#### **International Journal of Plant Science and Ecology**

Vol. 2, No. 2, 2016, pp. 15-22

http://www.aiscience.org/journal/ijpse

ISSN: 2381-6996 (Print); ISSN: 2381-7003 (Online)



# Response of Promiscuous and Non-promiscuous Soybean (*Glycine max* (L) Merill) Cultivars to Indigenous *Bradyrhizobium japonicum* Inoculation in Three Ghanaian Soils

Klogo Phanuel Y.<sup>1, \*</sup>, Ofori Johnson K.<sup>2</sup>, Glover Mawutor K.<sup>1</sup>, Avumegah Patrick K.<sup>1</sup>

#### **Abstract**

Soybean, a recently introduced leguminous crop in West Africa, does not usually nodulate satisfactorily unless it is inoculated with selected rhizobial strains. The development of the Tropical Glycine Cross (TGx) soybean varieties by IITA has made it possible for this promiscuous varieties to nodulate with the naturally occurring strains with source belonging to the cowpea bradyrhizobia spp. For many tropical countries particularly in Africa, Biological Nitrogen Fixation (BNF) continues to be the most promising alternative or supplement to the use of chemical Nitrogen fertilizers for sustainable Agriculture. Greenhouse experiments were carried out at the University of Ghana-Legon to determine the effect inoculation of soybean with bradyrhizobia has on nodulation, nodule dry weight %N and total N in three promiscuous soybean varieties, Bengbie, TGx 1903-7E and TGx 1910-2F, and the cultivar Bragg, anon-promiscuous genotype in three Ghanaian soil series, Oyibi (Sodic solunchaus), Hake (Eutric cambisol) and Nzima (Ferric acrisols). The Most Probable Number (MPN) technique was used to determine the bradyrhizobial population in these soils. Eighty four Bradyrhizobium isolates obtained from randomly selected nodules were assessed for effectiveness in nitrogen fixation. Three of the most effective Bradyrhizobium isolates were used in the inoculation studies. There were tremendous inoculation responses in these soils for both the promiscuous and nonpromiscuous cultivars, with even the promiscuous ones responding better to inoculation than the non-promiscuous Bragg. Inoculation gave rise to significant increases in nodule number, nodule dry weight, shoot dry weight and total nitrogen accumulation compared to the uninoculated control. However, the response of these soybean cultivars was higher in Hake soil series despite the high number of numerous indigenous bradyrhizobia strains in this soil which might have offset the inoculation response in this soil.

#### **Keywords**

Bradyrhizobium japonicum, Effectiveness, Inoculation, Non-promiscuous Soybean, Promiscuous Soybean

Received: May 5, 2016 / Accepted: May 17, 2016 / Published online: June 1, 2016

@ 2016 The Authors. Published by American Institute of Science. This Open Access article is under the CC BY license. <a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a>

### 1. Introduction

Soybean as an introduced crop often nodulate poorly in most Ghanaian soils hence it is necessary to inoculate it is effective bradyrhizobia strains for nitrogen to be fixed.

Following infection of soybean by bradyrhizobia to form nodules, N from the air is converted into a form readily available to the plant. The International Institute of Tropical Agriculture (IITA) developed promiscuous soybean varieties,

E-mail address: phanuely@yahoo.com (Klogo P. Y.)

<sup>&</sup>lt;sup>1</sup>Agro Enterprise Development Department, Faculty of Applied Sciences and Technology, Ho Polytechnic, Ho, Ghana

<sup>&</sup>lt;sup>2</sup>Agricultural Engineering Department, Faculty of Engineering, Ho Polytechnic, Ho, Ghana

<sup>\*</sup> Corresponding author

which are capable of establishing symbiotic relationship with indigenous bradyrhizobia, as a practical alternative to inoculation by African farmers [8]. Even though there were yield response by non-promiscuous soybean to inoculation, this was not significant in the promiscuous soybean lines [20]. This raised the question of the effectiveness of the bradyrhizobia 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 bradyrhizobia will out compete the inoculant strains. Recent studies with these promiscuous soybean lines, however, have shown considerable variability in the effectiveness and population communities of indigenous bradyrhizobia in a given location [23; 10]. [22] also found a direct relationship between bradyrhizobia cell counts and promiscuous soybean response. Thus promiscuous soybean may also need inoculation with exotic bradyrhizobia depending on effectiveness and indigenous bradyrhizobia in the locality [19], as well as the degree of promiscuity of the soybean variety [23]. There is therefore the need to examine the response of promiscuous soybean lines to inoculation in more soil series using different types of soybean varieties to assess whether effective bradyrhizobia strains capable of nodulating promiscuous soybeans occur in Ghanaian soils. Our objective of this study is to isolate bradyrhizobia from promiscuous and non- promiscuous soybean genotypes and to determine their effectiveness in nodulation and nitrogen fixation (as measured by total nitrogen accumulation in the plant).

## 2. Materials and Methods

The three soils used include Oyibi, Hake and Nzima. These soils were sampled from fields that had no history of soybean cultivation in order to establish the fact that no introduced crop enabled the soil to harbor the rhizobia strain. Soil samples were collected from 0-20cm depth to ensure that the soil series is actually a true representation of the area. Three promiscuous soybean namely Bengbie, TGx 1903-7E and TGx 1910-2F and a non-promiscuous variety, Bragg all obtained from the seed store of Crop Science Department, University of Ghana-Legon were used in the experiment

Soil pH was determined in both distilled water and in 0.01M CaCl<sub>2</sub> using pH meter. Available P was determined using a spectrometer [4]. Total soil N was estimated using Kjeldahl method [2]. The Most Probable Number (MPN) count was used to determine the number of infective rhizobia in soils [28]. Bradyrhizobia were isolated from randomly selected soybean and a loopful of the suspension was then streaked on yeast extract mannitol (YEM) agar plates and incubated at 28°C to assess their growth [26]. The bradyrhizobial isolates

were assessed for their effectiveness on sand obtained from the Densu river bed using soybean cultivar Bengbie as a reference crop described by [12]. There were three replicate jars for each Bradyrhizobial isolate. Uninoculated seedlings grown in jars supplied with nitrogen (70ugml<sup>-1</sup> KNO<sub>3</sub>) [26] and without nitrogen served as control. The inoculated plants and the uninoculated ones without nitrogen were supplied with N-free nutrient solution [3] Plants were harvested 42 days after planting and nodule number was counted, nodule dry weight and shoot dry weight were also recorded. Shoots were severed from their roots at the collar, put in labelled envelopes and oven-dried at 70°C for 72 hours after which their dry weights were taken.

The 'equation below was used to calculate the effectiveness index (Ej)

$$E_J = \frac{X_J - X_{TO}}{X_{TN} - X_{TO}} \times 100 \text{ [12]}.$$

Where, X is the mean dry weight of shoot, J is the shoot dry weight of inoculated test strain, TO is that of the uninoculated control and TN that of the nitrogen control. Plant dry weight value of each inoculation treatment was compared with those of the N-controls and the LSD at P = 0.01 level was used to delineate isolates significantly different from the N control [1]. Classes of effectiveness were defined from comparison with the N controls. Thus as criteria for grouping isolates, isolates with effective index more than 80% were classified as highly effective, effective index between 50% and 80% was classified as moderately effective and isolates with effective index less than 50% were classified as ineffective.

Three most effective indigenous bradyrhizobial isolates (Isolates 19, 37 and 57) were used for the inoculation. Each soil type was air-dried and the soil aggregates were gently crushed in a mortar using pestle to pass through a 2mm mesh sieve. The experiment was conducted in the Greenhouse of the Ecological Laboratory within the Geography department of University of Ghana with maximum and minimum day and night temperatures being 35°C and 23°C respectively. For inoculation, a 1ml, washed rhizobial suspension (containing about  $10^8$  cells) was diluted to 50 ml in sterile distilled water and mixed thoroughly with 1.5 kg soil contained in plastic pots (18cm high, 15cm wide at the top and 12cm at the base). Each pot was therefore inoculated with about  $6.0 \times 10^4$  cells g- $^1$  soil.

Uninoculated soil served as control. All soils were fertilized with essential macro and micro-nutrients except N [7]. Four surface—sterilized soybean seeds [27] were planted in each pot, and thinned to two after germination. The treatments were replicated four times in a completely randomized split-

block design with soils as the main blocks. Plants were watered daily with distilled water and were harvested six weeks of growth. The shoots were oven dried for 70°C for 48hrs and Kjeldahl N analysis [2] was done on ground samples (<0.2mm). Nodules were sun-dried and stored in

serum bottles. Statistical analysis was done using Genstat statistical software version 6.1 [13]. Significant differences were assessed at 5% level. Mean separation was carried out by Least Significant Difference (LSD) procedure.

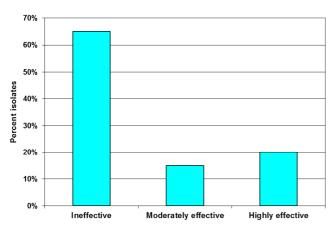
#### 3. Results

Table 1. Some physical and chemical properties of three soils used for growing inoculated soybean.

Soil Series	pH(in H <sub>2</sub> 0)	% N	Available P(mgkg-1)	PotassiumMe/100g	MagnesiumMe/100g
Oyibi	5.3	0.32	6.08	0.64	3.0
Hake	5.2	0.33	9.49	1.28	4.8
Nzima	4.8	0.23	4.40	1.28	8.2

Most Probable Number (MPN): The ascending order of MPN used to enumerate bradyrhizobial population in the soils include Nzima (2.5 x  $10^1$ /gsoil) <Oyibi (6.2 x  $10^2$ /gsoil) <Hake (6.0 x $10^3$ /gsoil)

Effectiveness grouping of 84 bradyrhizobial isolates: 55 were ineffective (65%), 12 (15%) were moderately effective while 17 (20%) were highly effective. The highest effectiveness index was detected in isolates number 37 (128%) and it also accumulated the highest nitrogen value of 177.85mgN followed by isolate 57 (114%) and 19 (111%)



**Figure 1.** Effectiveness grouping of 84 *Bradyrhizobium* Isolates obtained from soybeans grown in Ghanaian soils.

Nodulation, dry matter yield and nitrogen fixation: Fewer nodules developed on the uninoculated soybean in both promiscuous and non-promiscuous varieties in all the soil series tested than the inoculated ones. Both promiscuous and non- promiscuous soybean varieties failed to nodulate in Nzima soils but with the application of the inoculants strain nodulation occurred with the promiscuous variety TGx 1903-7E recorded the highest value (40 nodules plant<sup>-1</sup>) compared to the non-promiscuous soybean varieties in the Nzima soils. (Table 2). There were significant differences (P<0.05) among *Bradyrhizobium* isolates used with isolate 37 effecting the production of high nodule number in Oyibi (26 nodules plant<sup>-1</sup>) and Nzima (34 nodules plant<sup>-1</sup>). Nonetheless the highest mean

number of nodules per plant was recorded by Bradyrhizobium isolate 19 in Hake soil series (50 plant<sup>-1</sup>). Hake soils produced the highest nodulation in both uninoculated and the inoculated soils (18 and 49 nodules plant<sup>-1</sup>) respectively. There was significant difference (P<0.05) among the soybean varieties in Hake soils in terms of nodulation. While Bengbie recorded the highest nodule number (56 nodules plant<sup>-1</sup>), the least number of nodules recorded by Bragg in Hake soil (35 nodules plant<sup>-1</sup>) is higher than all the nodule number values recorded by the soybean varieties in Oyibi soil. The highest nodule value was produced in Bengbie by isolate 19 in Hake soil (61 nodules plant<sup>-1</sup>) compared to lowest value recorded by Davis (25). Surprisingly, all the soybean varieties in exception of TGx 1910-2F (22 nodules plant<sup>-1</sup>), all the other soybean varieties in Nzima soil recorded higher mean nodule numbers than all soybean varieties in Oyibi soils.

Nodule dry weight of inoculated soybean plants were significantly (P<0.05) greater than for the uninoculated counterparts. In exception of Nzima soil, Bengbie appeared a better nodule dry weight producer than all the soybean varieties (Table 3). Bengbie recorded the highest nodule dry weight (514mg plant-1) in Hake soil while the least nodule dry weight was recorded in Oyibi soil by TGx 1910-2F (103 mg plant<sup>-1</sup>). Bragg, the non-promiscuous soybean varieties produced the higher nodule dry weight (351mg plant<sup>-1</sup>) in Hake soil than all the soybean varieties in both Oyibi and Nzima soils. Significant difference (P<0.05) occurred among Bradyrhizobium isolates in nodule dry weight production. Isolate 57 produced the highest nodule dry weight (450 mg plant<sup>-1</sup>) in Hake soils; Isolate 37 recorded the highest nodule dry weight in both Oyibi (149 mg plant-1) and Nzima soils (302 mg plant<sup>-1</sup>). On the whole Bengbie maintained its superiority in terms of nodule dry weight production in both inoculated and uninoculated soils. Apart from the Oyibi soil in which the non-promiscuous Bragg produced nodule dry weight (145 mg plant<sup>-1</sup>) higher than the promiscuous TGx 1910-2F (103 mg plant<sup>-1</sup>) the non-promiscuous soybeans in both Hake and Nzima soils produced lower nodule dry weight (351 mg and 213 mg plant<sup>-1</sup> respectively) than their promiscuous counterparts. Even though TGx 1910-2F produced more nodules than Bragg, this did not reflect in their nodule dry weight production sincethe reverse was true as Bragg produced more nodule dry weight (145 mg plant<sup>-1</sup>) than TGx 1910-2F (103 mg plant<sup>-1</sup>)

Shoot dry weight of inoculated soybean plants also showed a greater and significantly higher yield (P<0.05) than the uninoculated controls. TGx 1903-7E produced the highest shoot dry weight in Hake soils (5.98 g plant<sup>-1</sup>) followed by Bengbie in Oyibi soil (4.19 g plant<sup>-1</sup>) (Table 4). Interestingly, Bragg superseded Bengbie and other two promiscuous soybeans in mean shoot weight production in one out of the three soils. This was recorded in Nzima soil (3.71 g plant<sup>-1</sup>) Inoculum 57 appeared better by producing more shoot dry weight in all the three soils. These soils are Hake (5.56 g plant<sup>-1</sup>), Oyibi soils (4.02 g plant<sup>-1</sup>) and Nzima soil (3.49 g plant<sup>-1</sup>). Without inoculation, the highest shoot dry weight was recorded in Hake soil by TGx 1903-7E (3.07 g plant<sup>-1</sup>)

Inoculation generally increased the total N fixation by way of nitrogen accumulation by soybean plants in all the three soils. Whilst significant differences (P<0.05) existed in some of the soybean varieties in terms of nitrogen fixation some soybean varieties are statistically similar. For example while TGx 1910-2E and Bragg are statistically similar in nitrogen

fixation (90.5 mgN plant<sup>-1</sup> and 88.2 mgN plant<sup>-1</sup>) respectively in Oyibi soil, TGx 1903-2F and Bengbie are statistically different in terms of nitrogen fixation (100.9 and 151.9 mgN plant<sup>-1</sup>) respectively. (Table 6).

TGx 1910-2E performed better recording the highest nitrogen fixation value in Nzima soil (150.2 mgN plant<sup>-1</sup>) with TGx 1903-7F recording the least N fixation (99.6 mgN plant<sup>-1</sup>). Surprisingly Bragg in Nzima soil fixed more N (109.4 mgN plant<sup>-1</sup>) than allthe soybean varieties in the Oyibi soil except Bengbie (151.9 mgN plant<sup>-1</sup>). Significant difference (P<0.05) existed among soybean varieties in Hake soil which also recorded the highest value in terms of N fixation by TGx 1903-7F (283.3 mgN plant<sup>-1</sup>) with the least nitrogen fixation being Bragg (126.4 mgN plant<sup>-1</sup>) in Hake Significant difference(P<0.05) existed between uninoculated and inoculated soybean varieties. The Bradyrhizobium inoculums 57 fixed the highest nitrogen in two out of the three soils recording 125.5 mgN plant<sup>-1</sup> in Oyibi and 218 mg plant<sup>-1</sup> in Hake soils. Inoculum 19 fixed the highest N in Nzima soil (141.3mgN plant-1). The highest N value was recorded in the uninoculated Hake soil by TGx 1903-7F (97.1mgN plant<sup>-1</sup>). Averagely the *Bradyrhizobium* isolates 19, 37 and 57 respectively performed better in the uninoculated and the inoculated Hake soils (68.2, 207.4, 205.4 and 218.7 mgN plant-1).

**Table 2.** Effect of inoculation with indigenous *Bradyrhizobium japonicum* isolates on nodule number per plant of four varieties of soybean in three different soils.

Soil Series	Varieties	No Inoculation	Inoculum				
			Isolate 19	Isolate 37	Isolate 57	Mean	
Oyibi	Bengbie	6.0	24.0	27.3	23.3	24.9	
	TGx 1910-2F	1.7	21.0	23.0	22.0	22.0	
	TGx 1903-7E	5.7	22.3	29.7	27.0	26.3	
	Bragg	1.3	22.7	22.0	20.7	21.8	
	Mean	3.7	22.5	25.5	23.4	-	
Hake	Bengbie	24.3	61.3	54.7	52.7	56.2	
	TGx1910-2F	23.7	55.7	52.7	53.3	54.0	
	TGx 1903-7E	24.3	44.3	49.3	58.3	50.6	
	Bragg	1.3	37.7	35.7	31.3	35.0	
	Mean	18.4	49.7	48.1	48.9	-	
Nzima	Bengbie	0.0	27.3	38.3	30.3	32.0	
	TGx1910-2F	0.0	22.7	24.0	19.7	22.1	
	TGx 1903-7E	0.0	42.7	40.3	35.7	39.6	
	Bragg	0.0	27.3	34.3	31.0	30.9	
	Mean	0.0	30.0	34.2	29.2	-	

LSD (P < 0.05); Soil =2.30, Variety =2.65, Inoculant= 2.65 Soil ×Variety =4.60 Soil x variety x inoculants =9.19

**Table 3.** Effect of inoculation with indigenous *Bradyrhizobium japonicum* isolates on nodule dry weight in mg per plant of four varieties of soybean in three different soils.

Soil Series	Varieties	No	Inoculum				
		Inoculation	Isolate 19	Isolate 37	Isolate 57	Mean	
Oyibi	Bengbie	100.0	186.7	150.0	186.7	174.5	
	TGx 1910-2F	40.0	110.0	100.0	100.0	103.3	
	TGx 1903-7E	103.3	146.7	160.0	163.3	156.7	
	Bragg	53.3	120.0	186.7	126.7	144.5	
	Mean	74.2	140.9	149.2	144.2	-	
Hake	Bengbie	323.3	530.0	453.3	560.0	514.4	
	TGx 1910-2F	220.0	466.7	430.0	460.0	452.2	
	TGx 1903-7E	276.7	426.7	430.0	446.7	434.5	
	Bragg	60.0	373.3	346.7	333.3	240.0	
	Mean	293.3	449.2	415.0	450.0	-	
Nzima	Bengbie	0.0	293.3	343.3	300.0	312.2	
	TGx 1910-2F	0.0	233.3	240.0	216.7	229.8	
	TGx 1903-7E	0.0	283.3	360.0	283.3	308.9	
	Bragg	0.0	260.3	263.3	213.3	245.6	
	Mean	0.0	292.5	301.7	253.3	-	

LSD (P < 0.05); Soil =19.3 Variety =22.3, Inoculant= 22.3Soil ×Variety =38.Soil x variety x inoculants =77.3

**Table 4.** Effect of inoculation with indigenous *Bradyrhizobium japonicum* isolates on shoot dry weight (g) per plant of four varieties of soybean in three different soils.

0.10	Varieties	No	Inoculum				
Soil Series		Inoculation	Isolate 19	Isolate 37	Isolate 57	Mean	
Oyibi	Bengbie	1.82	4.35	3.79	4.43	4.19	
	TGx 1910-2F	1.37	3.82	3.74	3.76	3.80	
	TGx 1903-7E	1.96	3.75	3.92	4.00	3.89	
	Bragg	2.15	3.88	3.83	3.87	3.86	
	Mean	1.83	3.95	3.82	4.02	-	
Hake	Bengbie	2.77	5.77	5.66	6.23	5.89	
	TGx 1910-2F	2.55	4.79	5.56	5.76	5.37	
	TGx 1903-7E	3.07	6.11	5.65	6.19	6.00	
	Bragg	2.40	4.54	4.99	4.06	4.53	
	Mean	2.70	5.30	5.47	5.56	-	
Nzima	Bengbie	1.63	3.28	3.08	3.48	3.28	
	TGx 1910-2F	1.67	3.67	2.94	3.26	3.29	
	TGx 1903-7E	1.69	3.26	3.38	3.43	3.36	
	Bragg	2.19	3.70	3.67	3.77	3.71	
	Mean	1.80	3.48	3.27	3.49	-	

LSD (P < 0.05); Soil =0.15 Variety =0.17, Inoculant= 0.17Soil ×Variety = 0.30Soil x variety x inoculants =0.60

Table 5. Effect of inoculation with indigenous Bradyrhizobium japonicum isolates on %N per plant of four varieties of soybean in three different soils.

Soil Series	Varieties	No Inoculation	Inoculum				
			Isolate 19	Isolate 37	Isolate 57	Mean	
Oyibi	Bengbie	1.45	3.52	3.09	4.23	3.61	
	TGx 1910-2F	1.28	2.37	2.15	2.61	2.38	
	TGx 1903-7E	1.14	2.65	2.31	2.82	2.59	
	Bragg	1.42	2.22	2.03	2.63	2.29	
	Mean	1.32	2.69	2.40	3.07	-	
Hake	Bengbie	2.72	4.25	3.75	4.58	4.19	
	TGx 1910-2F	3.15	4.01	4.03	3.84	3.96	
	TGx 1903-7E	3.20	4.50	4.20	3.85	4.18	
	Bragg	0.85	2.56	2.90	2.94	2.80	
	Mean	2.48	3.83	3.72	3.80	-	
Nzima	Bengbie	1.62	3.89	3.96	2.70	3.52	
	TGx 1910-2F	0.66	5.59	4.45	3.44	4.49	
	TGx 1903-7E	0.65	3.59	2.71	2.62	2.97	
	Bragg	0.81	2.81	2.88	2.98	2.89	
	Mean	0.94	3.97	3.50	2.91	_	

LSD (P < 0.05); Soil =0.42 Variety =0.49, Inoculant= 0.49, Soil × Variety = 0.84 oil x variety x inoculants =1.68,

Soil Series	Varieties	No	Inoculum				
		Inoculation	Isolate 19	Isolate 37	Isolate 57	Mean	
Oyibi	Bengbie	27.3	153.3	117.2	185.1	151.9	
	TGx 1910-2E	17.3	91.9	79.2	100.4	90.5	
	TGx 1903-7F	23.6	98.5	90.6	113.6	100.9	
	Bragg	29.2	85.2	77.4	102.1	88.2	
	Mean	24.3	107.2	91.1	125.3	-	
Hake	Bengbie	77.2	245.6	213.3	291.8	250.2	
	TGx 1910-2E	80.5	192.4	226.3	224.4	214.4	
	TGx 1903-7F	97.1	275.8	237.1	239.8	283.3	
	Bragg	18.0	115.6	144.7	118.9	126.4	
	Mean	68.2	207.4	205.4	218.7	-	
Nzima	Bengbie	25.6	128.9	120.7	90.6	113.4	
	TGx 1910-2E	10.7	205.4	130.8	114.4	150.2.	
	TGx 1903-7F	11.3	121.2	87.0	90.5	99.6	
	Bragg	17.6	110.6	106.0	111.5	109.4	
	Mean	16.3	141.5	111.1	101.8	_	

**Table 6.** Effect of inoculation with indigenous *Bradyrhizobium japonicum* isolates on Nitrogen fixationin mg N per plant of four varieties of soybean in three different soils.

LSD (P < 0.05); Soil =17.8 Variety =20.5, Inoculant= 20.5Soil ×Variety = 35.5Soil x variety x inoculants =71.0

## 4. Discussion

The fact that Nzima contained less than 50 bradyrhizobia cells per gram of soil supports the views expressed by [5] that where soybean has not been previously grown, there is generally a response to inoculation with bradyrhizobia especially for the non-promiscuous cultivars. The present study revealed that large bradyrhizobial counts occurred in soils such as Hake (6.0 x 10<sup>3</sup> cell/gsoil) and Oyibi (6.2x10 cell/gsoil) and itis not surprising that Hake supported the highest nodulation ranging from 51 to 56 nodules plant<sup>-1</sup> for the promiscuous soybean varieties in this study. This is supported by the suggestion by [6] that population range of 10<sup>3</sup> to 10<sup>4</sup> rhizobia per gram of soil should by most standard be adequate for high nodulation. The Nzima soil failed to nodulate without inoculation but did so well after inoculation suggesting that the indigenous bradyrhizobia isolate introduced to the soil medium demonstrated effectiveness and compatibility with the soybean varieties.

There were high increases in nodulation after inoculation for both the promiscuous (Bengbie, TGx1910-2F, TGx 1903-7E) and the non-promiscuous (Bragg) soybean varieties. A similar response to inoculation by cowpea brayrhizobia was assessed in the literature [11]. Nodulation of both promiscuous and non-promiscuous soybean varieties by indigenous bradyrhizobia was rather low and could not be supportive of high yield of soybean. Similar findings have been reported for promiscuous soybeans grown in the moist savanna of West Africa [19]. [16] from pot studies, also demonstrated that nodulation and N<sub>2</sub> fixation of promiscuous soybean may be increased by inoculation with effective bradyrhizobia. The current results on nodulation agree with the findings of [10] that bradyrhizobia numbers and effectiveness in N<sub>2</sub> fixation vary considerably among locations. In their study they found out that indigenous

cowpea bradyrhizobia was effective enough for inoculants production, a situation which is similar to this study that show soybean bradyrhizobia being effective for inoculants production. [23] found out that the need for inoculation of some elite promiscuous soybean breeding lines depended on effectiveness of indigenous bradyrhizobia in a given locality. However the good nodulation obtained by [18] and [20] on promiscuous soybean cultivars without inoculation in contrast to this study may support the evidence that West Africa bradyrhizobial populations may vary in number and effectiveness from one location to another and that promiscuous soybeans show considerable site-specific nodulation [21]. Many authors reported that non-promiscuous soybean varieties nodulate more than the promiscuous ones when they are inoculated [17, 9, 14]. These findings agree with the findings in this experiment where Bragg nodulated very well in Hake soils compared to the promiscuous soybean variety in Oyibi soils. Perhaps the ability of Bragg, the nonpromiscuous soybean to produce more nodules upon inoculation than the promiscuous ones in more of the soils used by those authors may be due to the fact that they used exotic bradyrhizobiainocula [20] which performed better than the indigenous ones used in this experiment. Even though soil series like Hake harbored a lot of indigenous bradyrhizobia, during the most probable number MPN count, it still responded to inoculation due to the fact that the introduced bradyrhizobia strains were more competitive than the indigenous strains in the soil. In this study, the low pH coupled with low available P in Nzima soil might have accounted for no nodulation in these soils unless inoculated. This is in agreement with available report that soil deficient in P limit the extent of nodulation and N2 fixation [15]. [24] also reported that phosphorus apart from its effect on the nodulation process and plant growth has been found to exert some direct effects on soil rhizobia.

# 5. Conclusion

It is concluded that inoculation of promiscuous and nonpromiscuous soybeans with effective bradyrhizobia may be a more important strategy for increasing nodulation, total N accumulation in soybean plants which is also an indication for nitrogen being fixed. High nodule dry weight produced by Bragg, a non-promiscuous soybean in some of the soils is an indication that its nodulation with compatible and effective Bradyrhizobium japonicum cannot be over emphasized and that where inoculants are not available it can nodulate with indigenous Bradyrhizobium for N2 fixation. The response to inoculation by the soybean lines is also an indication that the indigenous inoculants strains were so effective enough to compete with those Bradyrhizobium strains in the soil. This competitive ability of the Bradyrhizobium strains resulted in more nitrogen being fixed rendering importation of foreign inoculants for growth and yield of soybean unnecessary. It is also areliable solution for nitrogen fertilizer usage especially for peasant farmers who cultivate maize.

## References

- [1] Bell M. J., Wright, G. C., Suryantini, L., and Peoples, M. B., 1994. N<sub>2</sub> fixing capacity of peanut cultivars with different assimilate partitioning characteristics, Australian Journal of Agricultural Research. 45: 1455-1468.
- [2] Bremner, J. M., 1996. Nitrogen-Total. pp 1085-1121. In: methods of soil analysis. Chemical methods-SSSA Book series no 5. Soil Science Society of America and American Society of Agronomy. Madison Wisconsin, USA.
- [3] Broughton. W. J., and Dilworth, M, 1970. N-free nutrient solution. In: Methods in Legume Rhizobium Technology.
- [4] Chapman, H. D., 1965. Cation Exchange methods of soil analysis, part 2, chemical and microbial properties (Black, C. A., et, al., education), American Society of Agronomy, Inc Madison, Wisconsin, pp 8991-9000.
- [5] Cuttelan A. J and Hungria, M., 1994. Nitrogen nutrition and inoculation. In: Tropical Soybean Improvement and Production; Brazillian Agric. Research Ent, FAO of the United Nations. Rome.
- [6] Danso, S. K. A., 1992. Biological nitrogen fixation in tropical Agro-systems: twenty years of biological nitrogen fixation research in Africa. In Biological nitrogen fixation and sustainability of Tropical Agric. Pp 3-13. A wiley-sayce Co. Publication.
- [7] Danso, S. K. A and Owiredu J. D., (1988a). Competitiveness of introduced and indigenous cowpea *Bradyrhizobium* strains, for nodule formation on cowpea [*Vigna unguiculata* (L) Walp] in three soils. Soil Biol and Bioc 20:305-310.
- [8] Dashiell, K. E., Kueneman, E. A., Root W. R., and Singh S., 1983. Breeding tropical soybean for superior seed longevity and for nodulation with indigenous rhizobia. Pp 133-139. In: Shanmugasundaram, S and Sulzberger E. W (eds) Soybean in

- Tropical and Subtropical Cropping Systems. Fortune Printing Co. Ltd, Taiwan.
- [9] Djagbletey, D., 1995. The nitrogen fixing potential of soybean bradyrhizobia from three Ghanaian Soils. Mphil Thesis. Univ. ofGhana-Legon.
- [10] Fening, J. O. & Danso, S. K. A, 2002. Variation of symbiotic effectiveness of cowpeabradyrhizobia indigenous to Ghanaian soils. App Soil Ecol 21:23-29.
- [11] Fening J O and Danso S K A, 2001. Response of cowpea to inoculation with indigenous Bradyrhizobium strains. Trop. Sci. 41, 172-176.
- [12] Ferriera, E. M., and Marques, J. F., 1992. Selection of Portuguese *Rhizobium legumiosarum bv. trifoli* strains for production of legume inoculants. plant and soil 147: 151-158.
- [13] Genstat., 2000. Genstat for windows, Release 4.14<sup>th</sup> ed. VSN International Ltd Oxford.
- [14] Gyau, A. A., 2001. Nodulation promiscuity of soybean genotypes. Mphil. Thesis. Univ of Ghana-Legon.
- [15] Isreal, D. W., 1993. Symbiotic dinitrogen fixation and hostplant growth during development of and recovery from phosphorus deficiency. Physiol. Plant. 88: 294-300.
- [16] Kumaga, F. K, and Etu-Bonde, K., 2000. Response of two promiscuous soybean genotypes to bradyrhizobial inoculation in two Ghanaian soils. J. Ghana Association 2:99-104.
- [17] Kumaga, F. K, and Ofori, 2004. Response of soybean [Glycine max (L) Merill] to bradyrhizobial inoculation and phosphorus application. Journal of Agric. And Biol. 2: 324-327
- [18] Nangju, D., 1980. Soybean response to indigenous rhizobia as influenced by cultivar origin. Agron. J. 72: 403-406.
- [19] Okereke, G. U., Onochie, C. C., Onukwo, E., and Ekejundu, G. O., 2000. Response of introduced *Bradyrhizobium* strains infecting a promiscuous soybean cultivar. World J. Microbiol. Biotech. 16:43-48.
- [20] Pulver, E. L., Brockman, F., Wein, H. C., 1982. Nodulation of soybean cultivars with *Rhizobium*spp and their response to inoculation with *R. japonicum*. Crop Sc. 22:1065-1070.
- [21] Pulver, E. L., Kueneman, E. A., Ranga-Rao, V., 1985. Identification of promiscuous nodulating soybean. Efficient in  $N_2$  Fixation. Crop Sc. 25:660-663.
- [22] Sanginga, N., Abaidoo, K., Danshiell, R. Carsky, J. Okogun, A., 1995. Persistence and effectiveness rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. Appl. Soil Ecol., 3:16-24.
- [23] Sanginga, N., Thottapilly G., and Dashiell K., 1999. Effectiveness of prizobian odulating recent promiscuous soybean selection in the moist savanna of Nigeria. Soil Biol. Biochem. 32:127-133.
- [24] Singleton, P. W., Bohlool, B. B., Nakoo, P. L., 1992. Legume response to rhizobial inoculation in the tropics: myths andrealities. In myths and science of soils of the tropics. Soil Science Society of America. Special pub. 29:135-155.
- [25] Somasegaran, P., and Hoben, H. J., 1985. Methods in Legume Rhizobium Technology. Hawaii Institute of Tropical Agric. And Human Resources. Univ. of Hawaii.

- [26] Somasegaran P., and Hoben, H. J., 1994. Handbook for *Rhizobium* methods in Legume-*Rhizobium* Technology. Springer-Verlag, Heidelberg, Germany.
- [27] Vincent, J. M., (1970). A manual for the Practical Study of Root-Nodule Bacterial I. B. P. Handbook No. 15. Blackwell Scientific Publications, Oxford.
- [28] Woomer, P. W., Bennett, J., Yost, R., 1990. Overcoming the inflexibility of the most- probable-number procedures, Agron. Journal 82: 349-353.