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EVALUATION OF NODULATING AND N_2 FIXING CAPACITY OF SIX SOYBEAN (*Glycine max* (L) Merrill) CULTIVARS WITH AND WITHOUT INOCULATION WITH COWPEA *Bradyrhizobium spp*

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Abstract

The relative responsiveness of six soybean ***(Glycine max (L.)*** *Merrill) cultivars to inoculation with* ***Bradyrhizobium spp****. in terms of nodulation and N_2 fixation was evaluated in a factorial experiment conducted in the greenhouse at the Institute of Agricultural Research Training, Ibadan, using isotope dilution technique. The purpose was to generate empirical evidence to confirm or disprove the assumption that inoculation of soybean with rhizobia in order to enhance N_2 fixation may not be necessary since the crop nodulates in most Nigeria soils.*

The soil used for this experiment contained indigenous rhizobia (2.4×10^3 cell/g soil), and was inoculated with a water suspension of IRC 27 to give an estimated concentration of 2.3×10^6 cells/g soil. Four seeds of the soybean cultivars, namely, TGX 1447-3^D, TGX 1437-1^D, TGX 1448-2^C, TGX 1447-1^D, TGX 1019-2^E N and TGX 1455-2^E were sown in pots containing either inoculated or uninoculated soil .
The cultivar chippewa was used as a non-nodulating soybean isoline.

Results showed that nodulation and N yield of most of the cultivars were increased by inoculation while the ability of about 50% of the cultivars to fix atmospheric N_2 (% Ndfa) was improved. Lack of improvement in the ability of the others to fix N_2 showed that they did not benefit from inoculation of the soil with the ***Bradyrihizobium spp****. Thus, differential response of the cultivars to inoculation was evident.*

Introduction.

Soybean (*Glycine max* (L) Merrill) is fast becoming an important grain legume crop in Nigeria not only bocause of its ability to improve N fertility of the soil through the nitrogen fixation but, also from dietary point of view. A survey by the Nationally co-ordinated Research on soybean put the total area under soybean cultivation in 1991 (sole and intercrop) at about 80,000 hectares.

As much as 80% of the total soybean cultivation is done in the Savannah areas of Nigeria. According to the survey, yields of 744kg/ha (sole) and 502kg/ha (intercrop) were recorded. There appears to be a general notion that since soybean nodulates in many Nigeria soils, it may not respond to inoculation. Thus, the potentials of this grain legume to respond to inoculation in terms of N_2 fixation has not been adequately exploited. However, N_2 fixation does not necessarily occur following nodulation. Working on an acid tropical soil, for example, Fried and Middefleed (1977) found that a legume crop nodulated well but fixed N_2 poorly. Bromfieed and Ayanaba (1980) found that the inoculation of soybean with *Bradyrhizobium japonicum* in an acid tropical soil was necessary for the full exploitation of its N_2-fixing potential. The need for inoculation of soybean as a routine agronomic practice has been stressed even in the presence of indigenous strains (Morton et al, 1982: Thu *et al.*, 1986 Young *et al* 1988 Olufajo et. al 1989 and Rahman and Sanoria 1990).

In Nigeria, reliance on N fertilizer application for increasing soybeans yield may soon become too expensive for the resource-poor farmers because of the ever-increasing cost of the input. Research is, therefore, now required to exploit the N_2- fixing attribute of this crop through inoculation with rhizobia.

This approach may likely cut down on investment on nitrogen fertilizer. The aim of the experiment here reported was, therefore, to evaluate the nodulation and nitrogen fixing potentials of soybean cultivars with or without inoculation with *Bradyrhizobium spp.*

Materials and Methods

Location

The soil culture experiment was conducted in the greenhouse at the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan between March and May 1992. A bulked topsoil (0-15cm) sample of an alfsol (USDA nomen), a sandy clay loam, was obtained from a plot previously grown to maize at Ilora, situated in the Guinea Savanna. The sample was sieved through a 2mm mesh and analysed for biological, physical and chemical characteristics. The following values were obtained: total N, 0.09%; organic carbon, 0.565%, silt, sand, and clay, 23.4,54.4 and 22.0% respectively; pH (H_2 0), 6.25; Bray-1 P.3.65mgkg-1; CEC, 4.10cmol kg-1 and indigenous rhizobia, 2.4 x10^3 cells/g soil.

Preparation of *Bradyrhizobium* spp. Inoculum

A *Bradyrhizobium spp* (cowpea miscellany) IR_C 27 was obtained from the culture stock at the International Institute of Tropical Agriculture (IITA), Ibadan.

A seven – day old slant culture of the IR_C 27 was harvested into Yeast Extract Mannitol (YEM) broth incubated on a mechanical shaker for 7 days at 26°C.

The culture was centrifuged at 5000g for 20minutes and later, the cells were washed twice with sterile distilled water. The washed cells were resuspended in sterile distilled water to give a density of 3.4 x10^8 cells/ml as measured by a dilution plate count (Somasegaran and Hoben, 1985).

Soil Inoculation

Ten- milliliter (10ml) portions of the inoculum were diluted into 50ml water and were mixed thoroughly with 1.5kg portion of soil in each pot to give an estimated cell concentration of 2.3x 10^6 cells/g soil. Fifty milliliters (50ml) of sterile distilled water were mixed with the soil in the control pots.

Muriate of potash (60%k) and single superphosphate (18%P) fertilizer were applied to all pots to give 15 and 10ppm K and P, respectively.

The Treatments

There were twelve treatment combinations of two factors, namely, soybean cultivars and inoculation (with and without). The following were the cultivators: TGX 1447-3^D (V_1), TGX 1437-1^D(V_2), TGX 1448-2^E (V_3), TGX 1447-1D (V_4), TGX 1019-2^E –N (V_5) and TGX 1455-2^E (V_6). The cultivar Chippewa was employed as non-nodulating isoline for the estimation of the proportion and amount of N_2 fixed using the ^{15}N isotope dilution equation of Fried and Middelboe (1977):

$$\%Ndfa = (1 - \frac{^{15}N \text{ atom excess in fixing system)}}{^{15}N \text{ atom excess in non-fixing system}} \times \frac{100}{1}$$

$$\text{The amount of } N_2 \text{ fixed} = \frac{\% \text{ Ndfa x Total N}}{100}$$

Plant height was taken four weeks after planting (4WAP). Harvesting was done 42 days after planting (DAP) for the determination of nodule number. The nitrogen content of the dry matter of plant top was determined by kjeldahl digestion method (Eastin, 1978). and the atom % ^{15}N excess by emission spectrometry (Fiedler and Proksch, 1975). Statistical analysis was by ANOVA and treatment comparisons were made using the least significant difference (LSD) method (Steel and Torrie, 1960).

Results and Discussion

Plant Height and Nodulation

Plant height assessment of the soybean cultivars at the seedling stage (4WAP) showed a significant ($P \leq 0.05$) depressive effect of inoculation on V_5 (Fig. 1) In this trial, however, non- significant increases in heights of V_2 and V_2 were obtained through inoculation. Similar depression as in V_5 were obtained when cowpea seedlings (21DAP) were inoculated (Daramola, 1980). However, subsequent height measurements showed that the depression was transient. The results suggest that inoculation with rhzobia can produce varying degrees of depressive effect on height of soybean seedlings, depending on cultivars. With regard to nodulation, except for cultivar V_1, the inoculation of soybean with cowpea rhizobia (*Bradyrhizobium spp.)* stimulated increased nodulation (Table 1), and the increases were significant ($P \leq 0.05$).

Table 1: Nodule number of soybean varieties as affected by inoculation with *Bradyrhizobium spp.*

Cultivar	Uninoculated	Inoculated	Cultivar means
V_1	13.25^a	9.75^b	11.50
V_2	8.88^b	13.50^a	11.19
V_3	13.25^b	15.00^a	14.13
V_4	10.00^b	19.25^a	14.63
V_5	5.00^a	7.75^a	6.38
V_6	8.88^b	12.00^a	10.44
Inoculation means	9.88^b	12.88^a	

	Lsd (0.05)	Cv (%)
Cultivar	3.41	29.0
Inoculation	2.30	35.0

Means followed by same letter (row-wise for cultivar under inoculation regimes and for mean cultivar) are not significantly different ($P \leq 0.05$) according to Duncan Multiple range test.

Table 2: Total N productions (mg/ plant) of soybean varieties as affected by inoculation with *Bradyhizobium* spp

Cultivar	Uninoculated	inoculated	Cultivar means
V_1	25.35^a	31.30^a	28.58
V_2	34.30^b	77.42^a	55.86
V_3	60.88^b	87.09^a	72.49
V_4	49.56^a	53.07a	51.52
V_5	54.08^a	44.23^b	48.81
V_6	61.83^a	43.65^b	52.75
Inoculation means	47.82^a	55.74^a	

		Lsd (0.05)	Cv(%)
	Cultivar	14.99	25.0
	Inoculation	NS	33.4

Means followed by the same letter (row –wise for cultivar under inoculation regimes and for mean cultivar) is not significantly different ($P \leq 0.05$) according to Duncan Multiple range test.

Therefore, the assumption that soybean requires no inoculation for increased nodulation to occur has been disproved.

Total N and percent N derived from atmosphere.

Total N content of cultivars V_1, V_2, V_3, and V_4 were increased by inoculation (Table 2). The increases were significant ($P \leq 0.05$) in cultivars V_2 and V_3.

The N yield of V_2 grown in an inoculated soil was more than twice the N yield of the cultivar grown in a non –inoculated soil (Table 2). The significant increase in N yield of N_2, reflected in corresponding increase in pod yield indicating an apparent translocation of the N to the pod.

The proportion of N_2 derived from the atmosphere (%Ndfa) by V_2, V_4 and V_6 increased following inoculation. (Table 3).

The increase was, however, significant ($P \leq 0.05$) only in the case of V_2. Thus, the results obtained indicate the potentials of V_2, V_4 and V_6 for higher N_2 fixation when inoculated even with cowpea rhizobia. The enhanced %Ndfa of the three cultivars apparently led to a corresponding increase in pod yield with inoculation (Table 4).

Table 3: **Nitrogen derived from atmosphere (%ndfa) by soybean cultivars as influenced by *Bradyrhizobium* inoculation**

Cultivar	Uninoculated	Inoculated	Cultivar means
V_1	50.50[a]	47.40[a]	49.15
V_2	46.61[b]	57.27[a]	51.94
V_3	50.07[a]	45.93[a]	48.00
V_4	45.32[a]	49.57[a]	47.45
V_5	51.72[a]	37.36[b]	44.59
V_6	47.58[a]	52.60[a]	50.09
Inoculation means	48.70[a]	47.86[a]	

		Lsd (0.05)	Cv (%)
	Cultivar	NS	12.0
	Inoculation	6.89	14.0

Means followed by same letter (row-wise for cultivar under inoculation regimes and for means cultivar) are not significantly different ($P \leq 0.05$) according to Duncan multiple range test.

Table 4: **Pod yields (g/plant) of soybean cultivars as influenced by *Bradyrhizobium* inoculation**

Cultivar	Uninoculated	Inoculated	Cultivar means
V_1	2.44[a]	1.87[b]	2.16
V_2	3.92[a]	4.16[a]	4.04
V_3	2.72[a]	2.10[b]	2.41.
V_4	3.44[a]	3.72[a]	3.20
V_5	3.22[a]	2.66[b]	2.94
V_6	1.71[b]	2.88[a]	2.29
Inoculation means	2.91[a]	2.89[a]	

		Lsd (0.05)	CV (%)
	Cultivar	0.50	
	Inoculation	NS	29.0

Means following by same letter (row-wise for cultivar under inoculation regimes and for mean cultivar) are not significantly different ($P \leq 0.05$) according to Duncan multiple range test.

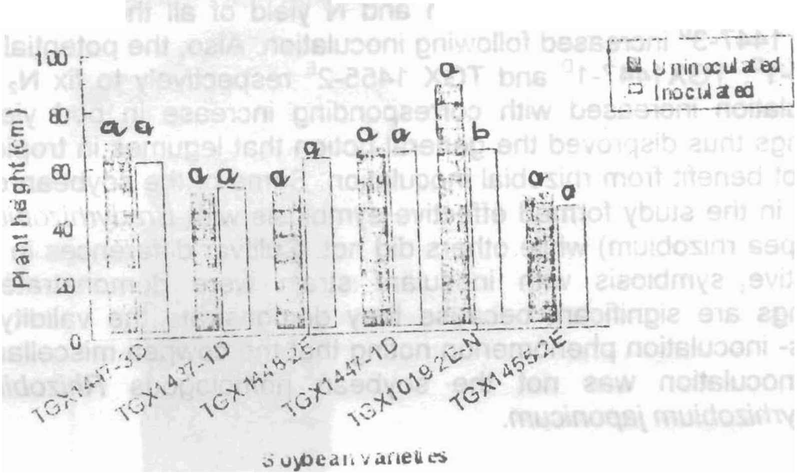

Fig 1

However, inoculation resulted in lower %Ndfa in V_3 and V_5 (Table 3) despite the increase shown in nodulation through inoculation (Table 1). Notably, V_5 which showed increased nodulation following inoculation had a significant ($P \leq .05$) reduction in %Ndfa, showing that profuse nodulation is not a reliable index of N_2 fixation. This finding agrees with the results obtained by Fried and Middlboe (1977) for bean in which profuse nodulation did not reflected in a correspondingly high nitrogen fixation. The depression in the height of V_5 (Fig 1) showed that it did not benefit from inoculation with the introduced cowpea rhizobia a non- homologous Rhizobium specie for soybean.).

It is apparent that the soybean cultivar V_5 symbioses better with the soil unsorted indigenous strain because it had the highest % Ndfa when grown in a non –inoculated soil (Table 3).

Pod Yield

The pod yields of the different soybean cultivars are shown in Table 4. Significant negative responses to inoculation were shown by V_1, V_3, and V_5. In contrast, increase in pod yield was observed for V_4. And V_6 as a result of inoculation. Pod yield was significantly greater in V_6 ($P \leq 0.05$) when grown in inoculated soil than in non-inoculated soil. The result showed that inoculation might contribute to pod yield depending on cultivar.

Conclusion

In this study, nodulation and N yield of all the cultivar except TGX 1447-3^D increased following inoculation. Also, the potential of TGX 1437-1^D, TGX1447-1^D and TGX 1455-2^E respectively to fix N_2 due to inoculation increased with corresponding increase in pod yield. The findings thus disproved the general notion that legumes in tropical soils do not benefit from rhizobial inoculation. Some of the soybean cultivars used in the study formed effective symbiosis with *Bradyrhizobium spp.* (cowpea rhizobium) while others did not. Culitvar differences in forming effective, symbiosis with inoculant strain were demonstrated. The findings are significant because they demonstrate the validity of the cross- inoculation phenomenon noting that the cowpea miscellany used for inoculation was not the soybean homologous *Rhizobium* i.e. *Bradyrhizobium japonicum.*

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