

Relationship between Indigenous Bradyrhizobia Population and the Degree of Nodulation and Nitrogen Fixation among Promiscuous Soybean (*Glycine max* (L) Merrill) Lines in Five Ghanaian Soils

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Abstract

In the light of conflicting reports, the earlier expectation that the promiscuous Tropical Glycine cross (TGx) varieties developed by the International Institute of Tropical Agriculture (IITA) in Ibadan would relieve farmers in Africa of the need to inoculate their soybean crops for optimum nitrogen fixation and yield has become questionable and calls for more verification. Soil must contain enough compatible bradyrhizobia population for optimum nodulation and nitrogen fixation of promiscuous soybean lines. The presence of indigenous bradyrhizobia nodulating promiscuous soybean was determined using the Most Probable Number (MPN) technique. Soil series such as Chichiwere contained 6.0×10^3 cells/g soil as against the minimum required level of 40×10^1 in Bekwai soils indicating that the soils contained enough bradyrhizobia for nodulation and nitrogen fixation. Greenhouse experiments were carried out at the University of Ghana-Legon to determine the effect of indigenous bradyrhizobia on nodulation, nodule dry weight %N and total N in five soils with local names Aveime (a Chromic cambisol) Hatso (a Gleic cambisol), Adenta (a Ferric acrisol), Chichiwere (a Dystric fluvisol), and Bekwai (a Ferric acrisol) in which nodulation, nodule dry weight, growth, nitrogen accumulation and nitrogen fixing abilities of three nodulating uninoculated soybean cultivars, comprising three promiscuous soybean lines bred in International Institute of Tropical Agriculture (IITA)-Ibadan, Nigeria such as Anidaso (TGx 813-6D), TGx 1448-2E and TGx 1903-8F soybean were examined using a non-nodulating soybean variety as the reference crop. Nitrogen fixed by the indigenous bradyrhizobia strains was measured by total nitrogen difference method between the fixing crop and the reference one. Chichiwere soil had the highest nodule number, nodule dry weight, shoot dry weight, total N accumulation as well as the highest N fixed recording an average of 66.8mgN/ plant equivalent to 86.4%N in the plant. TGx 1903-8F, the best symbiotic soybean cultivar in this study fixed as high as 91.0mgN/ plant indicating that even though more bradyrhizobia were found in Chichiwere soil series most were not compatible for nodule infection and nitrogen fixation. The nitrogen fixed by these soybean cultivars in descending order were as follows TGx 1903-8F < Anidaso < TGx 1448-2E.

Keywords: Anidaso, *Bradyrhizobium japonicum*, nitrogen fixation, non-nodulating soybean, promiscuous soybean.

INTRODUCTION

Between 1970s and the early 1980s IITA soybean scientists began breeding promiscuous soybean varieties so that they can nodulate freely with indigenous soil rhizobia. Nodulation of soybean by effective rhizobia can result in substantial amount of N being fixed by the symbiosis (Henson & Heibel, 1884; Legg and Slegger, 1975). Successful nodulation of these promiscuous soybeans depends on the presence of sufficient numbers of compatible and effective indigenous rhizobia. Because soybean is not indigenous to Africa, compatible *Bradyrhizobium japonicum* population are not widely distributed there. Soybean crops generally require large supplies of N to achieve their maximum yield potential (George & Singleton, 1992). The deficiency could be supplemented with N fertilizer or BNF in poor soils (Abaidoo, 1997). Naturally nodulating or promiscuous soybean variety are being widely adopted in some African countries such as Nigeria (Sanginga *et al.*, 1996). Zimbabwe (Javaheri, 1996; Mpeperekí *et al.*, 2000). Ghanaians are yet to adopt the promiscuous soybean varieties due to their total lack of confidence on the crop to supply its N requirement through BNF. Even though there are available data that soil N has a large influence on BNF (George *et al.* 1988) and factors such as temperature, soybean cultivar, bradyrhizobial strains, root nodules position and management practices also affects proportion of N derived from the atmosphere (Danso, *et al.*, 1987 ; Hardason, *et al.*, 1989), relationship between bradyrhizobial population in soil and N fixation(as measured by total amount of N in plant) is yet to be determined. Our study therefore was to determine nitrogen fixation potential of soybean genotypes in five uninoculated soils from two ecological zones in Ghana.

MATERIALS AND METHODS

Five soils series namely Bekwai, Chichiwere, Aveime, Adenta and Hatso were sampled from fields that had no

History of soybean growth. These soils were collected from 0-15cm depth. No *Bradyrhizobium* inoculant was applied to seeds or into any of these soils series, a move to see which of the soybean varieties would be most compatible with the indigenous bradyrhizobia. Soil pH was determined on a 1:2 soil to water ratio. Available soil P was determined (Olsen and Dean, 1965). Total soil N was estimated using the Kjeldahl digestion method (Bremner, 1996). The most probable Number (MPN) method (Woomer *et al.*, 1990) was used to estimate TGx bradyrhizobia populations in the five soils. Plastic pots (18cm high, 15cm wide at the top and 12 cm at the base) with four holes made at the bottom were each filled with 1.2 kg of 2mm size sieved soil. Seeds of three promiscuous soybean varieties, Anidaso, TGx 1448-2E and TGx 1903-8F were surface sterilized (Somasagaran and Hoben, 1994), pregerminated on 1% (w/v) water agar and incubated at 28°C for three days. A non-nodulating (non nod) soybean genotype was used as a reference crop. Four seeds were planted per pot and thinned to two at 8 days after planting. The experiment was conducted in a green house within the Geography Department of University of Ghana, Legon.

The plants were watered daily with tap water. Six weeks after planting, shoots were decapitated and nodules were separated from roots after careful washing and counted.

Nitrogen fixation by Total Nitrogen Difference (TND) method is calculated as follows:

N₂ Fixation = N_{total} (fixing legume) - N_{total}(reference crop). (Rennie, 1984)

Statistical Analysis: Genstat statistical software version 6.1 Genstat, 2000) was used to analyse the data. Significant differences were assessed at 5% level and mean separation was carried out by Least Significant Difference (LSD) procedure

RESULTS

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The chemical analyses conducted on the five soils used show that the Bekwai soil was the most acidic, followed by the Chichiwere soil, while all five soils had sub optimal levels of available P for plant growth (Table 1).

Most Probable Number (MPN): The MPN tests conducted indicated the presence of *Bradyrhizobium* sp. in all five soils with the numbers and ranking being as follows ($4.0 \times 10^1/\text{gsoil}$) < Aveime ($1.0 \times 10^3/\text{gsoil}$) < Hatso ($4.0 \times 10^3/\text{gsoil}$) < Adenta ($4.6 \times 10^3/\text{gsoil}$) < Chichiwere ($6.6 \times 10^3/\text{gsoil}$)

Nitrogen Fixation Measurement: There was a significant difference ($P < 0.05$) among the soil series, varieties of soybean and soil and variety interaction in terms of nodulation of soybean by indigenous bradyrhizobia strains in the soils (Table 2). With the exception of the Bekwai soil in which no nodules were formed on any of the four soybean varieties, nodules were observed on all varieties grown in the other four soils. Chichiwere soils recorded the highest average nodules of 18 with Aveime soil being the least in terms of nodule formation (8). With the varieties, Anidaso with the mean of 17 nodules plant⁻¹ supported highest nodulation but this was statistically similar ($P < 0.05$) to what was formed on TGx 1903-8F (16 nodules plant⁻¹) but higher than TGx 1448-2E (11 nodules). Similarly, significant differences ($P < 0.05$) occurred among the four soybeans varieties and the five soil series with regards to nodule dry weight, and with soil x variety interaction. The ranking among varieties for nodule dry weight was similar to that for mean number of nodules (Table 3). The highest nodule dry weight was recorded in Chichiwere soil series (158.8mg plant⁻¹) about two times as high as that in Aveime soil (117.5mg plant⁻¹). Mean nodule dry weight values for the intermediates, plants grown on the Hatso and Adenta soils (113.1 and 134.6 mg plant⁻¹) were statistically similar ($P < 0.05$).

Besides significant differences ($P < 0.05$) existed among shoot dry weight of soybean plants grown in different soils (Table 4). Chichiwere and Hatso soils appeared better in terms of mean shoot dry weight producing the highest values (3.33g and 3.01 respectively) while Bekwai soil which did not form any nodule recorded the lowest value of 0.96g plant⁻¹. Soybean varieties also affect growth. TGx 1903-8F soybean variety appeared best recording mean shoot dry matter yield of 3.03g plant⁻¹ while the non-nodulating soybean recording the lowest value (1.50g) in terms of mean shoot dry weight.

Although TGx 1903-8F overall accrued the highest plant N (57.3 mgN plant⁻¹). It is statistically similar ($P < 0.05$) to that of Anidaso but higher than TGx 1448- 2E (47.6). Soil effect appeared more pronounced than varietal influence on differences in the total N acquired by plants (Table 5). Hatso soils recorded the highest mean value in terms of N accumulation in plant (61.4 mgN plant⁻¹) with plant grown in Bekwai soil which did not support nodulation accumulating only about a ninth (6.8 mgN plant⁻¹). Significance difference ($P < 0.05$) did not exist neither in the four soil series (Hatso, Adenta and Chichiwere) except Aveime nor among soybean varieties except TGx 1448-2E and TGx 1903-8F. TGx 1903-8F did not only fix the highest N (by Nitrogen difference method) but also produce the highest score in terms of Nitrogen fixation (57.1mgN plant⁻¹). Chichiwere soil appeared best in terms of Nitrogen fixation (66.8 mgN plant⁻¹) while Aveime soil recording the lowest mean nitrogen being fixed (19.3 mgN plant⁻¹) (Table 6). There was significant difference ($P < 0.05$) in the %N fixed between Aveime soil and other three soil series (Hatso, Adenta and Chichiwere) but the varieties and variety x soil interaction were insignificant at the same probability level. (Table 7) Chichiwere soil recorded the highest mean % N fixation (86.4%) and the least mean % N fixation was recorded in Aveime

soil (64.4%). All the three promiscuous soybean varieties (TGx 1903-8F, TGx 1448-2E and Anidaso) were statistically similar. Anidaso soybean variety also recorded the highest % N in Chichiwere soil (89.8%) but was outcompeted by TGx 1903-8F in overall mean N fixation in all the five soils (78mgN plant⁻¹).

DISCUSSION

With no *Bradyrhizobium* cells inoculated unto seeds or into any of the soils used, any nodules formed on soybean in this study must have originated from infection by the indigenous bradyrhizobia in that soil. The extent to which nodulation occurred was therefore a reflection on the achievement of the objective for breeding for the TGx cultivars thus they would nodulate freely without artificial inoculation (Okogun and Sanginga 2003). The fact that the soybean varieties used in this study, attests to their being promiscuous. That the breeding programme must have given rise to range in promiscuity is the evident from the observed differences in nodulation between the soybean varieties. Optimum nodulation and high nitrogen fixation require the presence of high numbers of indigenous rhizobia (Abaidoo et al. 2007; Fening and Danso, 2002) and is a reliable indicator of whether or not the soybean will respond to added rhizobia or fertilizer (Thies et al. 1991a). However, the highest nodule number recorded in Chichiwere soil series reflected their more numerous indigenous bradyrhizobia over the other soil series. Since soils often vary considerably in the nature and abundance of their established bradyrhizobial populations (Singleton and Tavares, 1986; Owiredo and Danso, 1988), this could all things being equal contribute to differences in the nodulation of plants grown on different soils. A bradyrhizobial population of 10³g⁻¹ soil would normally be considered high, and enough for optimum nodulation of many tropical legumes (Danso and Owiredo, 1988). It is thus not surprising that with each of the Chichiwere, Adenta, Hatso and Aveime soils containing in excess of 10³ bradyrhizobia g⁻¹ soil, soybean grown in them nodulated well and that the generally lower nodulation observed on the Aveime and Hatso soils than on the Adenta and Chichiwere soils could possibly be attributed to soil factors. However, the report of Thies et al. 1991b indicated that soils subjected to constraints would require greater numbers of rhizobia to nodulate and fix N₂ than those without. It is therefore highly probable that the Bekwai soil being low in both pH and available P as shown in Table 1 may have raised the minimum requirement for the number of bradyrhizobia cells required for nodule formation in this soil. From the significant soil x variety interaction obtained, for nodulation, and nitrogen fixation, it may furthermore be inferred that no single variety was best suited for all soils, and that for best results, it may be prudent to select for varieties to suit different soils and environments.

In general, the entire promiscuous soybean lines nodulated fairly well with indigenous bradyrhizobia and this is in agreement with work done by Singh and Rachie (1987). This also confirmed findings of Pulver *et al.*, (1982 and 1985) and Nangju (1980). The poor performance in nodulation by TGx 1448-2E is a confirmation of earlier work done by Okogun and Sanginga (2003) in Nigeria. Anidaso soybean appeared best in terms of nodulation perhaps due to the fact that its earlier introduction into Ghanaian soils made it better adapted to cowpea rhizobia. Both %N and total N fixation estimated by the Total Nitrogen Difference method (TND) was highest in Chichiwere soil which had the highest bradyrhizobia population, nodule number and nodule dry weight. The lowest N fixed in Aveime confirmed its low number of bradyrhizobia and its high %N (Table 1). No N was fixed in Bekwai soil because of its inability to produce nodules on all soybean varieties. My results demonstrated a link between bradyrhizobia numbers, nodulation and nitrogen fixation. Of the three promiscuous soybean, the two (Anidaso and TGx 1903-8F) that accumulated higher N and produced higher shoot dry weights than TGx 1448-2E were also associated with higher nodulation and nitrogen fixation, suggesting that the higher N demand could have stimulated higher nitrogen fixation (Thies et al. 1991b). Mean N accumulated in Chichiwere (59.4mgN plant⁻¹) was more than twice as much as in Aveime (25.0mgN plant⁻¹). This might be due to differences in N accumulated in fixation as supported by the fact that for the same parameter, N accumulated by non-nodulating soybean (which was not expected to fix N₂) is slightly higher in Aveime soil (10.5mgN plant⁻¹) than in Chichiwere. It is interesting to note that although Anidaso was promising in terms of nodulation, its dry matter yield and N accumulation compared to TGx 1903-8F were poor. This may be due to the fact that many of Anidaso's nodule bradyrhizobia were not effective enough to form nodules and fix nitrogen. In this study TGx 1903-8F fixed 91.0mgN/plant in Hatso soil followed by Anidaso (89.0mgN plant⁻¹) in Chichiwere soil. This result contradicts Bezdicsek *et al.*, (1978) which showed that soybeans are capable of fixing over 300mg N/plant when effective strains of bradyrhizobia are supplied in high numbers and the soil is also low in available nitrogen. Averagely, TGx 1903-8F fixed the highest % N followed by Anidaso perhaps due to its higher dry matter yield compared to Anidaso.

CONCLUSION

It can be concluded that nodulation and nitrogen fixation were directly proportional to indigenous bradyrhizobia population in soils. For example Chichiwere soil had the highest bradyrhizobia population and recorded the highest nodule number and N₂ fixation as well as compared to Aveime soil having the lowest bradyrhizobia population and produced fewer nodules and suboptimal N₂ fixation as well. Either low bradyrhizobia population,

low pH might have been responsible for no nodulation and no N₂ fixation in Bekwai soil. Nonetheless Hatso soil also exhibited its superiority over Adenta soil over nodulation and N₂ fixation due to its numerous bradyrhizobia population. It can be confirmed therefore that nodulation and N₂ fixation had a direct relationship with bradyrhizobial population in the soil.

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Table 1. Some physical and chemical properties of five soils used for growing uninoculated soybean

Soil Series	pH(in H ₂ O)	% N	Total P(mgkg ⁻¹)	Available P(mgkg ⁻¹)	Calcium (Me/100)
Aveime	7.0	0.85	127	5.83	4.4
Hatso	6.5	0.43	52	7.81	4.2
Adenta	6.3	0.31	120	5.73	2.0
Chichiwere	6.0	0.33	126	9.49	4.8
Bekwai	5.9	0.23	173	4.40	5.4

Table 2: Mean nodule number per plant of four soybean varieties in five different uninoculated soils

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	15.8	16.8	23.5	26.3	0.0	16.6
TGx 1448-2E	7.0	16.0	15.8	20.3	0.0	11.2
TGx 1903-8F	8.3	24.5	20.8	26.0	0.0	15.9
Non-nod	0.0	0.0	0.0	0.0	0.0	0.0
Mean	7.8	14.3	14.9	18.3	0.0	-

LSD (P < 0.05); Soil =2.8, Variety =2.5, Soil ×Variety =5.6

FIELD DATA JULY 2015

Table 3: Mean nodule dry weight in mg per plant of four soybean varieties in five different uninoculated soils

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	117.5	162.5	180.0	262.5	0.0	144.5
TGx 1448-2E	55.0	122.5	112.5	140.0	0.0	86.0
TGx 1903-8F	72.5	167.5	142.5	232.5	0.0	123.0
Non-nod	0.0	0.0	0.0	0.0	0.0	0.0
Mean	61.2	113.1	108.7	158.8	0.0	-

LSD (P < 0.05); Soil = 17.7, Variety =15.8, Soil ×Variety =35.3

FIELD DATA JULY 2015

Table 4: Mean shoot dry weight in g per plant of four soybean varieties in five different uninoculated soils

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	2.37	3.44	2.65	3.81	1.50	2.75
TGx 1448-2E	2.19	2.63	2.51	2.67	0.89	2.18
TGx 1903-8F	2.75	3.76	3.16	4.55	1.10	3.03
Non-nod	0.97	2.19	1.68	2.31	0.37	1.50
Mean	2.02	3.01	2.50	3.33	0.96	

LSD (P < 0.05); Soil = 0.35, Variety = 0.31, Soil × Variety = 0.70

FIELD DATA JULY 2015

Table 5: Total N (mg/plant) accumulation by four soybean varieties in five different uninoculated soils

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	30.8	68.2	64.6	98.3	6.7	53.7
TGx 1448-2E	28.8	55.3	92.0	53.1	8.8	47.6
TGx 1903-8F	29.8	106.3	64.3	77.0	8.7	57.3
Non-nod	10.5	15.6	14.0	9.3	2.9	10.5
Mean	25.0	61.4	58.7	59.4	6.8	-

LSD (P < 0.05); Soil = 9.4, Variety = 8.4, Soil × Variety = 18.5

FIELD DATA JULY 2015

Table 6: Total N (mg/plant) fixed by three soybean varieties in five different uninoculated soils

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	20.3	52.6	50.6	89.0	-*	53.1
TGx 1448-2E	18.3	39.7	78.0	43.8	-*	45.0
TGx 1903-8F	19.3	91.0	50.2	67.7	-*	57.1
Mean	19.3	61.1	59.6	66.8	- *	-

LSD (P < 0.05); Soil = 11.2, Variety = 10.0, Soil × Variety = 106.9

Table 7: Percent N fixation by three soybean varieties in five different uninoculated soils.

Varieties	Soil series					Mean
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	
Anidaso	65.3	76.0	76.3	89.8	-*	77.1
TGx 1448-2E	62.2	69.9	84.4	82.9	-*	74.9
TGx 1903-8F	65.7	84.3	75.4	86.4	-*	78.0
Mean	64.4	77.0	78.7	86.4	-*	-

LSD (P < 0.05); Soil= 11.9, Variety =10.7, Soil x Variety =23.9

-* No % N fixation was assessed in Bekwai soil because soybean was not nodulated in this soil.

FIELD DATA JULY 2015