefore only two treatments i.e., soybean cultivars and growth periods were main and sub treatments, respectively. Each subplot corresponding to each soybean cultivar consisted of 4 rows, 10 m long with an interrow spacing of 0.75 m and 50 mm within rows. Plot size allowed for destructive sampling during the four harvesting periods and grain yield harvests. ^{15}N microplots were centrally located within each sub plot and measured 0.5 m by 1.5 m. There were four ^{15}N microplots per subplot corresponding to the four harvesting dates. Immediately after sowing and thinning (about one week after germination) the labelled N fertilizer was applied at the rate given above as 200 mL m^{-2}.

Harvest

Harvests were made at the indicated stages of development by gathering all aboveground plant material within the innermost 1.2 m of the two central rows of each subplot. Harvested plant samples were chopped into 10-to 20-mm pieces and sub sampled, and about 500 g fresh weight were oven-dried at 70 °C before grinding to pass through a 0.5 mm sieve. For the last harvest, plant samples were separated into reproductive (grains) and vegetative parts (shoots) before subsampling. Total N and the N isotope ratios were determined with the Automatic Nitrogen Analyser (1500 Carlo Erba), coupled to a SIRA mass spectrometer (Axmann, 1990). The proportion of N derived from the atmosphere was calculated using the isotope dilution equation of Fried and Middelboe (1977). Fixed N_2 values in different organ of soybeans were estimated using the corresponding organs in maize as the control. In addition to the above parameters, nodulation (number and weight) and dry weight of each plant organ (roots, nodules, stems, leaves and seeds) were recorded. Identification of nodule formed by introduced rhizobia was done using a modified ELISA technique using 50 nodules collected at random for each treatment (Asanuma et al., 1985).

Statistical analysis

Statistical analyses were done using SAS (Statistical Analysis System Institute Inc., 1989). Correlation and analysis of variance were done using 'PROC-CORR' and 'PROC GLM' to determine the statistical differences between the treatment and their interactions. Specific pair-wise comparisons of treatment levels were done using the Duncan tests or the Least Significant Difference (LSD) test at $p = 0.05$.

Results

Nodulation

Nodules were formed in all treatments but numbers and weight varied between lines (Table 1). The early maturing lines TGX 1519–1D had the highest number of nodules in 1994 followed by and TGX 1456–2E IAC 100 and the late maturing lines TGX 1660–19F and Br 17060. A similar pattern was noticed for the weight of nodules. Rhizobial inoculation increased both the number and weight of nodules except for cultivar Br 17060.

The inoculant strains induced on average 25% of the nodules formed but these varied according to the soybean lines. Recovery of the strain used in the inoc-

Table 1. Nodulation response of soybean lines to rhizobial inoculation in 1994 and 1995

Soybean lines	Nodule number (no plant^{-1})			Nodule weight (g plant^{-1})			% nodules formed by inoculant
	Io[a]		I1	Io		I1	1995
	1994	1995	1995	1994	1995	1995	
IAC 100	17	12	22	0.33	0.36	0.79	12
TGX 1519–1D	59	10	18	0.78	0.43	1.03	35
TGX 1456–2E	39	21	34	0.67	0.30	0.48	24
TGX 1660–19F	5	11	22	0.11	0.27	0.41	24
Br 17060	4	11	12	0.10	0.25	0.28	37
Means	25	13	22	0.40	0.32	0.60	25
LSD 5% (1)[b]	NA[c]		5	NA		0.12	NA
(2)	37		8	0.37		0.20	7

[a]Io–Uninoculated; I1–Inoculated.
[b](1) For comparing lines among inoculated and uninoculated treatments in 1995;
(2) For comparing lines within inoculated and uninoculated treatments in 1995.
[c]NA–Non applicable.

Table 2. Effect of lines and rhizobial inoculation on grain yield of soybean grown in the field Mokwa in 1994 and 1995

Soybean lines	Grain yield (kg ha^{-1})			% increase due to inoculation
	Io[a]		I1	I1/Io
	1994	1995	1995	1995
IAC 100	1142	1196	1432	20
TGX 1519–1D	884	1307	1374	5
TGX 1456–2E	951	1450	1539	6
TGX 1660–19F	2158	1559	1427	-9
Br 17060	1633	1202	1069	-12
Means	1142	1342	1368	1
LSD 5% (1)[b]	NA[c]		125	
(2)	695		233	

[a]Io–Uninoculated; I1–Inoculated.
[b](1) For comparing lines among inoculated and uninoculated treatments in 1995; (2) For comparing lines within inoculated and uninoculated treatments in 1995.
[c]NA–Not applicable.

ulation from nodules ranged between 12 and 37% for IAC 100 and Br 17060, respectively. Amongst the IITA lines, TGX 1519–1D had the highest nodule recovery (35%) followed by TGX 1456–2E and TGX 1660–19F (24%).

Grain yield

Promiscuous soybean line TGX 1660–19F had a higher grain yield than the other lines except Br 17060 in 1994. This was not the case in 1995 (Table 2). The year and soybean line interactions were significant; with lines, TGX 1456–2E and TGX 1519–1D having high grain yield in 1995 relative to 1994 while that of TGX 1660–19F and Br 17060 was lower in 1995 than in 1994. Grain yield of IAC 100 was not affected by the two years of cultivation.

Rhizobial inoculation treatment did not significantly affect mean grain yield of soybean lines in 1995, except that of the early maturing line IAC 100. This

Table 3. Total N accumulation (kg ha^{-1}) in inoculated and uninoculated soybean lines grown in the field in 1994 and 1995 at R_3/R_4

Soybean lines	Total N (kg ha^{-1}) Io[a] 1994	Io[a] 1995	I1 1995	Means	% increase due to inoculation I1/Io 1995
IAC 100	95	115	160	123	39
TGX 1519–1D	133	176	160	156	-10
TGX 1456–2E	181	145	254	193	75
TGX 1660–19F	307	186	179	223	-4
Br 17060	290	149	200	213	34
LSD 5% (1)[b]	NA[c]		28		
(2)	87		44		

[a] Io–Uninoculated; I1–Inoculated.
[b] (1) For comparing lines among inoculated and uninoculated treatments;
(2) For comparing lines within inoculated and uninoculated treatment in 1995.
[c] NA–Not applicable.

line had its grain yield increased by 20% due to inoculation. Inversely to IAC 100, the late maturing line Br 17060 had its yield reduced by about 10% due to inoculation (Table 2).

Total nitrogen accumulation

Uninoculated soybeans lines accumulated between 95 and 307 kg N ha^{-1} in 1994 and between 115 and 186 kg N ha^{-1} in 1995 (Table 3). The total N accumulation in inoculated plants in 1995 ranged from 160 to 254 kg N ha^{-1}. Similar to the grain yield, cultivar TGX 1660- 19F accumulated more total nitrogen than the other soybean lines in 1994. Inoculated plants accumulated on the average 46 kg N ha^{-1} (23%) more total N than uninoculated ones, but this depended on the lines. The early maturing lines IAC 100 and TGX 1456–2E had their total nitrogen increased by about 53% due to inoculation compared to a non significant reduction of 4% for the late maturing line TGX 1660–19F. Total N accumulation before reproductive stage (R_1/R_2) was low (about 12% of the cumulated plant N) and did not differ among the five soybean lines (Table 4). More than 42% of the total N was accumulated at R_3/R_4 with the late maturing lines TGX 1660–19F and Br 1706 having significantly higher total N than the early maturing IAC 100. Maximum N accumulation in plants was reached at R_1/R_2 for IAC 100 and TGX 1519-ID while TGX 1456 had its peak at R_3/R_4 together with the late maturing lines TGX 1660–19F and Br 17060. Total plant N accumulation dropped by about 70% between R_3/R_4 and R_8.

Sources of plant nitrogen

Atom % ^{15}N excess in the three maize reference crops did not differ significantly (data not shown) and therefore the average value of the three was used to estimate N_2 fixation in soybean lines. Nitrogen fixation was detected in all lines whether uninoculated or inoculated with the mixture of the two rhizobial strains (Tables 5 and 6). Soybean lines fixed an average amount of 84 kg N ha^{-1} representing about 46% of their total N. The proportion and amount of nitrogen derived from atmosphere (Ndfa), however, varied between inoculated treatments and soybean lines. A line effect was apparent with the late maturing TGX 1660–19F and Br 17060 deriving on the average 125 and 109 kg N ha^{-1}, representing 52% and 50% of the total N accumulated in the plant. These lines were closely followed by the early maturing TGX 1456–2E with 82 kg N ha^{-1} (50%) and the other two early maturing lines TGX 1519–1D and IAC 100 with 67 kg ha^{-1} (43%) and 37 kg N ha^{-1} (38%), respectively.

Nitrogen derived from soil (Ndfs) accounted for 43% of the plant total N representing an amount equivalent to 75 kg N ha^{-1}. Similar to Ndfa, the contribution from Ndfs differed significantly between the soybean lines ranging from 56 to 96 kg N ha^{-1} for cultivar IAC100 and TGX 1660–19F, respectively. Fertilizer N accounted for approximately 11% of the plant total N or about 20 kg N ha^{-1}. The amount of nitrogen derived from fertilizer (Ndff) also varied between lines ranging from 11 to 28 kg N ha^{-1}.

Table 4. Total N (kg ha^{-1}) accumulated during four growth stages of soybean lines in the field Mokwa in 1994

	I (V_2/V_3)	II (R_1/R_2)	III (R_3/R_4)	IV (R_8)
Soybean lines				
IAC 100	58^b	123^c	94^c	26^c
TGX 1519–1D	76^a	142^{ab}	132^{bc}	38^{bc}
TGX 1456–2E	53^b	141^{ab}	181^b	82^{ab}
TGX 1660–19F	52^b	196^a	306^a	61^{abc}
Br 17060	51^b	177^a	290^a	95^a
Means	58	156	201	60

Values within a column followed by the same alphabet are not significantly different at $p = 0.05$.

Total N distribution and translocation

Roots accumulated on average about 13% of total plant N and N_2 fixed between V_2/V_3 and R_3/R_4 (Table 7). Leaves and stems had 53 and 32% of the entire plant N (Table 7).

The proportion of grain N that was derived from fixation was on average 70% representing about 49 kg N ha^{-1} (Table 8). It was affected by both lines and rhizobial inoculation and their interactions. Proportions and amounts of Ndff and Ndfs in grain were small representing 2% or about 2 kg and 28% or 24 kg N ha^{-1} of the two sources, respectively.

Nitrogen harvest index and balance

When the quantities of N involved in plant growth, in N_2 fixation and seed are calculated for soybean lines (Table 9), it was apparent that the net N balance was low for the early maturing lines TGX 1519–1D and TGX 1456–2E (13 kg N ha^{-1}, average) and even negative for cultivar IAC 100 (-8 kg N ha^{-1}). The nitrogen harvest index of IAC 100 was the highest amongst all lines. The late maturing lines TGX 1660–19F and Br 17060 contributed more residual N, 30 and 43 kg N ha^{-1} respectively than the early maturing lines. These lines also had the lowest N harvest index. Inoculated plants had on the average a lower N harvest index and therefore contributed more to the net N balance than uninoculated plants. The interactions between inoculation treatments and the soybean lines for the N harvest index and the net N balance were significant. For example, inoculation increased the net N balance of lines Br 17060 and TGX 1456–2E but not that of IAC 100, TGX 1529–1D and TGX 1660–19F which had a higher N balance when uninoculated.

Discussion

The actual amount of N_2 fixed by soybean lines at Mokwa averaged 84 kg N ha^{-1} representing 46% of the plant total nitrogen. An average of 91 kg N ha^{-1} or 46% of plant total N was fixed by the promiscuous lines developed at IITA. Recorded values of amounts of N_2 fixed by soybeans based on the ^{15}N isotope dilution, in other countries are 85–154 in Brazil; 26–57 in Thailand; 78 in Australia (Peoples and Crasswell, 1992). Other values reported are 92 for Hungary; 114 for USA and 71 kg N ha^{-1} for Sri Lanka (Rennie et al., 1982). An average figure of 100 kg N ha^{-1} or 50% of N_2 fixed seems appropriate for soybeans. Thus, the IITA promiscuous soybeans are as efficient in supporting N_2 fixation and deriving benefit from fixed N_2 as other soybean lines grown in other countries. However, the proportion and amount of Ndfa differed significantly for the lines used in this study. Several reports have shown that N_2 fixed by a *Bradyrhizobium* strain is strongly influenced by the host plant (Hardarson et al., 1984) and that nitrogen fixation supporting traits often vary among different hosts. Although only 5 varieties were used in each study, the results showed a large spread in N_2 fixed (ranging from 38 to 126 kg N ha^{-1}) under identical soil N and *Bradyrhizobium* treatments. Results in Table 5 and 6 indicate however, that more than half of the plant total N was mainly from soil N, suggesting that the promiscuous soybean used cannot meet all their demand for growth and seed by only N_2 fixation. This indicates the need to adopt new strategies such as breeding new materials with high potential for N_2 fixation, continuing with the search for efficient *Bradyrhizobium* strains and adopt good management practices in order to improve N_2 fixation.

This study confirmed early observations by Patterson and LaRue (1982) indicating that N_2 fixation in soybean was related to the maturity group. Late maturing lines TGX 1660–19F and Br 17061 fixed more N_2 than the early maturing lines IAC100 and TGX 1519–1D. However, cultivar TGX 1456–2E could be considered as an intermediate between the above two groups, because inspite of being an early maturing cultivar it was as efficient in N_2 fixation as one of the late maturing cultivar Br 17061. This, however, does not invalidate the hypothesis that although TGX 1456–2E may be genetically capable of supporting high N_2 fixation,

Table 5. Sources of N (%) of inoculated and uninoculated soybean lines in 1994 and 1995 at R_3/R_4 growth stage calculated using the weighed ^{15}N atom excess

	Sources of N								
	% Ndfa			% Ndff			% Ndfs		
	Io[a]		II	Io		II	Io		II
Soybean lines	1994	1995	1995	1994	1995	1995	1994	1995	1995
IAC 100	26	34	48	11	12	7	63	54	44
TGX 1519 - 1D	34	53	41	11	13	16	55	31	43
TGX 1456 - 2E	59	43	47	8	11	11	33	46	42
TGX 1660 - 19F	55	64	36	8	14	14	37	22	50
Br 17060	50	47	52	9	11	11	41	42	47
LSD 5% (1)[b]	NA[c]		8	NA		2	NA		9
(2)	9		13	1		3	11		14

[a]Io–Uninoculated; I1–Inoculated.
[b](1)For comparing lines among inoculated and uninoculated treatments in 1995; (2) For comparing lines within uninoculated and inoculated treatments 1995.
[c]NA–Not applicable; ND–Not determined.

Table 6. Sources of N (kg ha^{-1}) of inoculated and uninoculated lines in 1994 and 1995 calculated using the weighed ^{15}N atom excess at R_3/R_4 growth stage excess

	Sources of N								
	Ndfa			Ndff			Ndfs		
	Io[a]		II	Io		II	Io		II
Soybean lines	1994	1995	1995	1994	1995	1995	1994	1995	1995
IAC 100	24	39	50	7	5	6	59	61	46
TGX 1519–1D	45	93	63	10	7	7	73	54	66
TGX 1456–2E	106	62	76	11	5	11	59	66	68
TGX 1660–19F	168	118	88	17	4	7	113	40	123
Br 17060	145	70	114	14	5	8	119	62	103
LSD 5% (1)[b]	NA[c]		17	NA		1	NA		20
(2)	20		28	0.8		2	27		31

[a]Io–Uninoculated; I1–Inoculated.
[b](1) For comparing lines among inoculated and uninoculated treatments in 1995; (2) For comparing lines within uninoculated and inoculated treatments in 1995.
[c]NA–Not applicable; ND–Not determined.

further enhancement of this capability could be realized by increasing the present yield potential. It is likely that by increasing yield further, not only would actual N_2 fixed be greater, but the proportion of N derived from fixation may also be higher, since photosynthates may be less limiting, and more carbohydrates could be made available to the nodules for N_2 fixation than under low yield or limited supply of photosynthates. In addition, results from 1995 experiments showed that the *Bradyrhizobium* strains used influenced the amount of N_2 fixed in some varieties, indicating the importance of *Bradyrhizobium* strain selection to optimise N_2 fixation.

Grain yields in 1995 of non-promiscuous soybean IAC 100 increased (20%) whereas the IITA promiscuous lines TGX 1519–1D and TGX 1456–2E gave only modest yield increase (average, 6%) in response to inoculation. Yield of the promiscuous line TGX 1660–

Table 7. Comparison of total N (kg ha^{-1}) of the different organs of soybean during the four sampling times in 1994

Plant organs	Sampling stage I (V_2/V_3)	II (R_1/R_2)	III (R_3/R_4)	IV (R_8)
Total N				
Leaves	36[a]	87[a]	99[a]	20.80[a]
Stems	13[b]	53[b]	69[b]	39.69[a]
Roots	8[b]	15[b]	32[b]	ND
Whole plant	58	156	201	ND
N_2 fixed				
Leaves	0[b]	20.57[a]	39.27[a]	11.88[a]
Stems	0[b]	5.85[b]	12.10[b]	6.00[a]
Roots	0.25[a]	5.56[b]	5.51[b]	ND
Whole plant	-5.59[a]	36.40[a]	76.55	ND

ND–not determined.
Values within a column followed by the same alphabet are not significantly different at $p = 0.05$.

Table 8. Proportions and amounts of N sources (Ndfa, Ndff and Ndfs) in grain of soybean lines at physiological maturity in 1995

Soybean lines	% Ndfa	% Ndff	% Ndfs	Amount (kg N ha^{-1}) Ndfa	Amount Ndff	Amount Ndfs	Total N (kg N ha^{-1})
Uninoculated							
IAC 100	54	1	45	40	2	31	114
TGX 1519–1D	64	2	34	53	1	27	177
TGX 1456–2E	59	3	48	59	2	43	144
TGX 1660–19F	87	0	13	70	1	12	188
Br 17060	83	1	16	61	1	11	149
Inoculated							
IAC 100	46	4	50	42	2	30	159
TGX 1519–1D	60	3	37	55	2	31	160
TGX 1456–2E	68	2	30	69	1	28	254
TGX 1660–19F	77	2	21	83	1	13	178
BR 17060	85	1	14	56	1	12	200
LSD 5% (1)[a]	5	0.8	4	11	0.4	6	27
(2)	9	21	7	18	1	10	43

[a](1) For comparing lines among uninoculated and inoculated treatments; (2) For comparing lines within uninoculated and inoculated treatments.

19F was even reduced by rhizobial inoculation (Table 2). Consistent yield responses where not observed in previous IITA promiscuous lines used by previous workers (Chowdhury, 1975; Pulver et al., 1982, 1984). The higher amount of Ndfa and yield for uninoculated TGX 1660–19F may either reflect the presence of effective indigenous rhizobial population in the soil at Mokwa and/or the promiscuous nature of this cultivar. For a line such as IAC 100, the local bacteria strains were ineffective in N_2 fixation and this was proven by its response to the introduced rhizobia. Results in Table 1, show that the inoculant used is very effective in increasing nodulation of all promiscuous soybeans but yield was not necessarily related to this especially with TGX 1660–19F. In many cases, especially for the late maturing lines such as TGX 1660–19F introduced

Table 9. Nitrogen harvest index and N balance of uninoculated and inoculated soybean lines grown in the field in 1995

	Nitrogen harvest index[a]			Net N BALANCE (kg N ha^{-2})[h]		
	Io	I1	Means	Io	I1	Means
	Io	I1	Means	Io	I1	Means
IAC 100	0.62	0.52	0.57	- 4	- 12	- 8
TGX 1519–1D	0.47	0.53	0.50	23	- 2	11
TGX 1456–2E	0.65	0.37	0.51	- 13	44	15
TGX 1660–19F	0.50	0.49	0.49	48	11	30
Br 17060	0.53	0.33	0.43	27	59	43
Means	0.55	0.45		16	20	18
LSD 5% (1)[c]	0.06				13	
(2)	0.10				20	

[a]Nitrogen harvest index: total N in seed divided by total crop N.
[h]Net N balance: Net contribution of legume residue N to soil N is equal to amount of N fixed minus N removed in seeds in kg ha^{-1}.
[c](1) For comparing lines among uninoculated and inoculated treatments; (2) For comparing lines within uninoculated and inoculated treatments.

bacteria recovery (24%) did not improve yield and even reduced total amount N_2 fixed, perhaps indicating that these rhizobia were inefficient and/or parasitic. In contrast to TGX 1456–2E and especially for IAC 100, the few nodules formed as a result of inoculation were able to support most of the fixed N_2 occurring in these lines. The problem of competition between the indigenous and the introduced rhizobia applied to soybean is real in Mokwa. This indicates that in order to improve N_2 fixation in promiscuous soybeans our strategy should emphasize cultivar effects on strain recovery in addition to the search of highly competitive and effective rhizobial strains. Consequently, it appears that inoculants, if effective and competitive, can be used on some promiscuous varieties. These data indicate the need for concentrating studies on ecological aspects and on the quantitative limitations of the soil populations i.e. whether the restrictive range of suitable strains in the soil is sufficiently numerous to causes enough nodulation. The quantitative aspect i.e. the effectiveness and competitive ability of the strains should be seriously addressed. Lines such as TGX 1660–19F and TGX 1456–2E with differential responses to rhizobial inoculation should be used as models to test the above hypotheses.

The economic benefit of grain legumes is usually expressed in terms of seed yield, and the N that these legumes contribute to soils is often ignored. After grain removal, the growth of soybean has been shown to result in the net depletion of soil N (Eaglesham et al., 1982; Johnson et al., 1974). In this study, assuming that only seeds of soybean were removed from the plots, it is estimated that the net N accrual to soil ranged between minus 8 kg N ha^{-1} (IAC 100) and 47 kg N ha^{-1} (Br 17060). The average for IITA promiscuous lines was 19 kg N ha^{-1} with cultivar TGX 1660–19F contributing 30 kg N ha^{-1}. We are also cognisant with the fact that the lines evaluated represent a very small sample of IITA promiscuous soybean germplasm and more variation in residual soil N contributed by soybean varieties can still be exploited. Thus it might be dangerous to generalize as done by Eaglesham et al. (1982) since lines within one crop will give different values of residual N. The estimates made for these promiscuous soybeans do not, however, take into consideration N contribution from roots, which was shown to be small (Table 7). Nevertheless, any N added to the soil through root turnover and nodule decay represents a potential N gain that is not accounted for. Since leaf fall during development of crop and the nodulated roots contain up to 40 kg N ha^{-1} (Bergersen et al., 1985; Buresh and De Datta, 1991; Kumar Rao and Dart 1987), it is desirable that they are both included when determining net N balance. However, only standing shoot N is often available and so net N balances are likely to be underestimated.

Levels of fixation achieved by many crops in the field may be high, but are not always sufficient to offset the N removed with the harvested seed. Clearly, if promiscuous soybeans are to contribute substantial amounts of N to the soil, the proportion of N_2 fixed must be considerably greater than the total nitrogen harvest index. A soybean cultivar such as IAC100 would require % Ndfa values greater than 60% or a low nitrogen harvest index to avoid a net loss of N from the system. To maximize contribution of soybean N to a following crop, it is necessary to maximize the total crop N at seed harvest, percentage of N_2 fixed and minimize the nitrogen harvest index. However, minimizing the N harvest index will reduce grain yield so, more effort should be made to maximize the two first characters. These conditions have more been fulfilled by the late than the early maturing varieties.

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