

Genetic Variability Among Soybean [*Glycine max* (Merrill)] Varieties in Nitrogen Fixation in Five Ghanaian Soils

Phanuel Y. Klogo^{1, *}, Patrick K. Avumegah¹, Seth K. A Danso², Mawutor Glover¹, Victor Owusu-Gyimah³

¹Agro Enterprise Development Department, Ho Polytechnic, Ho, Ghana

²Soil Science Department, University of Ghana, Legon, Accra

³Agricultural Engineering Department, Ho Polytechnic, Ho, Ghana

Abstract

The high diversity of bradyrhizobia in tropical soils offers great advantage in making the nodulation of an array of tropical legumes possible. The nodulation, N₂ fixed (using ¹⁵N) and growth of three uninoculated promiscuous soybean varieties, TGx 813-6D, TGx 1448-2E and TGx 1903-8F and Bragg, and a non-promiscuous American-type soybean variety were examined in five soil types in a greenhouse at the University of Ghana, Legon. Except for the Bekwai soil that contained 4.0 x 10¹ *Bradyrhizobium* (sp.) cells g⁻¹ soil, the other soils contained 10³ or more cells g⁻¹ soil (1.0, 4.0, 4.6 and 6.0 x 10³ cells g⁻¹ soil in Aveime, Hatso, Adenta and Chichiwere soils, respectively). Both plant genotype and soil type significantly influenced nodulation, with TGx 813-6D and TGx 1903-8F generally forming more nodules than TGx 1448-2E, whose nodulation was similar (P<0.05) to that of Bragg. However, step-wise regression indicated that the native *Bradyrhizobium* population was most important in determining the outcome of nodulation, accounting for 56.90% of the total variation in nodule numbers. The contribution of *Bradyrhizobium* population to N₂ fixation was even higher, accounting for 74.46% the variation in % N fixed. Highest nodulation and N₂ fixation occurred in Chichiwere and Adenta, followed by Hatso and Aveime soils in that order with the TGx varieties on average deriving > 60% N from fixation, compared to 30-46%, for Bragg in these soils. No nodulation and N₂ fixation occurred in soybean in the Bekwai soil with lowest bradyrhizobia population, and averaged for all five soils, N₂ fixation contributed less than 50% of the N in the TGx varieties. The ability of promiscuous soybean varieties to nodulate with indigenous bradyrhizobia was highly variable and was strongly influenced by the abundance of bradyrhizobia cells in soil. With average of less than 50% N fixed by indigenous strains, there is scope for enhancing the symbiotic performance of TGx varieties in many soils through inoculation with more effective *Bradyrhizobium* strains.

Keywords

Bradyrhizobium, ¹⁵N Isotope, Nitrogen Fixation, Nodulation, Promiscuous, Soybean Variety

Received: November 25, 2015 / Accepted: December 24, 2015 / Published online: February 24, 2016

© 2016 The Authors. Published by American Institute of Science. This Open Access article is under the CC BY-NC license.

<http://creativecommons.org/licenses/by-nc/4.0/>

1. Introduction

Many reports have indicated that while the promiscuous soybean varieties nodulate freely in most tropical soils, the non-promiscuous lines have often failed to nodulate unless

inoculated with *Bradyrhizobium japonicum* cells [22, 30]. Specificity in bradyrhizobial requirement for nodulation in the North American soybean genotypes has been attributed to breeding from a relatively narrow genetic base [12] and the absence of diverse compatible rhizobia in soil. Observations

* Corresponding author

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

of spontaneous nodulation of the Asian-type soybeans in African soils led to the establishment of an intensive breeding programme at the IITA in Nigeria [22, 30, 29] with the aim of developing high-yielding, adapted soybean germplasm able to nodulate with indigenous rhizobia [16, 29]. This effort resulted in improved soybean genotypes designated as Tropical Glycine Cross (TGx) [16], soybean genotypes that nodulate freely with the indigenous *Bradyrhizobium* spp and thus do not need to be inoculated. Despite these claims, some experiments conducted in Nigeria and Ghana with some of these newly developed high yielding TGx soybean varieties have indicated poor nodulation and low nitrogen fixation when they were not inoculated [23, 26].

Thus although the breeding programme at IITA achieved early success in identifying genotypes that could nodulate with indigenous rhizobia in Nigerian soils, making use of this trait has proved perhaps more complex than initially imagined. For example, the selected promiscuous lines have been found to nodulate with a rather more restricted range of the indigenous cowpea bradyrhizobia that occur in soils [7]. To resolve the uncertainty, more investigations into how widespread this promiscuity is in different soils and for the many promiscuous varieties developed, and whether the earlier expectation that farmers in Africa growing them might no longer need to purchase and use bradyrhizobial inoculants to ensure satisfactory nodulation and N₂ fixation are necessary. This will call for identifying the most promising TGx varieties assessing the conditions under which the promiscuity trait may or may not be expressed, and the ability of the nodules formed by the naturally occurring bradyrhizobia to satisfy the nitrogen requirements of different TGx varieties in various localities.

2. Materials and Methods

This greenhouse study was conducted with five soils, namely, Aveime (a Chromic cambisol), Hatso (a Gleyic cambisol), Adenta (a Ferric Acrisol), Chichiwere (a Dystric fluvisol) and Bekwai (a Ferric Acrisol). No *Bradyrhizobium* inoculant was applied to seeds or into any of these soil series. 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 [3]. The Most Probable Number (MPN) method [38] was used to estimate TGx bradyrhizobia populations in the five soils. Plastic pots (18 cm high, 15 cm wide at the top and 12 cm at the base) with four holes made at the bottom were each filled with 1.2kg of 2mm size sieved soil. Seeds of three promiscuous soybean varieties, TGx813-6D, TGx 1448-2E and TGx 1903-8F and one American-type soybean, Bragg, were surface sterilized [34], pre-germinated on 1% (w/v) water

agar and incubated at 28°C for 3 days. A non-nodulating (non-nod) soybean genotype was included as a reference crop. Four seeds were planted per pot and thinned to two at 8 days after planting. A solution of 10% ¹⁵N-labeled ammonium sulphate was prepared and applied in three splits (40 ml at planting, 30 ml at 3 as well as at 5 weeks after planting) to each soil to give a total rate equivalent to 20 kgN/ha. The experiment was conducted in the green house and each treatment replicated four times in a randomized complete block design. The plants were watered by capillary flow from tap water contained in saucers placed beneath each pot. At harvest, shoots were decapitated at soil surface level, nodules were separated from the roots after careful washing, and counted. Shoots and nodules were oven-dried for 24 hours at 70°C, and weighed. Dried shoots were milled for Kjeldahl N digestion [3] and analysis of ¹⁵N in the milled samples was performed at the IAEA Laboratories in Seibersdorf on a mass spectrometer [9].

The % N derived from N₂- fixation (%Ndfa) by each legume treatment was calculated using the ¹⁵N isotope dilution equation:

$$\%Ndfa = (1 - [R \text{ legume} / R \text{ reference plant}] \times 100),$$

(where R represents atom % ¹⁵N excess)

The total amount of N derived from fixation was calculated as

$$\text{Total N-fixed} = \%Ndfa / 100 \times \text{total N in legume} [10].$$

Statistical analysis was done using Genstat statistical software version 6.1 (Genstat, 2000). Significant differences were assessed at 5% level and Mean separation was carried out by the Least Significant Difference (LSD) procedure.

3. Results

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	Available P	Calcium
			(mg kg ⁻¹)	(Me/100)	(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

The chemical analyses conducted on the five soils used show that the Bekwai soil was the most acidic, followed by the Chichiwere soil, and all five soils had sub-optimal levels of available P for plant growth (Table 1). The MPN tests indicated the presence of *Bradyrhizobium* sp. in all five soils, with the numbers and ranking being as follows Bekwai (4.0 x 10³g⁻¹ soil) < Aveime (1.0 x 10³g⁻¹ soil) < Hatso (4.0 x 10³g⁻¹ soil) < Adenta (4.6 x 10³g⁻¹ soil) < Chichiwere (6.6 x 10³g⁻¹ soil).

soil). 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, ranging from 15 to about 27 nodules per plant on TGx1448-2E and Bragg, respectively, in the Aveime soil, 24 to 33 for TGx 1448-2E and TGx 1903-8F, respectively, in the Hatso soil, 21 to 32 for Bragg and TGx 813-6D, respectively in the Adenta soil, and 23 to about 35 for Bragg and TGx 813-6D, respectively, in the Chichiwere soil (Table 2). Thus while Bragg formed the lowest number of nodules in two of the four soils in which nodulation occurred, it also formed the highest number of nodules in the Aveime soil, compared to TGx813-6D with highest nodulation in two, and TGx 1903-9F in one soil. These data are indicative of the significant differences in nodulation ($P < 0.05$) that were observed among the soil series, among soybean varieties and also the significant soil x variety interaction. On average, the highest number of nodules were formed in the Chichiwere soil, with a mean of 30 nodules plant^{-1} compared to the 20 nodules plant^{-1} formed in the Aveime

soil, the lowest among the soils.

With the varieties, TGx 813-6D with a mean of 23 nodules plant^{-1} supported highest nodulation, but this was statistically similar ($P < 0.05$) to what was formed on TGx 1903-8F (22 nodules plant^{-1}), but higher than nodulation on Bragg (19 nodules plant^{-1}) and TGx 1448-2E (18 nodules plant^{-1}). Similarly, significant differences ($P < 0.05$) occurred among the four soybean varieties and the five soil series with regards to nodule dry weight, and with a significant soil x variety interaction (Table 2). The ranking among varieties for nodule dry weight was similar to that for mean number of nodules. Significant differences were also observed between soils, with mean nodule dry weight per plant being again highest for Chichiwere series (182 mg plant^{-1}), almost double that of the lowest (97 mg plant^{-1}), for Aveime. Mean nodule dry weight values for the intermediates, plants grown on the Hatso and Adenta soils (147.8 and 134.6 mg plant^{-1} , respectively), were statistically similar ($P < 0.05$).

Table 2. Number of nodules (plant^{-1}) and dry weights (mg plant^{-1}) of nodules formed on five uninoculated soybean varieties in five soils.

Varieties	Number of nodules					Nodule dry weight (mg plant^{-1})				
	Soil					Soil				
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	Aveime	Hatso	Adenta	Chichiwere	Bekwai
TGx-813-6D	23.8	24.8	31.5	34.8	0.0	119.0	164.0	181.5	264.0	0.0
TGx1448-2E	15.0	24.0	23.3	28.3	0.0	56.5	124.0	114.0	141.5	0.0
TGx1903-8F	16.3	32.5	28.8	34.0	0.0	74.0	169.0	144.0	234.0	0.0
Bragg	26.8	25.5	20.5	23.0	0.0	136.5	134.0	99.0	89.0	0.0

LSD ($P < 0.05$); Soil=3.2, Variety=2.8, Soil x Variety=6.3 LSD ($P < 0.05$); Soil = 21.0, Variety = 18.8, Soil x Variety = 42.0

Although TGx 1903-8F overall accrued the highest plant N (mean, 147.6 mg N plant^{-1}), there were not much statistical differences ($P < 0.05$) among the varieties, with the exception of the non-nodulating variety, for which the N accumulated (mean, 87.4 mg N plant^{-1}) was lower than for any other variety. Soil effect appeared more pronounced than the varietal influence on differences in the total N acquired by plants (Table 3). Chichiwere soil series registered the highest mean value for N accumulated in plants (158.6 mg N plant^{-1}), with plants grown in the Bekwai soil series which did not support nodulation, accumulating only about a third (57.1 mg N plant^{-1}) of that in the Chichiwere soil. Aveime series, which was second lowest, was only higher than for Bekwai series, while no significant differences were observed in

comparison to the other three soils.

Significant differences ($P < 0.05$) again existed among soybean varieties and soil series for shoot dry weight (Table 3). The TGx 1903-8F variety recorded the mean highest shoot dry weight (4.83g plant^{-1}), with TGx1448-2E producing the lowest mean shoot dry weight (3.98g plant^{-1}). Mean shoot dry weight of the non-nodulating soybean was statistically not different from those of Bragg and TGx 1448-2E that formed nodules. Soil type also affected plant growth, with Chichiwere and Hatso on average producing highest shoot dry weight, while the Bekwai soil in which soybean did not form any nodules being the lowest. Aveime and Adenta soils were intermediate and statistically similar in supporting soybean growth.

Table 3. Total N and dry matter yields of four soybean varieties in five soils.

Varieties	Total N (mg N plant^{-1})					Mean shoot dry weight (g plant^{-1})				
	Soil					Soil				
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	Aveime	Hatso	Adenta	Chichiwere	Bekwai
TGx.813-6D	111.4	188.1	139.9	223.4	53.4	4.17	5.24	4.45	5.60	2.59
TGx1448-2E	111.4	154.1	157.1	160.3	52.7	3.99	4.43	4.31	4.47	2.69
TGx1903-8F	121.2	185.6	169.6	202.8	58.6	4.36	5.58	4.96	6.35	2.90
Bragg	120.4	145.7	179.3	118.0	66.2	4.58	4.82	4.85	4.37	3.34
Non-nod	90.2	107.3	98.0	86.6	54.7	4.53	5.24	4.00	4.58	2.92

LSD ($P < 0.05$); Soil=22.8, Variety= 22.8, Soil x Variety=51.1 LSD ($P < 0.05$); Soil=0.36, Variety=0.36, Soil x Variety=0.80

There were significant differences ($P < 0.05$) among soil types with regard to the proportion of soybean's N that was derived from atmospheric N_2 fixation (Table 4). None of the soybean varieties derived any of its N from atmospheric N_2 in the Bekwai soil. Also, for plants grown in the Aveime soil, appreciable N_2 fixation occurred in only two varieties, TGx 813-6D and TGx 1903-8F, with none of Bragg's N and only a negligible proportion of the N in TGx 1448-2E attributable to fixation. Besides, the highest %N fixed in soybean in the Aveime soil was only about 39 in TGx 813-6D, making Aveime the second lowest after the Bekwai soil in terms of a soil supporting N_2 fixation. For soybean grown in the Hatso soil N_2 fixation ranged from 32.1 in Bragg to 48.2 in TGx 1903-8F, with no variety deriving up to half of its N from fixation. The situation improved in the Chichiwere and Adenta soils; two varieties, TGx 819-6D and TGx 1903-8F each derived over 60% of its N from N_2 fixation in the Chichiwere soil, while all three TGx varieties obtained in excess of 60% of their N from fixation in the Adenta soil,

with the highest being 64.4% in TGx 1903-8F. Bekwai soil was thus clearly the lowest in terms of supporting N_2 fixation, followed by Aveime with a mean of 15.8%, Hatso with 39.6%, Adenta and Chichiwere, 54.5% and 55.1%, respectively. Significant varietal effects ($P < 0.05$) on the proportion of soybean's N derived from atmospheric N_2 fixation were also recorded. The range in %Ndfa was wide, from 0 in each variety to the highest of 66.4% and 67.7% in TGx 813-6D and TGx 1903-8F, respectively. Treating the Bekwai soil as an unusual case and thus excluding the zero values recorded for the Bekwai soil in arriving at the mean values, then, the best fixing variety in this study, TGx813-6D derived on average 53% of its N symbiotically, followed closely by the 51% in TGx 1903-9F, while TGx 1448-2E derived significantly less, 35.8%, with Bragg recording the lowest %Ndfa of 26.4 and was besides the only variety in which %Ndfa never reached 50% in any of the five soils examined.

Table 4. Percent and total nitrogen fixed (mg plant^{-1}) in four soybean varieties in five soils.

Varieties	% N fixed					Total N fixed (mg N plant^{-1})				
	Soil					Soil				
	Aveime	Hatso	Adenta	Chichiwere	Bekwai	Aveime	Hatso	Adenta	Chichiwere	Bekwai
TGx- 813-6D	38.8	44.6	62.8	66.4	0.0	45.5	84.1	93.8	150.5	0.0
TGx1448-2E	0.5	33.9	62.7	46.2	0.0	0.7	57.1	98.4	74.1	0.0
TGx1903-8F	24.0	48.2	64.4	67.7	0.0	29.6	90.8	110.5	137.1	0.0
Bragg	0.0	32.1	28.7	44.9	0.0	0.0	50.5	51.1	53.3	0.0

LSD= ($P < 0.05$) Soil = 4.6, Variety = 4.6, Soil x Variety =10.2LSD ($P < 0.05$) Soil =15.47, Variety =15.47. Soil x Variety =34.59

Total N fixed like the other parameters examined, displayed wide variation within the varieties (Table 4). With or without the Bekwai soil, this variation was high. Even without the Bekwai soil, the values ranged from 0 and 0.7 $\text{mg N fixed plant}^{-1}$ in Bragg and TGx 1448-2E, respectively, to 150.5 $\text{mg N fixed plant}^{-1}$ in TGx 813-6D. These differences were evident among varieties grown in each soil; for example, the almost three-fold difference of 53.3 and 150.5 mg N fixed for Bragg and TGx 813-6D, respectively, in the Chichiwere, or the zero to 45.5 mg N plant^{-1} fixed in Bragg and TGx 813-6D in the Aveime soil. Highest mean for total N fixed across soils (if we should exclude the Bekwai soil) occurred in TGx-8136D and TGx 1903-8F (92.0 and 93.5 mg plant^{-1} , respectively) compared to the 38.7 and 57.6 mg N plant^{-1} for Bragg and TGx 1448-2E. Although the % N fixed values were substantially lower if Bekwai soil was included in striking the mean, the ranking remained the same. Soils continued to have a marked effect, as exhibited by the average of 19 $\text{mg N fixed plant}^{-1}$ in the Aveime soil to the corresponding 103.8 mg , i.e., more than 5-fold difference in the Chichiwere soil.

4. Discussion

With no *Bradyrhizobium* cells inoculated unto seed or into any of the soils used, any nodule 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 major objective for breeding of these TGx cultivars, i.e., that they would nodulate freely without artificial inoculation [24], in contrast to the generally poor nodulation exhibited by most uninoculated American-type soybean genotypes in tropical soils [22, 31, 29, 16, 4, 6, 28]. The fact that the TGx varieties used in this study were well nodulated in four of the five soils, attests to their being promiscuous. Also noteworthy is the fact that the non-promiscuous American-type soybean variety, Bragg, nodulated fairly well in three of the five soils, indicating that some American-type soybean varieties may take advantage of the high diversity of bradyrhizobia in tropical soils and nodulate with the indigenous *Bradyrhizobium* strains [17, 13]. That the breeding programme must have given rise to a range in promiscuity is

evident from the observed differences in nodulation between the TGx genotypes, with TGx 813-6D and TGx 1903-8F being better at nodulating with the indigenous bradyrhizobia than was TGx 1448-2E. Similar results were obtained by [24], who also found TGx 1448-2E to form the lowest number of nodules among four uninoculated TGx varieties examined. Similarly, [21, 20] reported on differences in the abilities of different TGx varieties to nodulate with the endemic bradyrhizobial populations.

Optimum nodulation of legumes and high nitrogen fixation require the presence of high numbers of indigenous rhizobia [8, 1], and is a reliable indicator of whether or not the legume will respond to added rhizobia or fertilizer N [35]. Besides, the occurrence of a diverse distribution of *Bradyrhizobium* strains although ensures greater chances for nodulation of different varieties of a particular or different legumes, it could be a disadvantage if a large proportion of the strains happen to be competitive in nodule formation and yet ineffective in N₂ fixation. Since soils often vary considerably in the nature and abundance of their established bradyrhizobial populations [33, 28], these could, all things being equal contribute to differences in the nodulation and N₂ fixation in legumes grown on different soils. A bradyrhizobial population of 10³ g⁻¹ soil would normally be considered high, and enough for optimum nodulation of many tropical legumes [37, 6]. It is thus not surprising that with each of the Chichiwere, Adenta, Hatso and Aveime soils containing in excess of 10³ bradyrhizobiag⁻¹ soil, soybean grown in them nodulated well, with the ranking for numbers of bradyrhizobia in these soils being similar to the abundance of nodules formed. Although some soils that contained as low as 10 rhizobia gsoil⁻¹ were reported to support optimal nodulation of soybean [19] while [35] observed few responses to inoculation when numbers of indigenous rhizobia were greater than 10cells g⁻¹ soil, this is in contrast to the complete failure of nodulation of all soybean varieties in the Bekwai soil that contained 40 times as much rhizobia. However, the report of [36] 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 initiation and formation in this soil. This would then explain why [13] observed that the TGX varieties form as many as 40 nodules per plant when any of seven *Bradyrhizobium* strains was inoculated into the Bekwai soil. Thus although numbers of bradyrhizobia cells influence the outcome of inoculation responses, serious consideration must be given to prevailing soil constraints and should not be based solely on numbers established elsewhere.

From the significant soil x variety interaction obtained for nodulation and nitrogen fixation, it may 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. For example, although Bragg, a non-promiscuous variety was the one with the lowest mean nodulation in all the soils, it yet out-nodulated all the TGx varieties in the Aveime soil, a soil that also supported the lowest nodulation among the four soils in which nodulation occurred.

Undoubtedly, the most valued agronomic function of legume nodules should not necessarily be in their numbers, but more so in their ability to fix nitrogen, two parameters that are not necessarily or always directly correlated [32]. Many attempts have been made to arrive at average estimates of nitrogen fixed in various legumes. [15] reported that most estimates of N₂ fixation in soybean fall between 25% and 75%, with others estimating an average of 50%. [5, 18] estimated that soybean on average derives about 55% and, 50%, respectively, of its nitrogen from fixation, with [14] reporting that soybean seldom derives more than 60% of its N from fixation. Using these values as yardstick, then the mean % nitrogen fixed by the native *Bradyrhizobiums* trains in all three promiscuous varieties in the Adenta and Chichiwere soils, 65% and 60%, respectively, could be considered as being high, while being medium to slightly below average in the Hatso soil (41.6%), low in the Aveime soil (21.1%) and poor in the Bekwai soil (0%). The TGx varieties varied in their symbiotic capabilities with the indigenous bradyrhizobia. For both % and total N fixed, the ranking was TGx 813-6D >TGx 1903-8F >TGx 1448-2E, and with each of them being significantly higher than for the non-promiscuous Bragg variety. The highest %N fixed in each of the genotypes, 62.7, 66.4, 66.7, for TGx 1448-2E, TGx 1903-8F and TGx 813-6D, respectively, are high enough to satisfy the objectives for incorporating nodulation promiscuity into soybean lines. However, when considered for the three TGx varieties in either the four soils that supported nodulation or in all five soils, these promiscuous nodulators on average sequestered more N from soil than what N₂ fixation provided. These results may not be strange, as shown by a careful examination of the N₂ fixation data reported by [27], for which of the 20 estimates of nitrogen fixed, we spotted 17 in which more N was accrued from soil compared to only three that fixed more than 50% of their total N in the two promiscuous soybean varieties. Such data seem to suggest that a large proportion of indigenous *Bradyrhizobium* spp. (TGx) populations occurring in tropical soils could be ineffective in fixing nitrogen, as reported by [1] who observed that of 258 isolates from TGx soybean nodules, only 27% were highly effective on soybean. [1] Further

suggested that indigenous *Bradyrhizobium* spp. populations that are truly specific for the TGx varieties are often not sufficient to meet the requirements for high yields of African soybeans. [7] in another study indicated that many of the bradyrhizobia which nodulate the promiscuous varieties in African soils are relatively ineffective, and may be a subset of the general population of Cowpea *Bradyrhizobium* which could be quite variable in its representation throughout African soils. Also, the relatively smaller differences between average number of nodules formed by the TGx varieties in the four soils in which nodulation occurred than the corresponding large differences in %N derived from fixation (>60% in the Chichiwere and Adenta soils, compared with the 42% and 21% in the Hatso and Aveime soils, respectively), seem to suggest the existence of marked differences in the distribution of effective strains in the various soils. Of the three promiscuous varieties, the two (TGx 813-6D and TGx 1903-8F) that accumulated higher N and produced higher shoot dry weights than TGx1448-2E) were also associated with higher nodulation and N₂ fixation, suggesting that the higher N demand could have stimulated higher N₂ fixation [36]. Another interesting observation was, that although total N in the non-nodulating soybean cultivar was only about 60% that of TGx 813-6D, their shoot dry matter yields were similar, indicating that the non-nodulating isolate used its N more efficiently in the production of plant material (20 mg N dry matter⁻¹) than TGx 813-6D (32 mg N dry matter⁻¹) and similarly than the other nodulated cultivars.

5. Conclusion

The ability of promiscuous soybean varieties to nodulate with indigenous bradyrhizobia was highly variable and was strongly influenced by the abundance of bradyrhizobia cells in soil. With average of less than 50% N fixed by indigenous strains, there is scope for enhancing the symbiotic performance of TGx varieties in many soils through inoculation with more effective *Bradyrhizobium* strains.

Acknowledgements

Authors are grateful to the staff of the Soils Section of the International Atomic Energy Laboratory in Seibersdorf for carrying out the ¹⁵N isotopic analysis, and to Prof. Emmanuel Owusu-Bennoah for reviewing the manuscript.

References

- [1] Abaidoo R C, Dashiell K E, Sanginga N, Keyser H and Singleton P W 1999. Time-course of dinitrogen fixation of promiscuous soybean cultivars measured by the isotope dilution method. *Biol. Fertil. Soils*, 31, 187-192.

- [2] Abaidoo R C, Keyser H H, Singleton P W, Dashiell K E and Sanginga N 2007. Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean genotypes in Africa. *Appl. Soil Ecol.* 35, 57-67.
- [3] Bremner J M 1996. Nitrogen-Total. *In: Methods of Soil Analysis. Part 3 Chemical Methods- SSSA Book Series no. 5.* Pp 1085-1121. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA.
- [4] Danso S K A 1988. Nodulation of soybean in an acid soil; the influence of *Bradyrhizobium* and seed pelleting with lime and rock phosphate. *Soil Biol. Biochem.* 20, 259-260.
- [5] Danso S K A 1992. Biological nitrogen fixation in tropical agrosystems: Twenty years of biological nitrogen research in Africa. *In: Biological Nitrogen Fixation and Sustainability of Tropical Agriculture.* Ed. K Mulongoy, M Gueye and D S C Spencer. pp3-13. John Wiley and Sons Chichester.
- [6] Danso S K A and Owiredu J D 1988. Competitiveness of introduced and indigenous *Bradyrhizobium* strains for nodule formation in three soils. *Soil Biol. & Biochem.* 20, 305-310.
- [7] Eaglesham A R J 1985. Comparison of nodulation promiscuity of US and Asian-type soybeans. *Trop. Agric. Trinidad.* 69, 105-109.
- [8] Fening J O and Danso S K A 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Appl. Soil Ecol.* 21; 23-29.
- [9] Fieldler R and Proksch G 1975. The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis. *A review. Anal. Chim. Acta* 78, 1-62.
- [10] Fried M and Middelboe V 1977. Measurement of amount of nitrogen fixed by a legume crop. *Plant and Soil.* 47, 713-715.
- [11] Genstat 2000. *Genstat for windows*, Release 4.14th ed. VSN International Ltd. Oxford.
- [12] Graham P H and Temple S R 1984. Selection of improved nitrogen fixation in *Glycine max* (L) Merrill and *Phaseolus vulgaris* L. *Plant and Soil* 82, 315-327.
- [13] Gyau A A 2001. Nodulation promiscuity of soybean genotypes. M. Phil. Thesis submitted to Soil Science Department, University of Ghana, Legon.
- [14] Herridge D F and Bergersen F J 1988. Symbiotic nitrogen fixation. *In Advances in nitrogen cycling in Agricultural Ecosystems.* Ed. J R Wilson pp 46-65. CAB International, Wallingford, UK.
- [15] Keyser H H and Li F 1992. Potential for increasing biological nitrogen fixation in soybean. *Plant and Soil* 141, 119-135.
- [16] Kueneman E A, Root K E, Hohenber J 1984. Breeding of soybean for the tropics capable of nodulating effectively with indigenous *Rhizobium* spp. *Plant and Soil* 82, 387-396.
- [17] La Favre A K, Sinclair M J, La Favre J S and Eaglesham A R J. 1991. *Bradyrhizobium japonicum* native to tropical soils: novel sources of strains for inoculants for US-type soya bean. *Trop. Agric. (Trinidad)* 68, 243-248.
- [18] LaRue T A and Patterson T G 1981. How much nitrogen do legumes fix? *Adv. Agron.* 34, 15-38.

- [19] Mpeperek S and Makonese F 1996. Promiscuous nodulation of soybean (*Glycine max* L Merrill). Potential in small scale cropping systems in Zimbabwe, 7th AABNF Conference, 2 to 7 September 1996. Yamoussoukro, Cote d'Ivoire.
- [20] Mpeperek S, Javaheri F, Davis P and Giller K E 2000. Soybeans and sustainable agriculture: Promiscuous soybeans in southern Africa. *Field Crops Res.* 65, 137-149.
- [21] Mwakalakombe B R 1998. Characteristics of indigenous rhizobial isolates from three soil types of the high rainfall zone of Zambia. *In: Harnessing Biological Nitrogen Fixation in African Agriculture. Challenges and Opportunities.* Eds. S Mpeperek and F T Makonesepp 124-129. University of Zimbabwe and CTA, Harare, Zimbabwe.
- [22] Nangju D 1980. Soybean response to indigenous rhizobia as influenced by cultivar origin. *Agron. J.* 72, 403-406.
- [23] Okereke G U and Uneagbu D 1992. Nodulation and biological nitrogen fixation of 80 soybean cultivars in symbiosis with indigenous rhizobia. *World J. Microbiology and Biotech.* 8, 171-174.
- [24] Okogun J A and Sanginga N 2003. Can introduced and indigenous rhizobia strains compete for nodule formation by promiscuous soybean in the moist savannah agro-ecological zone of Nigeria. *Boil. Fertil. Soils* 38, 26-31.
- [25] Olsen S.R., and Dean L A 1965. Phosphorus. *In: Methods of Soil Analysis Part 2. Chemical and Microbiological properties.* pp 1035-1048 Eds C A Black et al. American Society of Agronomy. Inc. Madison, Wisconsin
- [26] Olufajo O, Adu J K and Okoh P N 1989. Cultivar and *Bradyrhizobium* strain effect on performance of promiscuously nodulating soybean (*Glycine max* Merrill) in the Nigerian Savanna. *Biol. Agric. Horti.* 6, 57-88.
- [27] Osunde A O, Gwan S, Bala A, Sanginga N and Okogun J A 2003. Responses to rhizobial inoculation by two promiscuous soybean cultivars in soils of the Southern Guinea Savanna Zone of Nigeria. *Biol. Fertil. Soils* 37: 274-279
- [28] Owiredu J D and Danso S K A 1988. Response of soybean to *Bradyrhizobium japonicum* inoculation in three soils in Ghana. *Soil Biol. Biochem.* 20, 311-314
- [29] Pulver E L, Brockman F and Wein H C 1982. Nodulation of soybean cultivars with rhizobium spp and their response to inoculation with *R. japonicum*. *Crop Sci.* 22, 1065-1070.
- [30] Pulver E L, Kueneman E A and Ranga-Rao V 1985. Identification of promiscuous nodulating soybean. efficient in N₂-fixation. *Crop Sci.* 25, 660-663.
- [31] Rao V R, Ayanaba A, Eaglesham A J and Kueneman E A 1981. Exploiting symbiotic nitrogen fixation for increasing soybean yields in Africa. *In: GIAM VI Global Impacts of Applied Microbiology.* pp 153-167 Eds S O Emerjinaime, O Ogumbi and S O Sanni. Academic Press, London.
- [32] Sanginga N, Carsky R J and Dashiell K 1999. Arbuscular mycorrhizal fungi response to rhizobial inoculation and cropping systems in farmers' fields in the Guinea Savanna. *Biol. Fertil. Soils.* 80, 179-186.
- [33] Singleton P W and Tavares J W 1986. Inoculation response of legumes in relation to the number and effectiveness of indigenous rhizobium populations. *Appl. Environ. Microbiol.* 51, 1013-1018.
- [34] Somasegaran P and Hoben H J 1994. Handbook for rhizobia: Methods in Legume-Rhizobium Technology. Springer Verlag Publishers. New York, USA pp 450.
- [35] Thies J E, Singleton P W, and Bohlool B B 1991a. Influence of size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Appl. Environ. Microbiol.* 57, 19-28.
- [36] Thies J E, Singleton P W, and Bohlool B B 1991b. Modelling symbiotic performance of introduced rhizobia in the field by the use of indices of indigenous population size and nitrogen status of the soil. *Appl. Environ. Microbiol.* 57, 29-37.
- [37] Weaver R W and Frederick L R 1974. Effect of inoculum rate on competitive nodulation of *Glycine max* L Merrill. II Field studies. *Agron. J.* 66, 233-236.
- [38] Woomeer J E, Bennett J and Yost R 1990. Overcoming the inflexibility of the most-probable-number procedures. *Agron. J.* 82, 349-353.